PLANT GENOME EDITING – POLICIES AND GOVERNANCE

EDITED BY: Thorben Sprink, Ralf Alexander Wilhelm, Armin Spök, Jürgen Robienski, Stephan Schleissing and Joachim Hermann Schiemann

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PLANT GENOME EDITING – POLICIES AND GOVERNANCE

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frontiers in Plant Science

EDITORIAL
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Editorial: Plant Genome Editing – Policies and Governance

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Editorial on the Research Topic

Plant Genome Editing – Policies and Governance

Genome editing and modification techniques are tools for sequence-specific changes in the plant genome. These techniques enable breeders to introduce single point mutations or new DNA sequences at a specific location in the plant genome thus for the first time enabling the precise modulation of traits of interest with unprecedented control and efficiency. The advent of genome editing has evoked enthusiasm but also controversy, creating regulatory and governance challenges worldwide. In this scenario, the Research Topic "Plant Genome Editing—Policies and Governance" aimed at collecting articles on the latest advancements and future targets of genome editing, as well as contributions addressing the regulatory, social and socioeconomic aspects, the ethics, risk assessment, management, and biosafety researches. In the following, key ideas contributed to this Research Topic are summarized which serve to illustrate the broad and complex landscape of ideas that must be addressed for plant genome editing to succeed.

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THE CONTEXT—GENOME EDITING IN AGRICULTURE

The review article by Sedeek et al. provides a broad perspective on how plant genome editing can improve crop traits in a targeted manner. The paper highlights the TALEN and CRISPR/Cas approaches providing a general overview on the historical development of the techniques and the problems which have been addressed by targeted genome editing. It focuses on practical examples improving abiotic and biotic stress resistance as well as the improvement of yield and nutritional values. Furthermore, a short excurse provides a short overview on the regulation of genome edited crops in the US and Europe.

The paper by Nadakuduti et al. also deals with targeted improvement of crops with emphasis on improving clonally propagated crops—esp. polyploids—with a special focus on potato. It provides a general overview about the delivery of genome editing tools into plants and stresses special challenges associated with genome editing in clonally propagated crops with potato as a practical example. The authors further provide a list of clonally propagated crops which have been improved by genome editing and traits which have been addressed in the individual crops.

Metje-Sprink et al. present a special application of genome editing in crops in which no DNA is used for targeted genome modification. The authors present the different methods of performing DNA-free genome editing and current applications of DNA-free genome editing in the plant sector by providing a list of DNA-free genome applications based on a systematic literature search. Furthermore, an overview about the current and potential future delivery methods of DNA-free genome editing reagents is provided and a comprehensive overview on the current regulation of genome editing in a global perspective is given.

GENOME EDITING POLICY IN EUROPE

On 25 July 2018, the European Court of Justice ruled on the interpretation of the definition of the term "genetically modified organism" in the GMO Directive 2001/18/EC. It follows from the ruling that all organisms produced by genome editing are subject to the legal framework applicable to release, placing on the market, labeling, and traceability of GMOs. In their recently published statement "Toward a scientifically justified, differentiated regulation of genome edited plants in the EU" (https://www.leopoldina.org/uploads/tx_leopublication/2019_Stellungnahme_Genomeditierte_Pflanzen_web_02.pdf), German science academies and the German Research Foundation conclude that, "due to the mounting divergence between scientific progress and legal standardization, the primarily process-based European regulatory approach is no longer justifiable" and that "potential risks can only emanate from the modified traits of the organism as a product of the breeding process, and not from the process itself." Consequently, the statement proposes—as a first step—to amend the European genetic engineering regulation in the short term. "In a second, long term step, the legal framework should be fundamentally overhauled to place the focus on novel traits and features of an organism that are relevant to the environment, health, and nature conservation, not on the underlying breeding process."

Legal and procedural uncertainties regarding genome edited organisms and possible ways forward for European GMO policy are described by Wasmer. He proposes that in a first step "the authorization procedure for GMO release can be tailored to different types of organisms by making use of existing flexibilities in GMO law." Since European competitiveness and research in green biotechnology will suffer if the problems of current GMO law are ignored, in a second step "any way forward has to aim at amending, supplementing or replacing the European GMO Directive."

How the genome editing policy in Europe is obstructing the development of new traits and is negatively influencing governance decisions and trade worldwide is described by Jouanin et al. for wheat with hypoimmunogenic gluten and by Fritsche et al. for New Zealand. Wheat with hypoimmunogenic gluten exemplifies the potential of genome editing for improving crops for human consumption where conventional breeding cannot succeed. Due to strict regulation of unintended risks at the expense of reducing the existing immunogenicity risks of patients these healthy products may become available in other parts of the world but not in Europe. Jouanin et al. strongly recommend implementing the innovation principle and argue that "Responsible Research and Innovation, involving stakeholders including patient societies in the development of gene-editing products, will enable progress toward healthy products and encourage public acceptance." After discussing the potentials and the current regulation of genome editing in New Zealand, Fritsche et al. emphasize that for the global competitiveness of a predominantly food exporting country like New Zealand it is important that innovative technologies such as genome editing are supported by modern legislation.

With his opinion on the "politicization of the precautionary principle," Aerni has put his "finger in the wound" of the debate on genetic engineering in Europe, which is characterized more by fear than expertise. At the same time, he discusses which consequences it can have for Europe, also in view to world trade, when the precautionary principle in genetic engineering legislations is abused as an argument for avoidance and an instrument of prevention without a science-based risk assessment.

The controversial debate whether at all and how to regulate genome edited plants has essentially led to the formation of two opposing schools of thoughts. Those who consider (certain types of) genome edited plants of low or negligible risks and argue for no or less regulation and those who highlight uncertainties and knowledge gaps and ask for same or similar regulations as for GMOs. The contributions by, Eckerstorfer et al. and Agapito-Tenfen et al. follow the latter type of thoughts. Against the backdrop of calls for regulatory reform in the EU Eckerstorfer et al. argue in favor of establishing a case-specific risk assessment for genome edited plants within the existing regulatory and biosafety framework. They suggest the EFSA guidance documents on GMO risk assessment to be updated allowing the risk assessment to be tailored to the level of uncertainties to be expected—depending on the novelty of trait / plant-use combinations, depth of genetic intervention, etc. This might also allow for a "risk assessment light" in case of minimal changes and of familiarity with a given trait/plant-use. A similar view is held by Agapito-Tenfen et al.. They conclude that a broader societal consensus is necessary for proceeding with genome editing and that research and innovation need to be governed not only by biosafety but also by societal needs, ethical principles, and sustainable development.

By comparing existing regulatory frameworks in the EU and non-EU countries, Eckerstorfer Engelhard et al. conclude that genome edited plants pose challenges for both process-triggered regulations (such as in the EU) and product-triggered systems (such as in the USA) and that eventually judicial and/or political decisions are needed to clarify if genome edited plants are covered by existing regulations. These still ongoing decision-making processes, however, are heading in very different directions, resulting in complex geographical patterns of different regulations. As harmonization is likely to take time and in order not to hamper international trade, they suggest an international public register for all GMOs including also all nGM in all jurisdictions—whether they are regulated or not.

The analysis of Bartkowski and Baum focusses on two main types of public action to express dissatisfaction, purchasing decisions as consumers (exit) and expressing views in deliberative settings (voice). According to their analysis the criticism on genome edited plants could represent a delayed response on the part of consumer-citizens to previous grievances, specifically because of their previously limited options to express their views. Following their line of thoughts, calls from both science and industry to reduce options for exit (by arguing that labeling is not possible or not necessary) might increase the level of citizen-consumer dissatisfaction. The authors suggest to extend the options for deliberation when further developing the regulatory

framework with respect to genome edited plants. At the same time, they acknowledge the limitations and weaknesses of such practices, such as the constraints of power dynamics and the role of emotions. Further progress in application of the exit–voice framework can prove useful by, inter alia, helping to establish the preconditions and institutional forms necessary for such strategies to be able to effectively express (and resolve) the sources of popular dissatisfaction with the food sector.

ALTERNATIVE GOVERNANCE APPROACHES

The disruptive energy of genome editing in plant biotechnology initiated discussions about the appropriateness of legal frameworks in many countries. Wolt and Wolf provide a generic overview of the US Coordinated Framework for Biotechnology and implications for further decision making. Though in the USA products derived from biotechnology are widely not considered "risky" because of the technology, societal uncertainties about applications of genome editing led regulators "to seek ways whereby these uncertainties may be addressed through redefinition of those products of biotechnology that may be subject to regulatory assessments."

Societal uncertainty arises with regards to biosafety and biosecurity as reported by Fears and ter Meulen from a workshop in Hanover, Germany, in 2017. The workshop discussed potential benefits and biosecurity concerns associated with genome editing with regards to applications in human cells, agriculture, gene drives, and microbiology. The authors highlight that "it is crucial for the scientific community to share and implement good practice in self-regulation." Sharing perspectives, facilitating information exchange, and identifying priorities for further research in biosafety and biosecurity are suggested for the scientific and biosecurity communities.

Hudson et al. discuss that modern technologies such as genome editing are not necessarily incompatible with cultural concepts that include living in harmony with nature and a special sense of responsibility for the conservation of nature. Using the example of the Maori in New Zealand, they convey an indigenous perspective and the importance of including indigenous values in the acceptance of new technologies such as genome editing in this population group.

Regulatory uncertainty around new breeding techniques is described by Lassoued et al. The success of these techniques "is not guaranteed at the scientific level alone: political influences and social acceptance significantly contribute to how crops will perform in the market." Using survey data, Lassoued et al. report results from an international panel of experts regarding the institutional and social barriers that might impede the development of new technologies. "Survey results clearly indicate that regulatory issues, social, and environmental concerns are critical to the success of precision breeding."

DETECTION/ENFORCEMENT

Genetic modifications that occur with some likelihood through natural processes or conventional breeding efforts can hardly be distinguished from equal modifications derived by genome editing. As explained by Grohmann et al. there are several methods and approaches available to detect small differences between gene sequences (e.g., to a reference genome). But a mere sequence difference tells little about the underlying process or techniques. Extended (typical) detailed sequence information from genome edited reference organisms would be necessary to identify an underlying technical intervention with sufficient certainty. The actual accessible information, technical detection limits, natural variation in the field, and costs make it practically impossible to track and identify unwanted traces of genome edited plants in traded commodities.

TRIGGERS TO GUIDE APPROPRIATE AND PROPORTIONATE GOVERNANCE

In many jurisdictions the extent to which genome edited organisms fall under specific regulatory provisions depends on the genetic characteristics of the edited organism, and whether the changes introduced in its genome do (or do not) occur naturally. Custers et al. provide a number of key considerations to assist with this evaluation as well as a guide of concrete examples of genetic alterations with an assessment of their natural occurrence. "These examples support the conclusion that for many of the common types of alterations introduced by means of genome editing, the resulting organisms would not be subject to specific biosafety regulatory provisions whenever novelty of the genetic combination is a crucial determinant."

SOCIAL AND SOCIOECONOMIC ASPECTS

In their research paper "New Plant Breeding Techniques [NPBT] Under Food Security Pressure and Lobbying" Shao et al. show that more strict regulations on the approval and use of NPBT will have negative implications for food security and that the costs of food production increase, decreasing the overall supply of food. While decision makers are exposed to lobbying and lobby groups can influence the regulation, it is important to recognize that lobbying is not only done by one group. "The more policy makers consider implications for food security, the less they will be influenced by lobby groups. In the case of NPBTs, the implication is that supporters of the technology have to lobby less than opponents or if they lobby, they will stress the importance of NPBTs for food security."

ETHICS

Ethical deliberations on the regulation of genome editing reflect the social und normative conditions for the acceptance of molecular breeding technologies. This involves both the justification of normative principles and the analysis of life-world perceptions and different interests that play a role in the implementation of plant genome editing. The first aspect is dealt with in the article by Rippe and Willemsen. In response to the objection that the idea of precaution cannot be rationally justified in the end, the authors argue "for the ethical obligation to apply precautionary measures," provided that there is a plausible

scientific justification for the fear of serious damage to health and the environment. In contrast to this position, three other contributions emphasize the limits of a mere focus on risk issues in the question of social acceptability. Hamburger identifies the different interests of the stakeholders and discusses existing regulatory concepts "that are designed to facilitate a weighing and balancing of different interests or to achieve at least a mutual effectiveness of conflicting normative criteria." Bogner and Torgersen are skeptical about the existing instruments of the Precautionary Principle (PP) and the concept of Responsible Research and Innovation (RRI). While the PP stimulates above all the expert discourse on risk issues, RRI focuses on a participatory dialogue on values in agriculture, in which existing conflicts of interest nevertheless cannot be overcome. Rather than leaving political decisions to technical risk assessment or ethics and public awareness, they argue for "re-establishing a broad yet sober process of opinion formation and informed decision-making in agricultural policy." Bechtold is also critical of the narrow focus on risk issues in the discourse on genome editing. She argues for a comprehensive deliberation of values which allow for individual decisions within our value system. As an example, she refers to food labeling and consumer choice as "an institution to support communication about values and to broaden the perspective on the agricultural use of genome editing and its products."

Since agriculture faces major challenges to deliver food and nutrition security the more sustainable production of more food requires the development of crops that will contribute significantly to attaining multiple Sustainable Development Goals. Plant genome editing could play a key role in developing these crops provided that accompanying the rapid scientific progress also policy and governance problems will be solved on national and international level. This Research Topic will contribute to shape the technology and its future use.

AUTHOR CONTRIBUTIONS

All authors contributed equally to the preparation of this editorial.

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OPINION
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Assessing Security Implications of Genome Editing: Emerging Points From an International Workshop

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Genome editing, which includes the deliberate alteration of a selected DNA sequence in a cell using targeted nucleases, is greatly facilitating basic research in the life sciences. In particular, it is contributing significantly to our understanding of biological functions and disease mechanisms. The new genome editing tools are expected to empower innovation for societal applications in human and animal health, agriculture and food systems, and the bioeconomy. As with other tools, there may also be potential for misuse, either inadvertently and associated with biosafety concerns or deliberately and associated with biosecurity concerns.

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ASSESSMENT BY ACADEMIES WORLDWIDE

Because of the rapid development and widespread use of tools such as CRISPR-Cas9 in many countries with various, sometimes divergent regulation and governance of research, international dialog is essential for resolving contentious points and evaluating the implications for ensuring responsible research and innovation. Academies of science and medicine worldwide have already undertaken considerable analysis of the potential benefits and risks of genome editing as part of their broader interests in emerging technologies in the biosciences. For example, in Europe, the European Academies Science Advisory Council (EASAC) published a report (EASAC, 2017) last year providing a broad perspective on multiple genome editing applications, and in the US, the National Academies of Science, Engineering and Medicine (NASEM) have published several comprehensive reports, including on gene drives (NASEM, 2016) and human cell editing (NASEM, 2017).

Recently, in October 2017, EASAC and NASEM, together with the global InterAcademy Partnership (IAP) and the German National Academy of Sciences Leopoldina convened an international workshop of experts in genome editing, security studies, and public policy in Herrenhausen, Germany. This meeting addressed some of the emerging implications, for potential benefits as well as potential misuse, and what might be done to mitigate any potential harm. This workshop was designed to emphasize the pivotal role of transparent and inclusive dialog with stakeholders and the promotion of a research culture that builds trust through responsibility and integrity. Researchers cannot dissociate themselves from the uses of the new knowledge they generate and they must take into consideration the reasonably foreseeable consequences of their activities.

A report of this workshop has now (January 2018) been published (IAP, 2018) and our article here briefly draws attention to some of the key areas covered in detail in the report. Initial workshop sessions explored applications of societal value spanning medicine, plant and animal breeding in agriculture, microbial production, and gene drive systems that might transform an entire population of a selected species. Some of the potential opportunities are listed in **Table 1**. Participants in the workshop acknowledged the importance of doing more to share good practice in research policy and regulation worldwide to allow the flexibility to manage and enable innovation.

TABLE 1 | Examples of points emerging from workshop breakout session discussions.

Application of genome editing	Potential benefits	Potential security concerns
Human cells	Better understanding of disease; new targets and models in drug discovery and development; treatments for single or multiple gene disorders	Off-label use somatic editing for enhancement of individuals (for muscle mass, neurology), e.g., for military purposes; germline modification of future populations
Plants and animals in agriculture	Crops with higher yield, increased nutrient content, resistance to biotic and abiotic stress; improved livestock health, welfare, and productivity	No new categories of risk discerned but research locations may expand beyond traditional management frameworks; relative lack of traceability in edits challenges regulation and enforcement
Gene drive	Control of insect vectors of disease; correcting previous disturbances to vulnerable ecosystems (e.g., reversing rodent invasion of islands)	Potential threats to health and agriculture (although it is assumed there would be easier ways to cause harm); concern that controversy may undermine broader public confidence in science
Microbes	Novel pharmaceuticals, including antibiotics, high-value chemicals, biofuels, biosensors, and other applications in bioeconomy	Similar possibilities to other microbial manipulation methods, e.g., altered pathogens; digitalization of DNA increasingly important in widening access to results

See IAP (2018) for further detail.

ADDRESSING CONCERNS ON POTENTIAL MISUSE

A main focus of the workshop was to review concerns about misuse, appertaining to the possibilities that the widespread adoption of genome editing might expand research outside of regulated laboratory settings and, wherever located, might also elicit new national security concerns. For example, in the US, security concerns have been expressed by the President's Council of Advisers on Science and Technology and by the national intelligence community (EASAC, 2017; Fears and ter Meulen, 2017) and NGOs have also inferred (Friends of the Earth, 2017) that government funding of gene drive research denotes military or other national security interests. Although these security alarms lack detail, it is relevant to include consideration of possibilities for misuse when deriving principles for the responsible use of biotechnologies (Wolpe et al., 2017), including genome editing. Academies of science and their networks have been assiduous in these regards (NASEM, 2016, 2017; EASAC, 2017, and see IAP, 2018 for other academy sources). Because some of the security concerns may be application specific and because public anxieties about genome editing—whether relating to safety or security—tend to be about the specific application rather than the technology itself (Gaskell et al., 2017), the workshop was designed also to evaluate concerns in terms of specific sectors, although it became clear that some concerns crossed application boundaries (**Table 1**).

We emphasize that the focus of the discussions was on "potential." The science is advancing rapidly but timeframes are uncertain and, indeed, proof of principle for the application has not yet been established in many cases. Therefore, reaching consensus on which, if any, concerns are realistic will be challenging. More robust assessment of the feasibility and probability of concerns is warranted, and there is need for better understanding about the conditions that may repurpose technology for hostile use—evaluating intent as well as accessibility. Moreover, it can be difficult to separate safety from security consequences. Nonetheless, even though there is much more to be done in clarifying the evidence base, the academies through IAP, have already built good connections with policy-makers, particularly in the Biological and Toxins Weapons Convention, to inform about recent scientific developments (IAP, 2017).

There is more to be done to clarify what is new about the issues raised by genome editing, whether these new tools will facilitate outcomes that could already be imagined by other methods, and whether additional risks are conferred. Even if the advent of genome editing were to raise new issues, these should be set into a broader context. First, to appreciate that the success of new tools depends on the opportunities created by the accumulation of other modern biosciences research outputs (particularly those associated with declining costs of gene sequencing and synthesis) so that a much larger accrual of research advances is necessarily implicated in any concerns. Second, to appreciate that the wider use of such tools does not in itself promote intent to nefarious action.

What are the possibilities to prevent or mitigate security issues? When genome editing is viewed in the broader context, it can be seen that there is a wide range of legal, regulatory and policy strategies, norms of responsible behavior and voluntary guidelines, together with educational, scientific, and technical strategies already available to mitigate potential risks. The disparate elements in this framework of protection are discussed in detail in the report (IAP, 2018) and, to note just one of these elements, it is crucial for the scientific community to share and implement good practice in self-regulation. The German National Academy of Sciences Leopoldina has worked with scientific partners to develop model rules (DFG and Leopoldina, 2016) on scientific freedom and responsibility in handling security-relevant research. Committing to self-regulation, while minimizing bureaucracy, helps to address a common concern within the scientific community that additional governance measures would hamper responsible research without diminishing the likelihood of intentional misuse.

PUBLIC ENGAGEMENT AND GLOBAL COORDINATION

The workshop concluded with a discussion of the next steps required both to ascertain and clarify what is currently uncertain in the evidence base and to communicate about the continuing

responsibility of the scientific community to tackle these complex topics. As with other emerging technologies, a lack of communication about uncertainties may undermine public confidence in science. It is vital that younger scientists and researchers worldwide have a voice in the continuing public dialog. Standards of evidence are important. Scientists need to build trust with the security community as well as the public-at-large, recognizing that there are differing perceptions of threats and differing expectations of evidence. There should be balanced and open discussion of the potential benefits and any safety or security issues, particularly as they relate to consumers. In order to resolve uncertainties, the dimensions of security must be well defined: security concerns can apply to public health, food, national economies, data, and privacy, for example, as well as to biological weapons. Many consider that genome editing can itself assist in tackling security challenges, such as for health and food, and help to provide countermeasures.

It is important to develop international coherence in research management to enable innovation (Gaskell et al., 2017), and the workshop discussion emphasized opportunities for global coordination in responsible science guidelines and their monitoring, research standards, risk assessment, and management procedures. Risk assessment and mitigation are intrinsic to all scientific developments. The academy organizers regard this intensive and diverse workshop as a significant first step in an ongoing process. It is deemed highly desirable to develop a sustainable network encompassing the scientific and security communities, and others, to share perspectives, facilitate information exchange, identify priorities for further study, and serve as a basis for extending engagement more widely.

AUTHOR CONTRIBUTIONS

RF provided a first draft of this article and of the Herrenhausen report (IAP, 2018). VtM chaired the organizing committee of the Herrenhausen workshop and the earlier EASAC Working Group (EASAC, 2017) and revised this article.

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Regulatory Uncertainty Around New Breeding Techniques

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Emerging precision breeding techniques have great potential to develop new crop varieties with specific traits that can contribute to ensuring future food security in a time of increasing climate change pressures, such as disease, insects and drought. These techniques offer options for crop trait development in both private and public sector breeding programs. Yet, the success of new breeding techniques is not guaranteed at the scientific level alone: political influences and social acceptance significantly contribute to how crops will perform in the market. Using survey data, we report results from an international panel of experts regarding the institutional and social barriers that might impede the development of new plant technologies. Survey results clearly indicate that regulatory issues, social, and environmental concerns are critical to the success of precision breeding. The cross-regional analysis shows heterogeneity between Europeans and North Americans, particularly regarding political attitudes and social perceptions of targeted breeding techniques.

Keywords: innovation, uncertainty, gene editing, agricultural biotechnology, European Union, United States, new breeding techniques, food security

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INTRODUCTION

Modern crop biotechnology has been dynamically progressing through increases in the knowledge about, and applications of, genomics. Scientific advancements have yielded more sophisticated and targeted breeding techniques—known as new breeding techniques (NBTs)—resulting in plants with novel traits including pest and disease resistance, stress tolerance, and improved quality attributes (Sprink et al., 2016). In addition to their simplicity, many NBTs allow clear-cut and reliable mutations, setting them apart from previous genetically modified (GM) crops. The ability to improve crop varieties through the precise addition of useful traits or deletion of undesirable phenotypes (known as gene editing) has to the potential to lower technology development costs and reduce development time (Abdallah et al., 2015). Regardless of their scientific potential, NBTs have been, and are being viewed as a radically controversial innovation in some countries. While some jurisdictions have decided to treat some new plant technologies as simply a variation of existing conventional plant breeding and apply case-by-case assessment (e.g., United States, Canada, Argentina, Brazil, Chile, Columbia, China, Sweden and Australia), others remain mired in uncertainty, unable to determine what to do or how to proceed to regulate (e.g., the EU and France, which both are seeking to use the technology as a trigger).

Regional differences in public expectations and consumer attitudes toward the use of biotechnology in agriculture and its impact on food production and international trade have a lengthy history of examination between the United States and Europe (Gaskell et al., 1999; Jasanoff, 2015; Lau, 2015). Many studies have shown that Europeans' acceptance of agricultural biotech

products is low compared to Americans (Einsele, 2007; Aerni, 2014). As a result, production and consumption policies for transgenic products in the European Union (EU) and North America diverge (Smyth et al., 2013). While the EU endorses the precautionary principle and explicitly incorporates speculative discussion of uncertain risks in its review of GM crops, Canada and the United States focus on managing largely foreseeable risks (Wiener and Rogers, 2002). Why do the EU, Canada and the United States regulate the same technology differently despite their similar economic circumstances as high-income, food exporting nations? In part, the answer lies in public perception (i.e., the subjective assessment of risks and benefits). While Americans have a generally positive attitude on the safety and benefits of biotech crops, most Europeans have a negative opinion (Einsele, 2007). Thus, technology adoption for crop improvement will depend not only on the best scientific method and evidence, but also on effectively and appropriately engaging with the public and industry in the regulatory space (Chapotin and Wolt, 2007).

The innovation literature has largely covered technological and commercial uncertainties, but only superficially explored social debates (Hall et al., 2011). Genetic technology in agriculture has disrupted long-standing acceptance and motivated a range of third parties and stakeholders to engage in the debate. This paper reviews the socio-economic uncertainty triggered by the introduction of NBTs and assesses how this uncertainty influences regulatory assessment and social acceptance of emerging technologies in the agri-food context. Rather than exploring societal concerns from a public or a consumer perspective, we are interested in the cross-cultural differences in expert opinion and, more fundamentally, to what extent do country of origin or field of expertise influence opinions on innovation. We surveyed scientists in industry, government and universities, as well as social scientists. We test whether expert opinions on novel plant biotechnology are influenced by a respondent's home county as well as to their area of expertise (natural science vs. social sciences).

Using contingency analysis of survey data, this paper deepens the understanding of innovation-related uncertainties of the set of precision breeding tools that are expected to make a crucial contribution to the future of global food security. This paper has five parts: the next section provides a brief theoretical background on innovation and uncertainty; the third elaborates on the research methodology; the fourth presents and discusses the survey results; and this is followed by conclusions.

INNOVATION, REGULATION, AND UNCERTAINTY

Uncertainty is an intrinsic characteristic of innovation as the potential benefits of any specific innovative product or process might be achieved in the future (Jalonen, 2012). In fact, innovations can introduce a wide-range of unintended, often undesirable, health, environmental and social side effects. Risk assessment is a standard approach used to reduce innovation-related uncertainty (Peters et al., 2007). These requirements—with their costs and delays—do not necessarily increase public confidence in biotechnology. Extensive regulatory assessment of plant technologies subject to precautionary principles has led to relatively negative public attitudes to transgenic products (Einsele, 2007; Marchant and Stevens, 2015). Thus, more regulatory oversight might increase public skepticism toward agricultural biotechnology rather than build trust.

The success of agricultural and food innovations depends very much on acceptance by consumers, regulators, and non-governmental organizations (NGOs). The involvement of these secondary stakeholders with conflicting interests creates ambiguity and more complexity (Hall and Martin, 2005). As posited by Aldrich and Fiol (1994), the acceptance of innovation depends on its level of socio-political legitimacy, where cultural aspects and political influences matter. "An innovation thus establishes its legitimacy when its technical performance and social acceptance co-evolves and expands, thus reducing uncertainty" (Hall et al., 2011: 1149). Based on these insights, we emphasize that the legal environment and the social context can either enhance or hinder the success of precision breeding. That is, the success of NBTs is not guaranteed at the scientific level alone, but that political socio-cultural influences significantly contribute to how it will perform in the market.

In the context of plant breeding, in the last two decades scientific progress has created a range of new tools that fall between genetic engineering and conventional techniques (Sprink et al., 2016). Yet, application of NBTs (with its subset of gene editing) lacks legal clarity. One reason could be the large spectrum of NBTs under evaluation. Some techniques are a refinement of conventional breeding, and do not alter the genetic material such as the case of RNA-dependent DNA methylation (RdDM) (HLG-SAM, 2017). Some forms of gene-editing tools including clustered regularly interspaced short palindromic repeats (CRISPR), transcription activator-like effector nuclease (TALEN) and ***zinc-finger nucleases*** (ZFN) induce site-specific genome changes via the development of site-directed nucleases (SDNs). As these point mutations are precision alterations (SDN1 and SDN2), final products are transgene-free and might escape the GM rules (Araki and Ishii, 2015). Other gene editing tools involve gene insertions and are likely to yield transgenic products (SDN3). With the advent of various NBTs and their heterogeneity (e.g., different molecular processes, variety of derived products), countries differ in how they regulate the technologies (Lassoued et al., 2018).

Absence of institutional arrangements governing these new techniques will likely have detrimental effects for their development. In spite of the fact that many European researchers have been leading the development of new crop biotechnology (Eriksson et al., 2018), EU regulatory quandaries around agricultural biotechnology have harshly affected innovation by discouraging scientists from using novel techniques, rejecting research funding applications, and shifting research investment out of the EU (Sprink et al., 2016). In essence, new crops and new technologies cannot prosper without legal authorization. Legal uncertainty creates commercial uncertainty; the more ambiguous are the regulations surrounding NBTs, the more developers are uncertain. Thus, we focus on the regulatory and social uncertainties next.

Regulations and institutional constraints are typically used to protect public health as well as the environment. They may be developed to constrain or support innovation—limiting specific applications or uses through licensing or promoting commercialization through intellectual property rights. The diffusion of novel breeding approaches to crop-trait development depends crucially on appropriate governance of new technologies. Currently, the rules governing agricultural biotechnology do not necessarily directly apply to NBTs; as already noted, that is a policy decision, with different countries making different judgements. As many NBT derived products share phenotypic similarity with conventionally-bred counterparts, logic follows that they should not be classified as regulated forms of GM. Experts have judged that the potential risks of using techniques like gene editing are comparable to conventional and transgenic technologies (EFSA, 2012). And, therein lies the uncertainty around the legal status of NBTs. Except for Canada, most nations tend to assess novel plants based on the process employed rather than the product's new phenotype, which would likely exempt gene-edited varieties from extensive review. This is an ongoing process in Europe. The 2001 directive governing the release of GMOs in the environment is under interpretation by the European Court of Justice; there is some indication there might be a softening of gene-edited rules, especially when a technique such as CRISPR involves targeted changes to the genome (Abbott, 2018). In the meantime, decisions are made on a case-by-case basis in other parts of the world. In the United States, for instance, authorities exempted many gene-edited crops from GM regulations by providing guidance to product developers through responses to formal review letters (Jones, 2015; Wolt et al., 2016; USDA, 2018). In contrast, the EU has not provided any legal guidance yet for NBT applications (Eriksson et al., 2018). While many NBTs will fall outside the GM regulatory criteria, this may vary by region. We would expect that given the diverging regulatory processes in the United States and EU, that American and European experts might have different opinions on NBTs and their uses.

SOCIAL UNCERTAINTY

Social uncertainty is caused by incomplete information and "is located in the social field, where hesitancy, vagueness, ambiguity or lack of confidence is [are] reflexive characteristics of social objects or actors in a community" (Pillania, 2011, p. 1159). Social uncertainty related to technology refers to whether an innovative product aligns with public values, beliefs and interests. In a way, it is also a judgment of the perception of the performance as well as the competence of social institutions.

A gap exists between the wide-spread farming of biotech crops across the world and the low public acceptance (Lucht, 2015). Despite the historical record on the safety of GM products, consumer opinions around the world are mixed, and social acceptance of biotech products has been limited in many countries. In part, this is due to the reality that the media is the prime source of information available to many consumers. The focus on technological risks in the media, and the vested interests of political stakeholders holding extreme positions, has worked to stigmatize biotechnology in many markets (Aerni, 2002). In addition, European NGOs have been successful in framing biotechnology as a menace. Einsele (2007) argues that the negative reports in newspapers by anti-GM lobbies turned the public against plant biotechnology in Europe.

It is fair to note that global consumer perception of biotech products has been slowly becoming more favorable, especially for output trait (second-generation) GM products that offer consumer health benefits. Earlier studies found that (American) consumers supported transgenic products if they satisfied specific needs such as enhanced nutrition (Hossain et al., 2003), and that they were willing to pay premiums to buy them (Lusk et al., 2003; Kaneko and Chern, 2005). Recent studies have shown that consumers are willing to accept biotech products if transparent information of product safety is shared (Evans and Ballen, 2014). In the same vein, some assert consumers will welcome products of NBTs if labeling adheres to the "Right to Know" rule.

Agricultural biotechnologies such as gene editing might be viewed differently in different countries, resulting in different regulatory and market decisions. It is expected that the highest degree of uncertainty lies within the social dimension (which is arguably the most complex) as there are more groups to accommodate (e.g., local, national and international communities, environmental activists). In addition to a consumer's mindset, social uncertainty is affected when civil society movements question the safety or efficacy of novel technologies (Paarlberg, 2014). One example of this was in 2015, when the European Academies' Science Advisory Council (2015) advised EU regulators that NBT-derived products, which are free of foreign gene(s), do not require GM regulation. Anti-GMO NGOs called on the Commission to ensure that NBTs be regulated within the current GM legislation framework (NGO-coalition, 2015). Thus, adoption of precision breeding could be hampered by public understanding and social acceptance rather than by technological aspects (Araki and Ishii, 2015).

Awareness of and appreciation for the benefits of these viable alternatives to transgenic crop breeding methods for crop improvement might reduce regulatory oversight (Wolt et al., 2016). If novel plant traits are not understood and accepted by the public, political pressure to have them evaluated under GM biosafety rules will increase, decreasing the availability of NBTs to public breeders in many, if not most, countries.

MATERIALS AND METHODS

The data used for the analysis reported in this paper stems from two online surveys examining the socio-regulatory aspects of uncertainty as it relates to NBTs. The regulatory survey was emailed to an expert panel of 638 on January 2016, and the social survey was emailed to 630 participants in May 2017. Both surveys have run for a 4-month period each with biweekly reminders. The questionnaires have comparable structures, asking respondents to rank the limiting factors to the development of NBTs, and to identify their sources of confidence used to form opinions.

These surveys are part of a multi-year project investigating risk preferences among experts regarding innovative plant breeding[1]. The target population includes scientists, regulators, and business professionals with backgrounds and experiences in agricultural biotechnology. A contact database was constructed using emails of participants in from a number of conferences on GM technology organized by the researchers dating over the past 15 years, and of experts from online searches (university websites, biotechnology research institutions, governmental agencies websites, etc.). Recruiting a large panel of international experts online is a challenging task: this method allowed us to reach out to a large number of international experts in the field of study.

In October 2015, an introductory recruitment effort was conducted. Those that enrolled in the research panel provided socio-demographic information and answers to a series of decision-making questions (survey materials are available on the website). Prospective panelists were asked about their primary current job and to identify themselves as scientist, regulator, policy advisor, economist, etc. Based on the answers, the researchers grouped the panelists into scientists—mostly according to plant/natural sciences, and social sciences. An expertise variable was used in the analysis to compare groups of experts. Respondents were also asked about their country of residence (chosen from a drop-down menu). For analytical purposes, the countries were grouped into three regions: North America, Europe and the rest of the world.

Our study (BEH 97) was exempt from full ethics review by the Behavioral Ethics Board at the University of Saskatchewan on April 7, 2015. The exemption status was based on the fact that the participants are not themselves the focus of the research per the Tri-Council Policy Statement: Ethical Conduct for Research Involving Humans, December 2014, Exemption Article 2.1.

RESULTS AND ANALYSIS

This section reports survey results on the sample characteristics and the contingency analysis. The questionnaires on the regulatory and social uncertainties of NBTs were completed by 201 and 173 respondents, yielding response rates of 31.5 and 27.5% respectively. Tabulated statistics and Chi-square analysis are reported on two categorical variables: expertise and region. The variable expertise includes two groups: scientists or scientific experts (about 40% of the sample), and non-scientists and social scientists, including regulators and industry professionals (about 60%). Considering the size of our sample, we aggregated results to regions rather than countries, as the Chi-square statistic is sensitive to sample size (i.e., it needs large expected frequencies). The variable region includes North America (NA: Canada and United States: about 50%), Europe (25%), and the rest of the world (ROW: Asia, Africa, Oceania, Central and South America: 25%). We assess the differences in opinions between groups and regions with respect to NBT-related regulatory and social uncertainties.

The panel is dominated by males (79%), aged between 45 and 65 years (70%). As mentioned above, nearly half of the panelists reside in North America, a quarter in Europe, and the remainder in the ROW (5% from Central and Latin America, 5% from Australia and New Zealand and 3% from Africa). The majority of subjects hold a PhD degree (71%); 20% have a masters' degree. Eighty percent are employed and 14% are self-employed. Forty percent work for industry, 26% for university, and 20% for government. Panelists were asked about the type of crops and markets they work with. Main crops of interest include cereals (63%), oilseeds (43%), pulses (39%) and vegetables (25%). More than 70% of the sample works with both food and feed, 43% on fiber, 37% on industrial ingredients, and 29% on environmental services.

Below, we report survey results on the regulatory and social uncertainties. We would like to briefly mention that while we did not report the technical uncertainty of NBTs here, we conducted a survey on the topic. Key results show that intellectual property (IP) and patents, public funding and technological uncertainty were deemed the top three major hurdles to the development of most novel techniques. In addition, 60% of participants felt moderately confident answering the questions related to the scientific uncertainty of NBTs. A further 21% felt very confident. About one fifth lacked confidence. Results suggest that respondents have moderate to high confidence in their answers thus reflecting knowledge of new breeding techniques. We report detailed results about the regulatory issues as the highest degree of uncertainty lies within these dimensions.

REGULATORY UNCERTAINTY RESULTS

Participants were asked about the regulation of NBT techniques. As displayed in **Table 1**, over half of the sample

[1]https://research-groups.usask.ca/nbt-regulation/

TABLE 1 | Opinions of appropriate regulation of NBT derived crops, differentiated by region and type of respondent (% of total).

	Regions			Total	*Expert groups*	
	NA	**Europe**	**ROW**		**Scientists**	**Non-scientists**
NBT derived products should be regulated as GM technology	10	2	4	16	7	9
NBT derived products should not be regulated as GM technology	18	7	6	32	12	20
Some NBT derived products should be regulated as GM technology while others should not	20	16	17	52	23	29
Total	48	25	27	100	42	58
Chi-square statistic	$\chi^2 = 8.578$; $p = 0.073$				$\chi^2 = 0.797$; $p = 0.671$	

(52%) indicated that some crops generated via precision breeding should be regulated as GM products whereas 32% believe they should not be regulated as such. Only 16% consider NBT derived crops to be like, or similar to, transgenic crops. Survey results show that respondents believe products of synthetic biology and of targeted gene editing techniques involving gene insertions or substitutions should fall in the same regulatory space as products produced by transgenesis.

We conducted cross-tabulation for both region and expertise; those with *p*-values greater than 0.05 indicate statistical independence of the variables of interest. There is no statistically significant difference in the opinions about how NBTs should be regulated among the three regions. Indeed, the majority of the sample (52% that specifically includes 20, 16, and 17% of North Americans, Europeans and the ROW, respectively) agrees that some NBT products should be regulated as GM products while others should not. Similarly, expertise is not found to affect responses. Similar proportions of scientists and non-scientists share opinions about the regulation of NBT-derived products. Despite the diverging regulatory systems around the world that govern biotechnology (i.e., process-based system in Europe, product-based system in Canada, and a hybrid system in United States), experts did not differ about how NBT techniques should be regulated. According to Marchant and Stevens (2015), nations should move toward a product-based approach as it would be more sustainable for newer methods of crop breeding.

Respondents were provided with a list of factors that might explain innovation-related regulatory uncertainty. They were invited to rank up to five factors they thought were the most limiting to the development of NBTs. One-quarter of the sample indicated that political involvement in the regulatory process, followed by unsynchronized approval between countries, are the most limiting factors facing NBTs (See **Table 2**). Inconsistent international standards, incomplete national regulatory rules, high regulatory compliance costs, and regulatory delays were other critical factors affecting emergence of NBTs.

Participants were asked to rank seven proposed sources of confidence they might rely upon to form their answers about the regulatory uncertainty of NBTs. The survey revealed that half of the sample tended to rely on their personal experience (54%), information from regulatory agencies (48%) and from academic studies (42%). It is interesting to note that information from NGOs was mentioned by 24% of respondents (See **Table 3**). When asked how confident they felt in answering the regulatory uncertainty question, 40% were moderately confident and 36% were very confident. Less than a quarter of the sample was slightly confident and only 5% were not confident.

The panel was asked whether their domestic government would adopt policies in line with their views (**Table 4**). Respondents seem to fall into two main groups—those who think that their government will (*definitely* and *probably*) adopt policies in line with their views (57%), and those who think that their government will (*definitely* and *probably*) not align with their views (43%). The crosstabs show some regional divergence ($p < 0.001$). The majority of NA and ROW respondents, including 31% (representing 60% of NA respondents) and 18% (representing 75% of ROW respondents) respectively, think their governments will adopt policies in line with their views, while the majority of Europeans (16%, which represents 67%) think the opposite. This is not surprising given the rigid nature of EU legislation toward crop biotechnology. There was no evidence that experts diverged with respect to policy adoption: a majority of scientists (23%, which represents 58%) and of non-scientists (34%, which represents 57%) think that their domestic government will (*definitely* and *probably*) adopt policies in line with their views.

When asked about the likelihood of approving NBTs, 52% indicated that they are either optimistic or very optimistic, while 15% were pessimistic or very pessimistic. Almost a third were neutral in their views. Contingency analysis in **Table 5** shows that respondents exhibited different levels of optimism regarding the likelihood of approving NBTs depending on their home region.

TABLE 2 | Regulatory barriers to the development of NBTs.

Limiting factors	Percentage
Political involvement in regulatory process	24
Unsynchronized approval between countries	20
Inconsistent international standards	19
Incomplete national regulatory rules	17
High regulatory compliance costs	17
Regulatory delays	16
Lack of skilled staff among regulators	7
Lack of scientific evidence	7
Inadequate infrastructure to carry out experiments and/or field trials	4
Lack of baseline data	6
Shortage of staff among regulators	3
Inadequate funding	3
Overly rigorous confidential business information	3

The score is a weighted sum value of the 5 ranked responses. Items ranked first were multiplied by 0.5. Ranks 2, 3, 4, and 5 were weighted 0.4, 0.3, 0.2, and 0.1, respectively.

TABLE 3 | Trusted sources of information on regulatory matters.

Sources of confidence	Percentage
My personal experience	54
Information from national regulatory agencies	48
Information from academic studies	42
Information from international regulatory agencies	41
Information from advisory bodies	37
Information from companies	37
Information from NGOs	24

The score is a weighted sum value of the 7 ranked responses where 1st, 2nd, 3rd, 4th, 5tg, 6th, and 7th choices were weighted 0.7, 0.6, 0.5, 0.4, 0.3, 0.2, and 0.1, respectively.

TABLE 4 | Policy alignment between expert view and government regulation, by region and group (% of total).

	Regions			Total	*Expert groups*	
	NA	**Europe**	**ROW**		**Scientists**	**Non-scientists**
(Probably/Definitely) Yes	31	8	18	57	23	34
(Probably/Definitely) No	21	16	6	43	17	26
Total	52	24	24	100	40	60
Chi-square statistic	$\chi^2 = 15.278$, $\mathbf{p < 0.001}$				$\chi^2 = 0.042$, $p = 0.837$	

The scale options "Probably yes" and "Definitely yes" were recoded as "Yes" to increase the cell count. Same for "No." The recoding does not affect the result interpretation. Bold value indicates significant p-values at 0.05.

TABLE 5 | Opinions on likelihood of governments approving NBTs, differentiate by region and group (% of total).

	Regions			Total	*Expert groups*	
	NA	**Europe**	**ROW**		**Scientists**	**Non-scientists**
Optimistic/Very optimistic	29	6	17	52	23	29
Neutral	18	8	7	33	11	22
Pessimistic/Very pessimistic	5	9	1	15	6	9
Total	52	23	25	100	40	60
Chi-square statistic	$\chi^2 = 31.392$, $\mathbf{p < 0.001}$				$\chi^2 = 1.793$, $p = 0.408$	

To increase the cell count, the scale options "Very optimistic" and "Optimistic" were grouped together. Similarly for "Very Pessimistic" and "Pessimistic". Bold value indicates significant p-values at 0.05.

The majority of North Americans (29%, which represents 56%) were optimistic, while Europeans (17%, which represents 74%) were more pessimistic or neutral. The current strict EU legal regime governing agricultural biotechnology—mainly based on the precautionary principle—might contribute to this divergence. There is no evidence that experts diverge, as the majority of both groups of experts (52%) are optimistic about the likelihood of approving NBTs in the future.

SOCIAL UNCERTAINTY RESULTS

Participants were asked to rank a list of socially-related factors that could limit the success of precision breeding. About one-third of the sample (34%) ranked public perceptions—led by social objections—as the most critical obstacle to the development of NBTs, followed by food/human safety concerns (mainly toxicity and allergenicity) at 27%, and environmental concerns (e.g., increased use of chemicals in agriculture and loss of biodiversity) at 21%. Animal/feed safety concerns were identified as a limiting factor by only 12%.

Panelists were asked about the five most important sources of confidence they used to form their answers. Results of **Table 6** show that university scientists are the most highly trusted at 29%. Regulators (18%), farmers/farmer organizations (17%) and environmental groups (16%) were closely grouped. Retailers (2%), private firms (3%), ethics committees (3%) and medical doctors (4%) ranked quite low. This finding confirms the significance of scientific evidence on the subject of innovative breeding.

As shown in **Tables 7** and **8**, 70% of the experts think that people from their country perceive some benefits from products obtained via precision breeding, against 90% who think that people perceive some risks from these products. In **Table 9**, 54% of the respondents indicated that people believe that NBTs can (*definitely/probably*) improve global food security. Contingency analysis shows that Europeans do not agree with other countries about the perceived benefits of NBT products ($p < 0.001$); moreover, they do not believe NBTs have much potential to address food insecurity ($p = 0.006$). Specifically, 45% of non-European respondents, but only 9% of their European counterparts, said that people

TABLE 6 | Trusted sources of information and judgment on social aspects of NBTs.

Sources of confidence	**Percentage**
University scientists	29
Regulators	18
Farmers/Farmer organizations	17
Environmental groups	16
Industry associations	12
Consumers' organizations	12
Advocacy groups	12
Social media websites	8
Politicians	5
Medical doctors	4
Other	4
Firms	3
Ethics committees	3
Religious leaders	2
Retailers	2
Primary education system	2

TABLE 7 | Opinions of fellow citizens regarding perceived benefits from NBT derived products among regions and among experts (% of total).

	Regions			Total	*Expert groups*	
	NA	**Europe**	**ROW**		**Scientists**	**Non-scientists**
(Probably/Definitely) Yes	41	12	16	70	25	45
(Probably/Definitely) No	8	15	7	30	15	15
Total	49	27	23	100	40	60
Chi-square statistic	$\chi^2 = 18.069$, $\mathbf{p < 0.001}$				$\chi^2 = 2.592$, $p = 0.107$	

Bold value indicates significant p-values at 0.05.

TABLE 8 | Opinions of fellow citizens regarding perceived risks from NBT derived products among regions and among experts (% of total).

	Regions			Total	*Expert groups*	
	NA	**Europe**	**ROW**		**Scientists**	**Non-scientists**
(Probably/Definitely) Yes	47	25	19	90	33	58
(Probably/Definitely) No	2	3	4	10	7	2
Total	49	28	23	100	40	60
Chi-square statistic	$\chi^2 = 5.542$, $p = 0.063$				$\chi^2 = 9.364$, $\mathbf{p = 0.002}$	

Bold value indicates significant p-values at 0.05.

TABLE 9 | Opinions of fellow citizens regarding perceived food security among regions and among experts (% of total).

	Regions			Total	*Expert groups*	
	NA	**Europe**	**ROW**		**Scientists**	**Non-scientists**
(Probably/Definitely) Yes	32	9	14	54	17	38
(Probably/Definitely) No	17	18	10	46	23	22
Total	49	27	24	100	40	60
Chi-square statistic	$\chi^2 = 10.241$, $\mathbf{p = 0.006}$				$\chi^2 = 9.364$, $\mathbf{p = 0.016}$	

Bold value indicates significant p-values at 0.05.

in their countries (*Probably/Definitely*) believe NBTs could improve global food security. These findings demonstrate a great uncertainty on the future of precision breeding in Europe. The regional heterogeneity in opinions about NBTs is likely to affect the regulatory process as well as the global trade of crop commodities. Non-scientists believe people in general perceive almost no risks related to NBTs while 7% of scientists believe people do perceive some risks ($p = 0.002$).

Table 10 shows that more than half of the panelists agree that communicating the benefits and risks of NBTs to the public should be a shared responsibility among university scientists (85%), regulators (75%), farmers/farmer organizations (64%), consumer organizations (53%), and industry associations (52%). These responsible institutions were also the most trusted sources experts use to form their opinions on precision breeding. This refers to the congruity principle (Osgood and Tannenbaum, 1955) by which "we tend to trust institutions who share our attitudes" (Peters et al., 2007: 196).

When asked about the likelihood that people would willingly purchase NBT-derived products, over half of the respondents think that it is (*extremely/moderately*) likely that consumers in their country will buy such products when available on the market; 10% think it is unlikely. While the crosstabs of **Table 11** indicate no difference in opinions by background, there is some evidence of different views by region. NA and

TABLE 10 | Responsible institutions for sharing the benefits and risks of NBTs.

Institutions	**Percentage**
University scientists	85
Regulators	75
Farmers/Farmer organizations	64
Consumers' organizations	53
Industry associations	52
Environmental groups	47
Politicians	36
Primary education system	35
Ethics committees	31
Advocacy groups	26
Social media websites	25
Medical doctors	24
Firms	21
Retailers	17
Religious leaders	7

TABLE 11 | Likelihood of consumers buying NBT products, by region and group (% of total).

	Regions			Total	*Expert groups*	
	NA	**Europe**	**ROW**		**Scientists**	**Non-scientists**
(Extremely/Moderately) Likely	41	11	16	68	25	43
Neither likely nor unlikely	8	9	5	22	8	14
(Extremely/Moderately) Unlikely	1	8	1	10	6	4
Total	50	28	22	100	39	61
Chi-square statistic	$\chi^2 = 29.661$, $\mathbf{p < 0.05}$				$\chi^2 = 4.163$, $p = 0.125$	

Bold value indicates significant p-values at 0.05.

the ROW show higher likelihood of consumers purchasing NBT products. In the United States, and since the introduction of GM crops, many consumers were little to not concerned about biotech products and were willing to buy GM products despite their superficial knowledge regarding plant biotechnology (IFIC, 2006). Unlike NA, European respondents appear to be less positive about future purchases of NBT products. In fact, the majority (17% that represents 61%) is either neutral or thinks it is unlikely that consumers will choose such products. On the other hand, 40% of Europeans are likely to try NBT products. This suggests that not all Europeans exhibit resistance to biotech products obtained via modern plant breeding. This is in line with existing research showing that not all Europeans are suspicious about biotech products. For instance, Aerni et al. (2011) found that Swiss consumers purchased GM corn bread when having the opportunity to choose freely between GM and non-GM variants.

In summary, we found more statistical differences based on region than on expertise. The groups of experts (natural science vs. social science) disagree on the perceived risks posed by NBTs and their potential to address global food insecurity. Non-scientists hold attitudes that are more positive. Unsurprisingly, findings show that the European respondents have the perception that the EU is socially and politically more precautionary about the application of new plant gene technology compared to the rest of the world; other studies of public attitudes and regulatory decisions tend align with that view. Europe seems to be less positive about the likelihood of approving, and adopting, NBTs. In addition, expert opinions in the EU indicate that consumers are less likely to purchase NBT-derived products due to the lack of perceived benefits.

CONCLUSION

Scientific innovation in the world of biology, particularly new techniques for breeding plants, are advancing rapidly. The ability to move from random mutation through the application of chemical or radiation mutation breeding to the precision of point-specific mutation offered through new breeding techniques is challenging regulatory systems to respond in a timely manner. The results presented and discussed above offer insights into the challenges of resolving this regulatory gap.

The regulatory uncertainty pertaining to products of NBTs is not due to scientific concerns, but rather political interference in the regulatory approval process. As identified above, the top reasons for uncertainty regarding regulatory approval of varieties produced by innovative plant breeding have no connection to science. The first scientific concern identified in the list of uncertainties was ranked by only 7% of respondents. The experts are clearly indicating that if the regulation of gene-edited technologies was to occur strictly based on scientific risk assessment principles, that these products would safely receive approval. But with political interference in the regulatory approval process, most notably in the EU, many express concerns that there will be few successful approvals.

Experts in the EU are less confident than are experts in other parts of the world, most notably North America, that consumers will accept NBT products. Some of our results support the fact that the EU is often described as being inflexible to the adoption of gene technology, including transgenic crops. Yet, we recognize there is variation among the EU countries regarding both political and public attitudes to plant gene technology. About 8–10 countries (of the EU-28) tend to be highly restrictive while 8–10 (e.g., Scandinavian and northern European) have a more pragmatic, science-based approach to GM applications (see Eriksson et al., 2018). These differences in opinions are not grounded in science, but rather in politics.

The results of our expert surveys reveal that trust in science is strong, while trust in social structures lags considerably. Our expert panel is not confident that politicians will not interfere in the regulatory approval for the products of new breeding technologies, thus increasing the uncertainty regarding the successful use of the technology. Given the highly competitive market for strategic agricultural and food investments, the level of uncertainty that exists within the EU has the potential to divert potential research and development investment away from the EU to markets with greater regulatory certainty.

DATA AVAILABILITY

The raw data supporting the conclusions of this manuscript are not publicly available because academic survey policy at the University of Saskatchewan states that all personal survey

data will be protected and held confidential to ensure responder anonymity. Requests to access these datasets should be directed to Dr. Stuart Smyth at stuart.smyth@usask.ca.

AUTHOR CONTRIBUTIONS

RL developed the method, performed the analysis, and wrote the paper. SS helped developing the method, writing the paper, and supervised the study. PP and HH helped developing the method and writing the paper.

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A New Zealand Perspective on the Application and Regulation of Gene Editing

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New Zealand (NZ) is a small country with an export-led economy with above 90% of primary production exported. Plant-based primary commodities derived from the pastoral, horticultural and forestry sectors account for around half of the export earnings. Productivity is characterized by a history of innovation and the early adoption of advanced technologies. Gene editing has the potential to revolutionize breeding programmes, particularly in NZ. Here, perennials such as tree crops and forestry species are key components of the primary production value chain but are challenging for conventional breeding and only recently domesticated. Uncertainty over the global regulatory status of gene editing products is a barrier to invest in and apply editing techniques in plant breeding. NZs major trading partners including Europe, Asia and Australia are currently evaluating the regulatory status of these technologies and have not made definitive decisions. NZ is one of the few countries where the regulatory status of gene editing has been clarified. In 2014, the NZ Environmental Protection Authority ruled that plants produced via gene editing methods, where no foreign DNA remained in the edited plant, would not be regulated as GMOs. However, following a challenge in the High Court, this decision was overturned such that NZ currently controls all products of gene editing as GMOs. Here, we illustrate the potential benefits of integrating gene editing into plant breeding programmes using targets and traits with application in NZ. The regulatory process which led to gene editing's current GMO classification in NZ is described and the importance of globally harmonized regulations, particularly to small export-driven nations is discussed.

Keywords: gene editing, New Zealand, regulation, traits, industry

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INTRODUCTION

Primary exports are critical to New Zealand's (NZ's) economy providing both employment and export revenue. In 2017, this totalled NZ$38 billion of which the dairy industry contributed NZ$ 14.6 billion, red meat and wool NZ$8.4 billion, forestry NZ$5.5 billion and horticulture NZ$5.1 billion (Ministry for Primary Industries, 2018b). New Zealand's pasture-based dairy industry is the world's largest dairy exporter and accounts for a third of the world's dairy trade (Chobtang et al., 2017a). Sheep and beef make up the majority of animal-based exports but venison and wool are significant contributors. The NZ sheep and beef sector exports close to 90% of its production.

Forestry, based around exotic plantation forests (primarily radiata pine and Douglas-fir), covers 1.751 million hectares—approximately 7% of NZ's land area (Ministry for Primary Industries, 2018a). The horticultural sector is predominately fruit based and led by kiwifruit, of which 95% of production is exported, wine, apple and pear are also exported in significant volumes. The main destinations for primary exports are China (NZ$9.1 billion), Australia (NZ$4.3 billion), and the US (NZ$4.0 billion), with Japan, South Korea and Europe also being significant markets.

Nations with small domestic markets like NZ face pressure to continuously adjust and innovate in order to maintain global competitiveness (Vitalis, 2007). To support this, NZ has a long history of implementation of agritech innovation (Easton, 1997; Vitalis, 2007; Hedley, 2015) including the use of genetic technologies (Harris et al., 2009; Kumar et al., 2012). In order to maintain NZ's position whilst providing sustainable solutions to the challenges of global food security and climate change a step change in productivity beyond that which has been possible through conventional breeding will be required (Williams et al., 2007). Solutions are also urgently required for the increased threat from pests and diseases. In the last decade the kiwifruit and forestry industry have suffered considerable losses from emerging diseases (Vanneste, 2012; Scott and Williams, 2014). Myrtle rust, which has caused worldwide damage to both agricultural and native ecosystems, arrived in NZ in 2017 (Office of the Minister of Conservation, 2017). Biotechnology-based improvements have the potential to be an important tool in delivering this. The unprecedented uptake of genetically modified (GM) crops over the last 20 years, such that 189.8 million hectares of GM crops were planted in 24 countries in 2017 (ISAAA, 2017) is testimony to this. GM crops are now cultivated on more than 10% of the worlds farmland and comprise 80% of global cotton and 77% of soybean plantings (ISAAA, 2017; Taheri et al., 2017).

Currently no GM crops are grown in NZ. The globally traded cash crops (corn, soybean, canola and cotton) that make up the majority of current GM plantings are not widely grown and do not provide a compelling value proposition for NZ. In contrast, NZ aims to supply high value innovative products that are not cultivated on a global large scale e.g., kiwifruit and radiata pine. The time and cost of developing and gaining regulatory approval for GM versions of these for the NZ market is prohibitive. The lack of relevant GM crops has meant that there has not been recent nationwide debate on the merits of these technologies in NZ (Bryan and Roberts, 2015).

Over the last decade genome editing methods based on Zinc finger nucleases (Urnov et al., 2010), TALENs (Chen and Gao, 2013), CRISPR/Cas (Doudna and Charpentier, 2014) systems have rapidly revolutionized both basic and applied biology. The wide-ranging applications of this technology have been extensively reviewed elsewhere (Voytas, 2013; Carroll, 2014; Wang et al., 2016a; Brooks and Gaj, 2018). In this review, we will focus on the use of gene editing to carry out targeted mutagenesis on plant species where no DNA template is used. We believe this technology has the ability to encourage a paradigm shift in the incorporation of biotechnology into NZ plant breeding programmes. Particularly if, as seems likely, it is ultimately regulated in a less burdensome way than GM technology. Here, we give examples of the traits that could be modified to give NZ relevant outcomes, describe the current regulatory landscape, and discuss the implications of this on the future innovation in NZ plant-based primary industries.

POTENTIAL APPLICATIONS OF GENE EDITING IN NEW ZEALAND

Gene editing offers the potential to produce a step change in NZ primary industry productivity, biosecurity and speed of innovation. This is particularly the case for perennial crops with slow or complex breeding cycles that are a feature of NZ's plant-based exports. Although gene editing has already been demonstrated for a number of NZ relevant crops (**Table 1**), it is still to be implemented for a number of important species particularly conifer forestry species. This review focuses on plant-based applications, however, uses in animal breeding (Wei et al., 2018) and control of introduced pests via gene drive technology (Dearden et al., 2018) are also in development. Below, as examples, we describe possible scenarios where plant-based gene editing could have an impact on primary production and innovation.

Control of Invasive Conifers by Manipulation of Reproduction

NZ faces serious ecological, economic and cultural challenges from invasive tree species that have "escaped" by seed dispersal from planted forests and shelter belts (Richardson and Rejmánek, 2004). Several exotic conifer species that have become established outside plantations now occupy ∼1.8 million ha of land, and are expanding by 6% annually (Froude, 2011). The government has declared these to be the most significant weed problem facing NZ (The New Zealand Government, 2016a) with control of the existing population costing an estimated NZ$15 million each year. The social and economic costs of these escapes is challenging the ability of forest owners to carry out new plantings with commercially advantageous, but potentially invasive species such as Douglas-fir. The capability to generate trees that are unable to reproduce would allow control programs to focus on the existing populations and give freedom to operate for new plantings. Prevention of cone development is also predicted to increase growth and wood development by the redirection of energy and nutrients toward vegetative growth (Santos-del-Blanco and Climent, 2014).

Gene editing provides an attractive approach to prevent the generation of new escapees via targeted mutagenesis of genes essential for normal sexual reproduction. Genes involved in the transition from the juvenile to reproductive growth phase, cone initiation or development, and pollen formation and development are potential targets (Strauss et al., 1995). If transgene-free edited trees are required, DNA-free delivery methods would be necessary because the long breeding cycles of conifers would prevent timely segregation of transgenes from edited genes.

TABLE 1 | Examples of species relevant to New Zealand's plant-based primary industries that have been modified using genome editing technologies.

Species	Method	Tissue	Purpose	Result	References
WOODY SPECIES					
Apple	CRISPR/Cas9 RNPs	Protoplasts	Mutate *DIPM-1, DIPM-2*, and *DIPM-4* to increase resistance to fire blight disease.	No plants regenerated	Malnoy et al., 2016
Apple	CRISPR/Cas9	Agrobacterium-mediated transformation of leaf disks	Mutate *phytoene desaturase (PDS)* gene	First generation albino plants regenerated with mutated PDS gene	Nishitani et al., 2016
Apple	ZFN	Agrobacterium-mediated transformation of leaf disks	Activation of a mutated *UidA* gene	First generation plants expressing GUS regenerated	Peer et al., 2015
Grape	CRISPR/Cas9	Proembryonal mass	Increased resistance to *Botrytis cinerea*	First generation plants regenerated with increased resistance to *Botrytis cinerea*	Wang et al., 2018a
Kiwifruit	CRISPR/Cas9	Agrobacterium-mediated transformation of leaf disks	Mutate *phytoene desaturase (PDS)* gene	First generation albino plants regenerated with mutated PDS gene	Wang et al., 2018b
Sweet Orange	CRISPR/Cas9	Agroinfiltration of leaf	Mutate *phytoene desaturase (PDS)* gene	No plants regenerated	Jia and Wang, 2014
Poplar	ZFN	Agrobacterium-mediated transformation of leaf disks	Mutate *LEAFY and AGAMOUS* orthologs in poplar	First generation plants regenerated	Lu et al., 2016
Poplar	CRISPR/Cas9	Agrobacterium-mediated transformation of leaf disks	Mutate *phytoene desaturase (PDS)* gene	First generation albino plants regenerated	Fan et al., 2015
Poplar	CRISPR/Cas9	Agrobacterium-mediated transformation of leaf disks	Mutate *4-coumarate:CoA ligase* (*4CL*) gene	First generation plants regenerated with decreased lignin	Zhou et al., 2015
FORAGE CROPS					
Alfalfa	CRISPR/Cas9	Agrobacterium-mediated transformation of leaf disks	Mutate *squamosa promoter binding protein like 9 (SPL9)* gene	First generation plants regenerated	Gao et al., 2018
VEGETABLE CROPS					
Tomato	CRISPR/Cas9	Agrobacterium-mediated transformation of cotyledon segments	Mutate tomato *ARGONAUT7* (*SlAGO7*)	First and second generation plants were produced	Brooks et al., 2014
Tomato	CRISPR/Cas9	Agrobacterium-mediated transformation of cotyledon segments	Mutate *SlIAA9*, *SLAGL6*	Parthenocarpy	Klap et al., 2017; Ueta et al., 2017
Potato	CRISPR/Cas9	Agrobacterium-mediated transformation of stem segments	Mutate *StIAA2* encoding an Aux/IAA protein	First generation plants regenerated	Wang et al., 2016b
Potato	Geminivirus mediated CRISPR/Cas9	Agrobacterium-mediated transformation of stem segments	Mutate *ACETOLACTATE SYNTHASE1* (*StALS1*)	Herbicide tolerant plant generated	Butler et al., 2016
Cucumber	CRISPR/Cas9	Agrobacterium-mediated transformation of cotyledon segments	Mutate *eIF4E* to develop resistance to virus	Plants exhibited immunity to Cucumber vein yellowing virus (Ipomovirus) infection and resistance to the Zucchini yellow mosaic virus and Papaya ring spot mosaic virus-W	Chandrasekaran et al., 2016
Lettuce	CRISPR/Cas9 RNPs	Protoplasts	Mutate *BRASSINOSTEROID INSENSITIVE 2* (*BIN2*) gene	Whole plants with mutated *BIN2* regenerated from protoplasts	Woo et al., 2015

Rapid Breeding in Apple

Breeding of new apple varieties is a slow process limited by a long-lasting juvenile stage taking more than two decades to bring a new variety into the market (Flachowsky et al., 2009). Shortening the juvenile stage has been the subject of intensive research and is a major objective in breeding (Meilan, 1997). Early flowering has been demonstrated in apple through the overexpression of beech *MADS4* and Arabidopsis *FT* gene (Flachowsky et al., 2007; Yamagishi et al., 2011). This technology has been used to rapidly breed fire blight resistance into apple within 7 years (Schlathölter et al., 2018). A similar result has been obtained using antisense-based silencing of *MdTFL1* expression (Kotoda et al., 2006). Gene editing could be used to knock out the expression of *MdTFL1* to reproduce this early flowering phenotype. This would allow rapid breeding of new cultivars through several cycles after which the edited gene could be crossed out to restore the non-engineered flowering phenotype without any trace of the modification.

Improved Pasture Quality

The dairy, meat and wool industries in NZ draw a significant market advantage from the predominantly pasture-based feed. Limiting environmental impacts whilst meeting the increase in global demand for dairy products requires improvements in pasture productivity (Chobtang et al., 2017b). Forage pastures generally consist of ryegrass, alfalfa and clover. Of these, annual and perennial ryegrass are most common. Gene editing provides tools to improve productivity and reduce disease either through the direct manipulation of forage crops or via manipulation of endophytes. The incorporation of herbicide tolerance (Butler et al., 2016) and easier digestibility (Li et al., 2018) have both been successfully introduced into plants by gene editing and research to increase energy values is underway. These are likely to offer routes to both increased productivity and a reduced environmental footprint.

Forage grasses like ryegrass are usually infected with symbiotic fungal endophytes (Latch et al., 1984) which produce secondary metabolites that protect the plant from invertebrate pests (Mortimer and Di Menna, 1983), give higher growth rates, tolerance to abiotic stress (West and Gwinn, 1993), and produce more dry matter than non-infected plants (Popay et al., 1999). These benefits can be compromised by the production of high levels of indole-diterpenes and alkaloids that have negative impacts on livestock e.g., ryegrass staggers in sheep (Fletcher and Harvey, 1981; Thom et al., 2007). To minimize the toxicity of these symbionts, strains of endophytes were selected that produced low levels of these alkaloids and indole-diterpenes (Davies et al., 1993). Molecular analysis revealed these lower levels were due to deletions within the coding sequence of genes in the biosynthetic pathway (Young et al., 2009). Gene editing will allow the modification of biosynthetic pathways to decrease or eliminate toxins and increase the production of desirable metabolites without the need to screen for extremely rare natural variants.

REGULATION OF GENE EDITING IN NEW ZEALAND

The global social and regulatory landscape surrounding GM crops remains complex with many different regulatory systems in place (Wolt et al., 2016; Davison and Ammann, 2017). The primary difference being whether a process or product driven framework is used (Ishii and Araki, 2017). As yet there is not a global consensus on the regulation of gene editing which was developed after current regulatory frameworks were put in place. Several nations, including the USA, Canada and Argentina, have decided that gene editing technologies where the final plant does not contain introduced DNA will not be regulated (Whelan and Lema, 2015; Ishii and Araki, 2017; Waltz, 2018). In contrast the European Union recently decided that all gene editing technologies will be regulated in the same way as conventional GM organisms (Callaway, 2018; Kupferschmidt, 2018). Others, including the two main destinations for NZ's primary exports, China and Australia, are yet to decide on their regulatory approach.

New Zealand regulates GM organisms using a stringent process driven regulatory framework—the Hazardous Substances and New Organisms (HSNO) Act 1996. The Act defines a GMO very broadly as any organism where the genes or genetic material have been modified by *in vitro* techniques (**Table 2a**). A number of technologies that were in use at the time the Act was passed are captured by this broad definition e.g., somaclonal variation, cell fusion, and chemical and physical mutagenesis. To counter this, a number of technologies that meet the definition of generating a GMO are excluded from being regulated by the HSNO (Organisms Not Genetically Modified) Regulations 1998 (**Table 2b**).

Application to Determine Status of Gene Editing

The HSNO Act, under section 26, provides a mechanism for an applicant to ask for a determination by the Environmental Protection Authority (EPA) as to whether, an organism is regulated as a GM in NZ (Kershen, 2015). In 2012, Scion, a forestry-focused Crown Research Institute, used this procedure to seek a determination on how gene edited organisms would be regulated. The HSNO definition (**Table 2a**) includes a clause specifying that genetic modifications "inherited or otherwise derived, through any number of replications" would be classed as GMOs. Scion's application, which was submitted before CRISPR/Cas9 technology was developed, thus sought to determine "*whether the use of custom Zinc Finger Nucleases and custom Transcription Activator-Like Effectors results in organisms classed as genetically modified organisms*" when the editing complex was delivered without the use of a transgene to carry the editing machinery.

Scion's application argued that gene editing technologies that did not include the insertion of a transgene into host genome were similar in process and outcome to chemical mutagenesis. As such they should be included within the HSNO regulations exception of "*chemical or radiation treatments that cause changes*

TABLE 2 | The regulation of GMOs and gene editing in New Zealand.

(a) HSNO Act – Definition of GMO	**Section 2** Genetically modified organism means, unless expressly provided otherwise by regulations, any organism in which any of the genes or other genetic material- (a) have been modified by *in vitro** techniques; or (b) are inherited or otherwise derived, through any number of replications, from any genes or other genetic material which has been modified by *in vitro* techniques (* the term *in vitro* is not defined by the Act)
(b) HSNO (Organisms Not Genetically Modified) Regulations 1998	**3 Organisms not genetically modified** (1) For the purposes of the Act, the following organisms are not to be regarded as genetically modified: (a) organisms that result solely from selection or natural regeneration, hand pollination, or other managed, controlled pollination; (b) organisms that are regenerated from organs, tissues, or cell culture, including those produced through selection and propagation of somaclonal variants, embryo rescue, and cell fusion (including protoplast fusion or chemical or radiation treatments that cause changes in chromosome number or cause chromosome rearrangements); (c) organisms that result solely from artificial insemination, superovulation, embryo transfer, or embryo splitting; (d) organisms modified solely by (i) the movement of nucleic acids using physiological processes, including conjugation, transduction, and transformation; and (ii) plasmid loss or spontaneous deletion; (e) organisms resulting from spontaneous deletions, rearrangements, and amplifications within a single genome, including its extrachromosomal elements.
(c) HSNO (Organisms Not Genetically Modified) Amendment to Regulations - 29 September 2016	**Regulation clause 3(1)(b)** (1) For the purposes of the Act, the following organisms are not to be regarded as genetically modified: (b) organisms that are regenerated from organs, tissues, or cell culture, including those produced through selection and propagation of somaclonal variants, embryo rescue, and cell fusion including protoplast fusion: (ba) organisms that result from mutagenesis that uses chemical or radiation treatments that were in use on or before 29 July 1998

The HSNO Act definition of a GMO (a), and regulations excluding certain technologies from being regulated in the original (b) and revised (c) regulations are given. The unorthodox use of the word including at the beginning of the list of except techniques in section (b) is underlined.

in chromosome number or cause chromosome rearrangements" (**Table 2b**). Scion noted that the list of techniques that were excluded from regulation was preceded by the word included (underlined for emphasis in **Table 2b**) suggesting that these were example techniques and not a closed list.

EPA Decision

In their decision of April 2013, the EPA concluded that the non-transgenic gene editing approach proposed by Scion had similarities to both chemical mutagenesis and genetic manipulation. However, because the changes involved the use of a chemical agent (in this case, a protein) without the introduction of foreign DNA it is more similar to chemical mutagenesis (Environmental Protection Authority, 2013). The EPA further stated that the Regulations (**Table 2b**) exclude products of chemical mutagenesis from being regulated as GMOs under the Act and that the proposed modifications were sufficiently similar to those listed in the Regulations and should also be excluded, and organisms arising from them should not be considered GMOs.

High Court Challenge

The EPA decision was appealed by the Sustainability Council of New Zealand in the High Court and the case was heard in November 2013. The key consideration of the judgement, issued on the 20th May 2014, was "*whether the specific techniques (listed in **Table 2b**) are a closed list of techniques that are exempted, or whether they describe a category of the kind of techniques that are excepted (so that other techniques which are sufficiently similar to those techniques are also exempted)*" (The High Court of New Zealand, 2014). The Court concluded that the list of techniques listed in the HSNO (Organisms Not Genetically Modified) Regulations 1998 (**Table 2b**) are a closed list and that adding to the exceptions list is a political decision and not an administrative decision (Kershen, 2015). On this basis the EPA's original decision was quashed and all gene editing is currently regulated as a GM procedure in NZ.

Implications of the Decision

In the court ruling the judge pointed out that the regulations are not well drafted, brackets are in the wrong place and the grammar poor. This reinforced her interpretation that the unorthodox use of the word "including" before start of the list of techniques that do not produce GMOs (**Table 2b**) does not constitute a list of examples but rather a closed list. She also highlighted that the regulations exempted only "*chemical or radiation treatments that cause changes in chromosome number or cause chromosome rearrangements*" from regulation as GMOs. Some long-standing *in vitro* chemical treatments do not have these effects, but are caught by this definition. Thus, techniques such as EMS mutagenesis that cause point mutations rather than changes in chromosome number or chromosome rearrangements are regarded technically as GMOs.

In response to these inconsistencies the government held a review of the not genetically modified regulations. The review, which included a public consultation process, resulted in changes intended to maintain the intent of the 1998 regulations and address the drafting errors present in the original regulations. The wording was changed such that mutagenesis techniques that were in use before 1998 were not regulated whilst those developed later are regulated as GMOs. This was done by simply excluding from regulation "*mutagenesis that uses chemical or radiation treatments that were in use on or before 29 July 1998*" (**Table 2c**) (The New Zealand Government, 2016b). Mutagenesis techniques developed later, including gene editing, however similar they are to the original excluded techniques are regulated as GMOs.

FUTURE OUTLOOK

Gene editing continues to rapidly evolve with developments such as new enzyme capabilities (Yin et al., 2018), base editing (Komor et al., 2016) and simultaneous multi-target approaches, (Svitashev et al., 2015; Chilcoat et al., 2017; Shen et al., 2017) increasing the scope and applicability of the technology. The recent demonstration of rapid *de novo* gene editing-based domestication of wild type relatives of domestic crops without the need for a long breeding programme (Zsögön et al., 2017) has particular applicability in NZ. Particular examples are kiwifruit and radiata pine which are relatively undomesticated and/or where a large number of wildtype genotypes are available (Ferguson, 2007) but require the introduction of essential commercial traits such as longer post-harvest storage and shelf life.

Recent decisions in USA (Waltz, 2018) and the UK (Rogowsky and Wilhelm, 2018) indicate that crops produced using gene editing-based targeted mutagenesis will be able to go to market without going through a time-consuming and burdensome regulatory process required for GMO crops. This regulatory approach will drastically reduce the time to market and compliance costs for gene edited crops. The recent US Department of Agriculture (USDA) approval of *Camelina sativa* edited for enhanced omega-3 oil was completed in 2 years at a much lower cost than the estimated US$30-50 million and 6 years plus that would have been required to fulfill the full USDA process (Waltz, 2018).

In contrast, NZ has adopted a wait-and-see-approach with regard to the regulation of gene editing. The government indicating that a cautious approach is appropriate because as an exporter of billions of dollars of food products we need to be mindful of market perceptions as well as the science (The New Zealand Government, 2016b). It should be noted that the three largest importers of NZ primary products, China, Australia and USA all currently grow GM crops and Australia and China seem likely to follow the lead of USA in not regulating gene edited crops. The current NZ approach prevents rapid implementation of non-transgenic gene editing and also places the extremely high regulatory compliance costs associated with GM research on developers of such technology.

For NZ to maintain its current global competiveness it is essential that industry is able to continue to implement innovative solutions. For this to happen with gene editing, it will be necessary for the government to be proactive in ensuring NZ is in step with global competitors and that innovation is not stifled by the current outdated regulations. Despite the opinion released in January, by the advocate-general of the European Court of Justice, that gene edited crops that did not contain foreign DNA could be exempted from the GMO regulations, the EU has recently decided to adopt a similar regulatory approach to that of NZ. All gene edited crops will be subject to the same stringent regulations as conventional genetically engineered organisms (Callaway, 2018). This makes a global consensus on regulation of gene editing impossible in the immediate future. Although it is too early to judge the long-term impacts of this decision on the global uptake of gene editing or the regulatory approach that will be taken by currently undecided nations, the existence of different regulatory systems will undoubtedly create many challenges, particularly for those nations with strong trading links with the EU.

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All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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New Plant Breeding Techniques Under Food Security Pressure and Lobbying

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Different countries have different regulations for the approval and cultivation of crops developed by using new plant breeding technologies (NPBTs) such as gene editing. In this paper, we investigate the relationship between global food security and the level of NPBT regulation assuming a World Nation Official (WNO) proposes advice on global NPBT food policies. We show that a stricter NPBT food regulation reduces food security as measured by food availability, access, and utilization. We also find that political rivalry among interest groups worsens the food security status, given the NPBT food technology is more productive and the regulatory policy is influenced by lobbying. When the WNO aims to improve food security and weighs the NPBT food lobby contribution more than the non-NPBT food lobby's in the lobbying game, the total lobbying contributions will be the same for the WNO, and the NPBT food lobby will be more successful in the political process. The NPBT food lobby, however, under food security loses its advantage in the political competition, and this may result in a strict NPBT food policy. Under food security problems implementing stricter NPBT food regulations results in welfare losses.

JEL Code: D04, D43, D72, P16

Keywords: food policy, food security, gene editing, lobbying, political economy

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INTRODUCTION

After the 2008 food crisis, the potential fragility of the global food system returned as a major topic in the debate on global food security. Politicians and researchers have suggested several solutions, such as reduction in trade barriers, food aid for food insecure regions, and improving productivity through new agricultural technologies. Modern biotechnology has been considered one of the main contributors to food security (e.g., Ruane and Sonnino, 2011; Sastry et al., 2011; Qaim and Kouser, 2013). However, the importance of the contribution to food security is under debate (e.g., Dibden et al., 2013). Although the topic of this paper is new plant breeding technologies (NPBTs), at several places we refer to experiences gained from the regulation of genetically modified organisms (GMOs) as they bear a number of similarities with NPBTs from a political economy perspective.

The debate about GMOs illustrates that the application of modern biotechnology is not just a scientific problem, but equally a political one involving several interest groups (Miller and Conko, 2004; Graff et al., 2009; Qaim, 2009; Freedman, 2013; Herring and Paarlberg, 2016). This applied to previous technologies, but also applies to NPBTs (e.g., Sprink et al., 2016).

Biotechnology scientists and companies emphasize higher yields and environmental benefits of NPBTs. Opposing organizations, such as Greenpeace and Friends of the Earth, emphasize

the potential human health and environmental risks (Rausser et al., 2015; Clancy, 2017), even though there is currently no evidence that proves that NPBTs pose higher risks to either human health or the environment and that rather the opposite can be expected.

International organizations are also involved in the debate. For example, the State of Food and Agriculture report of 2003 on "Agricultural Biotechnology: Meeting the Needs of the Poor?" by the FAO (Food and Agriculture Organization of the United Nations) has been heavily criticized for its "pro-GM" view. Similarly, the report of 2009 on the International Assessment of Agricultural Knowledge, Science and Technology for Development (IAASTD) has been criticized for not paying enough attention to the possibilities of modern biotechnology to address food security: "*But, partly due to the way in which the authors were selected and the main reports were translated into the summaries, the overall message which emerged from the IAASTD was a more restrictive, exclusionary message with an undercurrent against new technology, GMOs, and input-intensive agriculture.*"(McIntyre et al., 2009, p. 38).

In a similar vein, Urs Niggli, Director of the Swiss Research Institute of Organic Agriculture (FiBL), was heavily criticized for his statement NPBTs offer a great potential for organic agriculture (Maurin, 2016). The outcome of the debate on NPBTs, whatever it is, can be expected to affect food policies and therefore, food security.

In **Figure 1**, the importance of food policies is illustrated in the food system framework (Modified from Ericksen et al., 2009, p. 28). The NPBT food policy if regulated similar to GMOs will influence the whole food system through production and consumption decisions and will finally result in the changing of prices. The effect will trickle down, affecting food system outcomes. For example, farmers have to comply with food regulations and their labeling standards (Gruère et al., 2009) and coexistence rules (Wesseler and Punt, 2016), seed companies with environmental and food safety regulations (Smart et al., 2017), and countries with international trade agreements (Punt and Wesseler, 2016). In addition, consumers' preferences toward NPBT and non-NPBT food products are influenced by labels and advertisements on food products, media reports, and more (Lusk et al., 2014). Food regulations can also influence the acquisition of food products in the market by implementing stringent or lenient sanitary and phytosanitary standards for food imports. All these policies influence the food system outcomes with impacts on food security and social welfare.

Political differences in the use of NPBTs widen the productivity gap between developing and developed countries by setting barriers on the application of new agricultural technology. As Shiferaw et al. (2011) argue the "hard technology" of genetic modification alone is not enough to improve food security. It needs to be complemented with the "soft technologies" of the development of an appropriate food policy and the establishment of proper institutions that ensure that smallholder farmers can use the technology and profit from it. Biotechnology policy not only influences social welfare directly, but also generates environmental benefits and costs. Many positive environmental effects from GM crops have been observed, such as a reduction in pressure on habitats and biodiversity through increased productivity (Wesseler et al., 2011). Growing GM crops is also less harmful to the environment and human health (Bennett et al., 2004). Similar effects are expected for crops derived from NPBTs.

Several authors applied the political economy theory to study policies on agricultural biotechnology (e.g., Graff et al., 2009; Wesseler and Zilberman, 2014; Tosun and Schaub, 2017; Wesseler et al., 2017). Apel (2010) claims that there are substantial policy and financial benefits that GM food opponents gain from their opposition to GM food technology, i.e., donations, membership fees, and nationally funded policy programs. Some donors provide financial support to NGOs that campaign against GMOs and NPBTs in developing countries (Paarlberg and Pray, 2007). At the same time, the GM food R&D institutes and some seed companies lobby for less strict regulations of biotechnology across countries. The strict GM food regulation in the EU is regarded as a lobbying success of anti-GM food lobby groups (Graff et al., 2009; Qaim, 2009). These conflicting public attitudes and interests in biotechnology manifest in the GM food policy of each country. Therefore, a political economy analysis can offer important insights into the policy formation (Josling et al., 2004).

In this paper, we discuss NPBTs food policies that influence the food system, and thereby the three aspects of food security (food utilization, food access and food availability) from a global political perspective. The three pillars of food security follow the World Food Summit (1996)'s definition (Thomas and Morrison, 2006) and the FAO added stability as the fourth pillar in 2001, which refers to the first three aspects over time. Since our model is static, we only focus on the first three pillars. We quantify food availability by food production, food access by food prices, wages and food demand, and food utilization by consumer surplus from food consumption. The political economy model follows the classic model of Grossman and Helpman (1994) and investigates the NPBT food policy effects on food security in a global context. We follow Weitzman (2001) and model the World Nation Official's (WNO, such as FAO) advice on global GM crop policies. The crucial assumption is that NPBT food regulations are supplementary to the regulations on non-NPBT food products and do not generate additional social benefits, such as higher levels of food or environmental safety. They are treated as safe as crops derived using "conventional" breeding. The WNO maximizes the sum of a weighted social welfare function and contributions from two lobby groups, an NPBT and a non-NPBT food group, who have contradictory interests toward the NPBT food technology. Some consumers have strong preferences for or against NPBT, while many other consumers are indifferent to either NPBT or non-NPBT food products or demand variety. Consequently, in the model we divide consumers into three groups (for, against, and indifferent). This helps us to integrate consumer preferences into the conflict of interest analysis.

We find that a stricter NPBT food regulation has negative effects on all three aspects of the global food security. This influence gets more negative when interests groups get involved. If the NPBT food technology is argued to be more efficient in

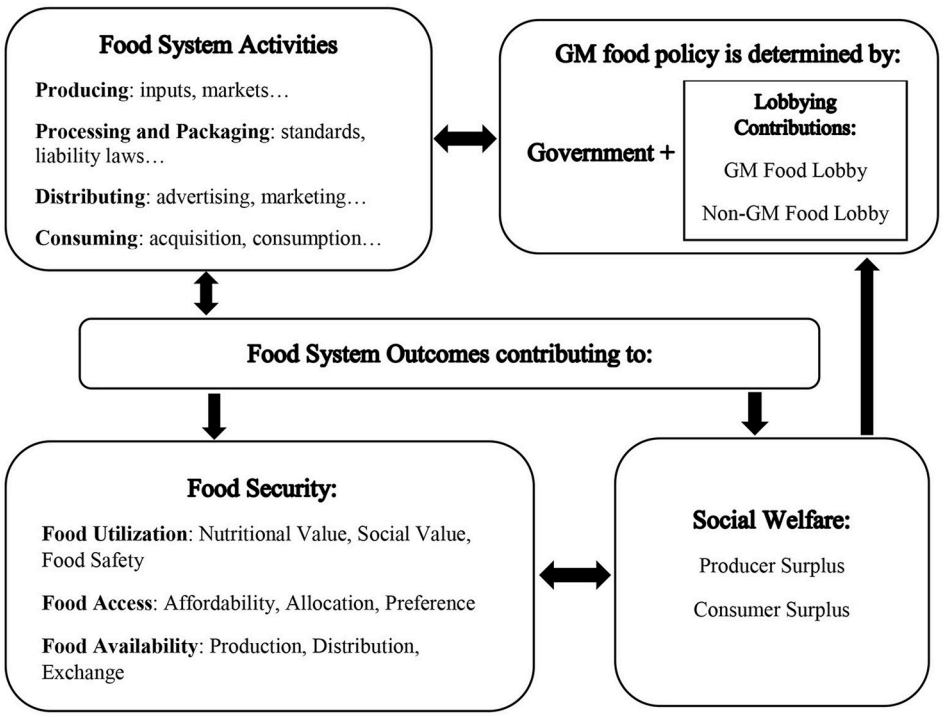

FIGURE 1 | Politically influenced main food system (modified from Ericksen et al., 2009).

production than the conventional technology, then the NPBT food lobby is more successful in the lobbying process when the WNO aims to improve the food security status. But if the non-NPBT food lobby group is very large, the NPBT food policy would stay strict. Therefore, the existence of a more powerful lobby group in the policy making process, be it the NPBT or the non-NPBT, makes that international organizations have difficulties in providing clear statements in favor of or against NPBTs, because these organizations depend on contributions from many sources.

THE MODEL

We model the world as a closed economy, the world. There are two sectors in the economy, an agricultural food sector and a numeraire sector (z). Even though there are many farmers for NPBT and non-NPBT food production, we assume there are only two firms in the food sector in our model, a firm producing NPBT food x_G (henceforth: NPBT food firm, subscript G) and a firm producing non-NPBT food x_N (henceforth: non-NPBT food firm, subscript N). Labor and capital are the inputs for production[1]. The NPBT food firm uses the NPBT food technology as an additional input in its production process and receives benefits such as improved yield and/or reduced production costs, whereas the non-NPBT food firm only uses conventional agricultural technology for its production. The WNO, however, implements restrictions on the use of NPBT food technology to regulate NPBT ingredients, such as specific regulations for NPBT approval, regulations on cultivation, and private sector policies on NPBT-free food products. Coexistence policies, for instance, could require minimum distance, buffer zones, and/or rotation intervals when planting NPBT crops with reference to the conventional farming. Such regulations raise the cost of using NPBT food technology (Beckmann et al., 2006, 2011). We translate these policies into a single variable θ, ($\theta \geq 0$), which represents an additional cost for the firm using the NPBT food technology; a stricter NPBT food policy means a higher NPBT food compliance cost.

We normalize the overall population to one and classify consumers into three types, denoted by superscripts α, β, γ, depending on their preferences. Fraction α of the population owns the NPBT food firm and shares the NPBT food profits. For example, NPBT food R&D researchers, producers, and retailers belong to this group. Consumers in this group have a strong preference for NPBT food and only consume NPBT food products. They are in favor of innovative technology and are convinced of its environmental and health benefits. Fraction β of the population belongs to the non-NPBT food group. It consists of people who own the non-NPBT food firm and earn the non-NPBT food profits. The anti-NPBT food organizations, conventional and organic food farmers, and anti-NPBT food consumers belong to this group. Consumers belonging to this group have a strong preference for non-NPBT food products and only purchase non-NPBT food products. The rest of consumers belong to fraction γ ($= 1 - \alpha - \beta$). This group considers NPBT

[1]We only focus on the agricultural sector and take the labor and capital price exogenous.

and non-NPBT food products as imperfect substitutes. Its members do not worry much about the potential risks of the NPBT food technology; therefore, we label them henceforth as "indifferent"[2]. The two food firms, NPBT and non-NPBT, engage in Bertrand competition, that is, they compete for the γ consumers by setting a lower food price.

Consumers in the different groups purchase food products and numeraire goods subject to their income. Following Singh and Vives (1984), the quasi-linear utility functions of the three groups are[3]:

$$\begin{aligned} U^{\alpha} &= z^{\alpha} + ax_G^{\alpha} - \frac{1}{2}b(x_G^{\alpha})^2 \\ U^{\beta} &= z^{\beta} + ax_N^{\beta} - \frac{1}{2}b(x_N^{\beta})^2 \\ U^{\gamma}\left(x_G^{\gamma}, x_N^{\gamma}\right) &= z^{\gamma} + ax_G^{\gamma} + ax_N^{\gamma} \\ &\quad - \frac{1}{2}\left[b\left(x_G^{\gamma}\right)^2 + 2hx_G^{\gamma}x_N^{\gamma} + b\left(x_N^{\gamma}\right)^2\right] \\ s.t\ \ I^i &= z^i + \sum_j p_j x_j^i \ \ for\ i = \alpha, \beta, \gamma \ \ and\ \ j = G, N \end{aligned} \tag{1}$$

where z^i is the utility from consuming the numeraire product with a price of one and p_j is the price of the food product. Given that a, b and h are positive parameters, we assume $b > h > 0$. For the indifferent food consumers, NPBT and non-NPBT are substitutes, when $h = 1$ they are perfect substitutes. γ consumers demand a mix of both NPBT food and non-NPBT food products. A price change of NPBT food has an effect on the demand for the non-NPBT food products by γ consumers. For α and β consumers, the total income consists of wage and a share of either NPBT or non-NPBT food profits. Consumers belonging to group γ only have income from their wages. The total demand for NPBT food products is $x_G^{\alpha} + x_G^{\gamma}$, and the total demand for non-NPBT food products is $x_N^{\beta} + x_N^{\gamma}$.

The NPBT food policy influence on the food market is modeled as a two-stage game. First, the WNO sets the NPBT policy level, and second the firms choose their prices. The NPBT and non-NPBT food firms have monopolies on their production. We use backward induction to identify the effects of the policy compliance cost. The firms' profits are:

$$\pi_G = p_G x_G - [w + (1 + \theta)\phi r]\, x_G \tag{2}$$

$$\pi_N = p_N x_N - (w + r)\, x_N \tag{3}$$

where $p_i (i = G, N)$ is the price of either the NPBT or non-NPBT food product, w is the unit labor cost (wage rate), and r is the unit capital cost. ϕ is the productivity parameter of using NPBT technology, and $0 < \phi < 1$ represents the technology and is capital saving for food production. The unit costs for the NPBT and non-NPBT food firms are assumed to be independent of the level of output and are given by $w + (1 + \theta)\phi r$ and $w + r$.

In equilibrium, the NPBT food firm produces a sufficient quantity to meet the NPBT food demand, that is, $x_G = x_G^{\alpha} + x_G^{\gamma}$, and the non-NPBT food firm produces $x_N = x_N^{\beta} + x_N^{\gamma}$. The demand functions for both products are derived from consumers' maximization problems. These demand functions are (**Appendix A**):

$$\begin{aligned} x_G = x_G^{\alpha} + x_G^{\gamma} &= \frac{a - p_G}{b} + m - np_G + \delta p_N \\ &= \frac{a}{b} + m - \left(\frac{1}{b} + n\right)p_G + \delta p_N, \\ x_N = x_N^{\beta} + x_N^{\gamma} &= \frac{a - p_N}{b} + c + \delta p_G - dp_N \\ &= \frac{a}{b} + c - \left(\frac{1}{b} + n\right)p_N + \delta p_G. \end{aligned}$$

where $m = \left(ab - ah\right)/\left(b^2 - h^2\right), n = b^2/\left(b^2 - h^2\right), \delta = h/\left(b^2 - h^2\right)$. Using the demand functions, we can solve for the reaction functions of the firms (see **Appendix B**):

$$p_G = \frac{\frac{a}{b} + m + \delta p_N + \left(\frac{1}{b} + n\right)[w + (1 + \theta)\phi r]}{2\left(\frac{1}{b} + n\right)} \quad \text{and}$$

$$p_N = \frac{\frac{a}{b} + m + \delta p_G + \left(\frac{1}{b} + n\right)(w + r)}{2\left(\frac{1}{b} + n\right)}.$$

Using these we can solve for the equilibrium price for the NPBT food product:

$$p_G^* = \frac{1}{b^2\delta^2 - 4b^2n^2 - 8nb - 4} \begin{pmatrix} -2\left(1 + bn\right)^2 (w + (1 + \theta)\phi r) \\ -b\delta\left(\left(1 + bn\right)(w + r) + \left(bm + a\right)\right) \\ -2\left(a + bm\right)\left(1 + bn\right) \end{pmatrix},$$

where $\partial p_G^*/\partial\theta > 0$. The NPBT food compliance cost influences the NPBT food price directly, and the non-NPBT food price indirectly. We solve for the equilibrium non-NPBT food price from the reaction function and find $\partial p_N^*/\partial\theta > 0$, but $\partial p_G^*/\partial\theta > \partial p_N^*/\partial\theta$. The NPBT food firm prefers a low NPBT food policy cost and more NPBT food technology, whereas the non-NPBT food firm prefers a high NPBT food price to attract more γ consumers to purchase non-NPBT food products.

The inverse demand functions for food products are: $p_G^{\alpha} = a - bx_G^{\alpha}$ for the NPBT food consumers, $p_N^{\beta} = a - bx_N^{\beta}$ for the non-NPBT food consumers, and $p_G^{\gamma} = a - bx_G^{\gamma} - hx_N^{\gamma}$ and $p_N^{\gamma} = a - hx_G^{\gamma} - bx_N^{\gamma}$ for γ consumers. In the equilibrium, the consumer surplus is $cs_G^{\alpha} = \int_0^{x_G^{\alpha *}} p\left(x_G^{\alpha}\right) dx_G^{\alpha} - p_G^* x_G^{\alpha *}$ for α consumers and $cs_N^{\beta} = \int_0^{x_N^{\beta *}} p\left(x_N^{\beta}\right) dx_N^{\beta} - p_N^* x_N^{\beta *}$ for β consumers. γ consumers demand both NPBT and non-NPBT food products, so $cs^{\gamma} = cs_G^{\gamma} + cs_N^{\gamma} = \int_0^{x_G^{\gamma *}} p\left(x_G^{\gamma}\right) dx_G^{\gamma} - p_G^* x_G^{\gamma *} +$

[2] γ consumers are indifferent to the NPBT food technology, not the NPBT or the non-NPBT food products.

[3] We assume the same parameters a and b for the three groups, because i) all consumers demand food products, no matter NPBT food or non-NPBT, ii) we want to simplify the analytical calculation and identify the policy effects.

$\int_0^{x_N^{\gamma*}} p\left(x_N^{\gamma}\right) dx_N^{\gamma} - p_N^* x_N^{\gamma*}$. The aggregate social welfare of each group is given by:

$$\begin{aligned} W^{\alpha} &= \pi_G(\theta) + cs_G^{\alpha}(\theta) \\ W^{\beta} &= \pi_N(\theta) + cs_N^{\gamma}(\theta) \\ W^{\gamma} &= cs_G^{\gamma}(\theta) + cs_N^{\gamma} \end{aligned} \tag{4}$$

Aggregate social welfare is the sum of the three groups' welfare in Equation (4):

$$W(\theta) = \pi_G(\theta) + \pi_N(\theta) + cs_G(\theta) + cs_N(\theta) \tag{5}$$

Thus, we can find the socially optimal NPBT food regulation by letting

$$\frac{\partial W(\theta)}{\partial \theta} = \frac{\partial W^{\alpha}(\theta)}{\partial \theta} + \frac{\partial W^{\beta}(\theta)}{\partial \theta} + \frac{\partial W^{\gamma}(\theta)}{\partial \theta} = 0 \tag{6}$$

NPBT FOOD POLICY EFFECTS ON FOOD SECURITY

We investigate the NPBT food regulation effects on availability, access and utilization of food security. Food security is a multi-aspects issue. To obtain specific results, we interprete the three dimensions of food security in our model in economic terms. As we stated earlier, the change of NPBT food regulation influences the NPBT food group directly and the non-NPBT food group indirectly. In addition, it influences the consumption distribution across NPBT and non-NPBT food products for the indifferent consumers. The marginal effects in **Table 1** (derivation: **Appendix C**) shows the NPBT food policy effects.

In **Table 1**, food availability is the production of food in the economy, i.e., $x_G + x_N$. A stricter NPBT food policy will reduce the production of NPBT food products because a higher NPBT food regulation compliance cost increases the price of capital input for the NPBT firm. As a result the price of NPBT food products increases and consequently the NPBT food demand from the α and γ group decreases. The non-NPBT food demand from the β group is not influenced by the NPBT food policy change, but if the demand for NPBT food products from the indifferent group decreases, the demand for non-NPBT food products will increase. Hence, a change of the NPBT food policy level has an indirect effect on the non-NPBT food demand. There are two opposing policy effects on both the NPBT and non-NPBT food production, but the policy effect on the overall food production is negative. The reason is that a higher NPBT food policy cost directly decreases the demand of both the NPBT food consumers and a portion of the indifferent consumers, which outweighs the positive effect on the non-NPBT food production, which is driven by only a part of the indifferent consumers.

The NPBT food regulation influences food access, which includes food affordability, food allocation, and consumer choices. We quantify the food access by food prices, the total income of consumers, and their food demand. The NPBT food consumers are directly influenced by the change of NPBT food price and income. If the NPBT food compliance cost increases, the NPBT food price increases, hence more indifferent consumers choose non-NPBT food. The increasing demand for non-NPBT food drives the non-NPBT food price up. The NPBT food firm's profit decreases under a stricter NPBT food policy defined in Equation (2), but the non-NPBT food profit increases from a higher demand and the resulting higher equilibrium price of non-NPBT food products. Wage rate does not change, so the total income is smaller for the NPBT food group, larger for the non-NPBT food group and the same for the indifferent group. The price increase of both NPBT and non-NPBT decreases the average households' affordability and access to food.

TABLE 1 | Marginal policy effects due to an increase in regulation on food security.

	Availability	Access			Utilization	
	Production	Price	Total income*	Demand	Total Utility	Consumer surplus
NPBT	−	+	−	−	−	−
Non-NPBT	+	+	+	+	+	−
Indifferent	N/A	N/A	N/A	−	−	−
Total	−	+	−/+	−	+/−	−

*"−" denotes a decrease and "+" an increase (see **Appendix C**); *Total income constitutes profits and wages.*

Food utilization comprises nutritional value, social value, and food safety. We measure this by total food demand and consumer surplus from food consumption. Consumers choose food products according to their preferences (see Equation 1); furthermore, they believe the food they choose is of higher value. Higher NPBT food regulation costs decrease the NPBT food production and total income of the NPBT food group and the demand for NPBT food products, hence nutrient intake decreases. If the NPBT food policy becomes stricter, consumer surplus of the NPBT food group will be reduced as well. The non-NPBT food consumers also lose from a higher non-NPBT food price induced by a higher demand from the indifferent group. Since the policy effect on the NPBT food price is larger than on the non-NPBT food price and the effect on the NPBT food production is opposing that on the non-NPBT food production, the policy effect on the total consumer surplus is negative.

Thus, we conclude that

Proposition 1 *A more stringent NPBT food regulation has a negative impact on global food security measured by its influence on food availability, accessibility and utilization.*

THE POLITICAL PROCESS

We endogenize the NPBT food policy in the policy-making process. The NPBT and non-NPBT food groups have opposing interests toward the level of NPBT food policy. The NPBT food group lobbies for lower NPBT food regulation costs in order to reduce the NPBT food firm's production costs, whereas the non-NPBT food group lobbies for a stricter NPBT food regulation.

Members in either NPBT or non-NPBT group have strong incentive to lobby, whereas those who have incentive to "free-ride" on the efforts of others are consumers in the indifferent group in the model (Olson, 1971). The indifferent group does not make any contribution to the WNO. Lobby groups influence the regulation in several ways. For example, they can make contributions, endorsements and committed votes to the WNO so as to influence the policy outcome. For simplicity, we model these contributions as monetary equivalents from the interest groups. We follow Grossman and Helpman (1994)'s model and define the WNO payoff function as a maximization of a weighted sum of aggregate social welfare plus contributions from the lobbies. The WNO payoff is given by:

$$G\left(\theta; C^{\alpha}, C^{\beta}\right) = qW(\theta) + \left(1 - q\right)\left[C^{\alpha}(\theta) + C^{\beta}(\theta)\right] \quad (7)$$

where q is the weight parameter, $0 < q < 1$, that the WNO attaches to the social welfare. $C^{\alpha}(\theta)$ and $C^{\beta}(\theta)$ are the differentiable truthful contribution schedules of the two lobbying groups like in Grossman and Helpman (1994), which means the NPBT food policy effects on the groups' contribution always represent the lobbies' policy preferences. We show this with two levels (high and low) of NPBT food regulations in **Figure 2**. For example, the negative effects resulting from higher NPBT food regulation costs induce the NPBT food lobby to contribute less. The non-NPBT food contribution reaches the maximum at a high level of NPBT food regulation. The maximum contribution that any lobby can make is its gross income, which include wages and firms' profits.

The political process is a three-stage non-cooperative game. Two lobbies simultaneously announce their contribution schedules to the WNO in the first stage, and the WNO decides the NPBT food policy that maximizes its payoff in the second stage. In the third stage, firms choose prices and lobbies pay their contributions.

The NPBT and non-NPBT food groups make the total contribution $B^{i}(\theta)$ from their income for lobbying. The amount of the contribution from each group depends on the number of consumers and the share of their donations. The net income of each group is their gross income minus the lobbying costs:

$$\begin{aligned} I_P^{\alpha} &= \alpha wL + \pi_G(\theta) - B^{\alpha}(\theta) \\ I_P^{\beta} &= \beta wL + \pi_N(\theta) - B^{\beta}(\theta) \\ I^{\gamma} &= \gamma wL \end{aligned} \quad (8)$$

where $I_P^{i}\,(i = \alpha, \beta)$ denotes the group's net income in the political game. The indifferent group does not lobby, they only choose the food product available in the market, so their net income does not change. We assume that lobbying is costly (Laffont and Tirole, 1993); and that a one dollar contribution costs $\left(1 + \lambda^{i}\right)$ dollars in donations for lobby i. That is, $B^{i}(\theta) = \left(1 + \lambda^{i}\right) C^{i}(\theta)$, where λ^{i} is nonnegative and represents the efficiency of lobbying. B^{i} is the total money collected for lobbying from group members. A group with a large membership collects a higher sum of contributions. But the lobbying efficiency also matters for the political outcomes. A higher λ^{i} implies less efficient lobbying or, equivalently, higher lobbying cost. The WNO may have different preferences for interest groups. One group may have a higher efficiency and hence lower costs than the other group in the lobbying process. Lemma 2 of Grossman and Helpman (1994) provides the micro-foundations for lobbying and implies the optimal contribution level $C^{i*}(\theta)$ for each group, which is determined by:

$$\frac{\partial W^{i}(\theta)}{\partial \theta} = \left(1 + \lambda^{i}\right)\frac{\partial C^{i*}(\theta)}{\partial \theta} \quad for \quad i = \alpha, \beta \quad (9)$$

In the above equation, we can see that due to lobbying costs $\left(\lambda^{i}\right)$, the marginal effect of NPBT food policy on the contribution is smaller than the marginal effect of NPBT food policy on welfare. It is, therefore, costly to lobby. The optimal political NPBT food policy is determined by:

$$\frac{\partial G(\theta)}{\partial \theta} = q\frac{\partial W(\theta)}{\partial \theta} + \left(1 - q\right)\left[\frac{\partial C^{\alpha}(\theta)}{\partial \theta} + \frac{\partial C^{\beta}(\theta)}{\partial \theta}\right] = 0 \quad (10)$$

We substitute Equation (9) into Equation (10), and find that the first order condition for NPBT food policy can be expressed as:

$$\begin{aligned} \frac{\partial G(\theta)}{\partial \theta} = &\left(\frac{1 - q}{1 + \lambda^{\alpha}} + q\right)\frac{\partial W^{\alpha}(\theta)}{\partial \theta} + \left(\frac{1 - q}{1 + \lambda^{\beta}} + q\right) \\ &\frac{\partial W^{\beta}(\theta)}{\partial \theta} + q\frac{\partial W^{\gamma}(\theta)}{\partial \theta} = 0 \end{aligned} \quad (11)$$

Equation (11) is different from Equation (6), which means that the politically determined NPBT food policy is a deviation from the social optimum, unless λ^{i} is extremely high or $q = 1$. We can see that lobby groups will not make contributions if the lobbying is extremely costly (i.e., λ^{i} is high). Similarly, the WNO will not consider the contribution from groups if it only considers welfare (i.e., $q = 1$).

Lobbying influences NPBT food policy and the food security status because the lobby contribution is taken from the income, according to Equation (8). The two groups spend B^{i} for lobbying, so the budget constraint shifts inward, which decreases the demand for both NPBT and non-NPBT food products as well as numeraire goods. The inwardly shifting budget constraint directly influences food security due to food access. The reduction in food demand decreases the amount of food consumed in equilibrium. More lobbying efforts from the NPBT food lobby may push the NPBT food compliance cost down and improve the overall food security, but food security will be improved only if the benefits from lower policy costs compensate the lobbying costs of the two groups. But, if the policy is stricter under lobbying, the food security will decline. To summarize,

Lemma 1 *The politically determined NPBT food regulation is a deviation from the socially optimal NPBT food regulation due to the unbalanced lobbying power of interest groups. Political rivalry among interest groups worsens the food security status unless the benefit from a lenient NPBT food regulation compensates for the lobbying costs.*

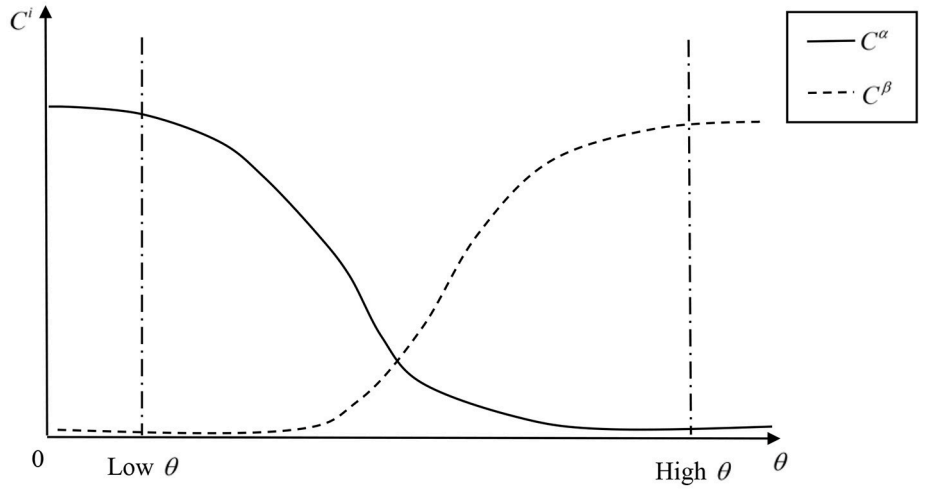

FIGURE 2 | Truthful Contribution Schedules and the Level of GM Food Regulations.

FOOD SECURITY AS A POLICY TARGET

NPBT food technology is a possible solution to improve food productivity and security (e.g., Paarlberg, 2010; Vigani and Olper, 2013). In this section, we discuss the political rivalry concerning NPBT food policy formation if the WNO wants to improve the food security level. We aggregate the three food security aspects (availability, access, and utilization) into a single variable s, which denotes the world's food security level.

Suppose the world has a target food security level to reach and allows NPBT food technology to be used in the agricultural food production. If the food security level is below the target level, the WNO would like to increase its food output by using more of the productive technology. Therefore, we define $\mu = \bar{s}/s$, where $\bar{s}$ is the target food security level and s is the current level. $0 < \bar{s} < 1$ and $0 < s < 1$. We use μ to indicate the inverse of the current progress toward the food security level of the world, $\bar{s}$. The non-NPBT food consumers constitute a significant part of social welfare, but an increase of s through more NPBT food input does not increase their welfare directly. Therefore, we use an indirect way of including food security in the WNO's objective function, namely by changing the weights of the different lobbying contributions based on progress toward food security. Although μ is an exogenous variable for the lobbying groups and does not depend on the groups' lobbying efforts, it will influence the lobbies' contribution behaviors in the political process. In this case, the WNO payoff function becomes

$$G_s = qW + (1 - q)\left(\mu C^\alpha + C^\beta\right) \tag{12}$$

We can use backward induction to find the optimal lobbying schedule for the two lobby groups. If the NPBT food lobbying group knows that the WNO will try to increase the food security level in the second stage, it will change its optimal lobbying schedule in the first stage. That is,

$$\frac{\partial W^\alpha}{\partial \theta} = \left(\frac{1 + \lambda^\alpha}{\mu}\right)\frac{\partial C_s^\alpha}{\partial \theta} \tag{13}$$

The NPBT food group spend $B_s^\alpha = \left(\left(1 + \lambda^\alpha\right)/\mu\right) C_s^\alpha$ for lobbying, which is smaller than in the absence of a food security improvement target (section The Political Process). The NPBT food group contribution weighs more in the policy-making process when $\mu > 1$ (i.e., food insecure). NPBT food consumers spend less of their income, which improves food affordability under a constant NPBT food price of food consumption.

Comparing Equation (13) with (9), we can see that the NPBT food group is more efficient in the political process. One unit of welfare gain in the lobbying process needs $\left(1 + \lambda^\alpha\right)/\mu$ units of contribution instead of $(1 + \lambda^\alpha)$. From the WNO perspective, the income from lobby groups stays constant because one unit of NPBT food group contribution counts for more in the WNO payoff function. Lobby groups would spend less than when food security is not a policy issue for a lenient NPBT food regulation, according to Equation (13).

From the above discussion, we determine that

Lemma 2 *When the food security status is an important part of a WNO policy, the NPBT food group will be more efficient in the political game, but the WNO will not be worse off because it has the same total contribution income.*

When the production level reaches its target food security level, the WNO resorts to its old weights. In this case, the WNO does not weigh the NPBT food lobby heavier than the non-NPBT food lobby; lobbies compete equally in the policy game. If the non-NPBT food lobby has a large membership and is more powerful in the political process than the NPBT food lobby, the non-NPBT food contribution will be high, and finally the NPBT food regulation will be strict. In this case, the WNO could also weight the non-NPBT food lobby heavier than the NPBT food lobby without decreasing its payoff.

DISCUSSION OF IMPLICATIONS

The results of the model show more strict regulations on the approval and use of NPBTs will have negative implications for food security following standard definitions of food security. The costs of food production increase by more stringent regulations decreasing the overall supply of food. Further, the fact that decision makers are exposed to lobbying and lobby groups can influence NPBT regulation. This may seem rather trivial, but the important message is that lobbying is not only done by one group. The more policy makers consider implications for food security, the less they will be influenced by lobby groups. In the case of NPBTs, the implication is that supporters of the technology have to lobby less than opponents or if they lobby they will stress the importance of NPBTs for food security.

One of the important assumption being made is that NPBTs provide an improvement in crop yield and increase food security. Readers have to bear in mind that this is one of the important assumptions in our model and further discussions rely on that assumption. The applications of NPBTs, however, suggest this will indeed be the case. Some of the already available applications include herbicide resistant oilseed rape and sunflower cultivated in France, non-browning apples, mushrooms and potatoes, late-blight resistant potatoes and more (Sprink et al., 2016; CAST, 2018). It is reasonable to expect the use of NPBTs will generate environmental as well economic benefits similar to GMOs increasing food security via higher yields and safer food. As the discussion about the safety of NPBTs shows, this is for most cases a reasonable assumption (Sprink et al., 2016).

Many low food security countries often implement strict food policies for GMOs (e.g., Paarlberg, 2009; Wesseler et al., 2017). Similar results can be expected for NPBTs. The results of our model suggest that policy makers are strongly influenced by lobby groups and that in the context of GMOs anti GMO lobby groups have been more successful. This supports the argument made by Paarlberg (2009) that some policy makers in Africa orient their policies more toward the policies in the European Union than being guided by the needs of their own populations. Similar observations have been reported for the case of insect resistant cotton (Herring, 2008) and Vitamin A enriched rice (Wesseler and Zilberman, 2014) in India.

For the case of NPBTs the possibility exists that the outcome for the case of GMOs can be changed if supporters for NPBTs increase their lobbying efforts and combine this with stressing the importance for food security. We use the parable of a World Nation Official as a benevolent dictator that has the power to decide about regulatory policies. The more food security will be considered as being important by the WNO the less influential lobby groups trying to block the introduction of NPBTs will be. Food security has become an important policy agenda item as part of the Sustainable Development Goals (SDGs) under Goal 2: end hunger, achieve food security and improved nutrition and promote sustainable agriculture. This increases the possibility that food security will receive more attention than before and according to our model results, the impact of lobby groups blocking the use of NPBTs will be reduced.

Looking at the European Union where there is an on-going debate about the regulation of NPBTs the results of our model provide some important insights. First, groups gaining and losing from NPBTs will both lobby and try to influence the policy outcome. Many environmental groups oppose the use of NPBTs and lobby for regulations similar to regulations for GMOs (Smart et al., 2015; Sprink et al., 2016; Purnhagen et al., 2018a). Their impact on regulatory policies in the EU can be expected to be stronger as in comparison to their impact on policies at e.g., FAO as decision making bodies within the European Union can be expected to care less about food security considering the supply of food within the European Union, relatively speaking. This finds support by the recent judgment of the Court of Justice of the European Union (CJEU, 2018).

The challenge for regulators is to take the implications of their regulatory policies for food security into considerations. A more stringent regulatory system reduces food security under the assumption of food safety. A stringent regulatory policy not only includes the requirements for safety assessments, which can already be substantial (e.g., Smyth et al., 2017), but also the time-length (Smart et al., 2017). There exist a number of opportunities in the European Union and the United States for improving regulatory policies (Wesseler and Kalaitzandonakes, 2011; CAST, 2018; Purnhagen et al., 2018b). A clear regulatory policy that aims at reducing regulatory costs without compromising food safety can have a positive "lobbying" effect for policy makers in particular in Africa who look for guidance and are exposed to different lobby groups (Falck-Zepeda et al., 2013).

CONCLUSION

NPBTs are an advanced technology to improve agricultural production. They are regarded as one of the options for improving global food security. The dispute about the effects of the technology on humans and nature impede its application as e.g., for the case of Vitamin A enriched rice (Wesseler and Zilberman, 2014). This debate also applies to NPBTs and as a consequence the level of NPBT food regulation is also a political game.

This paper develops a standard political economy model of NPBT regulations, modeling the NPBT food policy as the outcome of a NPBT and non-NPBT food group lobbying game. We find that a stricter NPBT food policy has negative effects on three aspects of food security: availability, access, and utilization. The politically determined NPBT food policy worsens the food security situation under the costly lobbying assumption. We also discuss when the WNO weighs the NPBT and non-NPBT food lobbies' contributions differently depending on the food security status. The NPBT food lobby becomes more efficient in the political game than the non-NPBT food group when the WNO commits to improving food security. If the non-NPBT food lobby is large and strong, it will make large lobbying contributions for a stricter NPBT food policy, even when the world is food insecure. The pro-NPBT food lobby group will be more effective if the WNO policy reflects concerns about food security. Linking the results to international debates on

NPBTs in the case where the opposition to the NPBT food technology is more successful, either the opposition has more financial resources available for lobbying or the governing bodies are less concerned about food security. What in this case is the most dominating factor will be an empirical question. Considering the importance of the issue, this warrants further research.

High-income countries, such as some European countries, can afford to implement a strict NPBT food policy without worsening the food security condition, but for more than two-thirds of low-middle-income countries, the food security issue remains (Economist Intelligence Unit, 2015). The NPBT food policy in many developing countries, such as Southern Asian and African countries, is still under debate, whereas many of them experience food shortages and malnutrition. The various countries could have tailored NPBT food policies according to each of their domestic food supply and demand, but they also need to take food security into consideration while making food policies.

The model presented provides an economic explanation for the observed lobbying activities. For many, it is obvious that the input supply sector of the technology will gain from lobbying for less strict regulations. But, there are also some private gains from lobbying against the technology, as claimed explicitly by Apel (2010) and more indirectly by Paarlberg (2009), when referring to projects funding biotechnology regulations in Africa. The political rivalry between contradictory interest groups offers an additional explanation why new technologies often have faced resistance, not only GMOs (Juma, 2016; Moses, 2016). Our model explains the market competition of the NPBT and non-NPBT food products and the driving force of lobbying competition that drives opposition to new technologies. The challenge is to identify what are the private economic gains of lobby groups that oppose new technologies. One obvious benefit is reducing stakeholder losses from being displaced by the new technologies. In the case of NPBT food technologies, environmental and other non-governmental organizations are more vocal within the European Union. Within the European Union, the group for non-NPBT food products is much larger than the group supporting the NPBT food technology (Clancy, 2017). Again, this raises the question of what do they gain? Or do EU policy makers care less about food security?

AUTHOR CONTRIBUTIONS

QS, MP, and JW contributed conception and design of the study. QS developed the model and simulation. QS and MP developed the game theoretical model. QS wrote the first draft of the manuscript. QS, MP, and JW wrote sections of the manuscript. All authors contributed to manuscript revision, read and approved the submitted version.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpls.2018.01324/full#supplementary-material

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Development of Wheat With Hypoimmunogenic Gluten Obstructed by the Gene Editing Policy in Europe

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Coeliac Disease (CD) is an auto-immune reaction to gluten in 1–2% of the human population. A gluten-free (GF) diet, excluding wheat, barley, and rye, is the only remedy. This diet is difficult to adhere to, partly because wheat gluten is added to many processed products for their viscoelastic properties. In addition, GF products are less healthy and expensive. Wheat products containing only hypoimmunogenic gluten proteins would be a desirable option. Various gluten peptides that trigger CD have been characterized. A single wheat variety contains around hundred gluten genes, producing proteins with varying numbers of epitopes. Gene editing using CRISPR/Cas9 can precisely remove or modify the DNA sequences coding for immunogenic peptides. Wheat with hypoimmunogenic gluten thus exemplifies the potential of gene editing for improving crops for human consumption where conventional breeding cannot succeed. We describe here, in relation to breeding hypoimmunogenic wheat varieties, the inconsistencies of applying GM regulation in Europe for gene-edited plants while mutation breeding-derived plants are exempted. We explain that healthy products derived from this new technology may become available in the United States, Canada, Argentina and other countries but not in Europe, because of strict regulation of unintended GM risk at the expense of reduction the existing immunogenicity risks of patients. We argue that regulation of gene-edited plants should be based on scientific evidence. Therefore, we strongly recommend implementing the innovation principle. Responsible Research and Innovation, involving stakeholders including CD patient societies in the development of gene-editing products, will enable progress toward healthy products and encourage public acceptance.

Keywords: coeliac disease, mutation breeding, new plant breeding technique, public acceptance, innovation principle, GM regulation, genetic modification, risk assessment

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WHEAT GLUTEN AND COELIAC DISEASE

Bread wheat (*Triticum aestivum*) is a staple crop consumed worldwide. The properties that make wheat flour suitable for bread-making are conferred by gluten, the glutenin and gliadin storage proteins present in the grain. High molecular weight (HMW) glutenins provide dough with elasticity, which is the most important property for bread quality, while gliadins provide viscosity (Shewry et al., 2009).

Wheat gliadins, and to a lesser extend low molecular weight (LMW) glutenins, carry immunogenic peptides that can cause Coeliac Disease (CD) in 1–2% of the human population (Fasano, 2006). CD leads to an inflammation of the small intestine, which affects nutrient absorption and causes diverse symptoms (Husby et al., 2012).

A gluten-free (GF) diet, excluding wheat, barley, and rye, is the only way CD patients can avoid symptoms. It is difficult to adhere to as wheat gluten is added to many food products (Atchison et al., 2010). Furthermore, current GF products are low in proteins and nutrients, high in salt and contain many additives to emulate the rheology of gluten-based dough (Caponio et al., 2008; Capriles and Arêas, 2014; Belz, 2016; Horstmann et al., 2016). Hence, healthier but safe products for CD patients are needed.

BREEDING TOWARD HYPOIMMUNOGENIC WHEAT: A COMPLEX CHALLENGE

Breeding wheat without immunogenic epitopes (Gilissen et al., 2008, 2014) would be a definitive solution for CD patients (Shewry and Tatham, 2016). Developing "hypoimmunogenic gluten" wheat varieties that retain baking quality is, however, very challenging. Firstly, gluten proteins are encoded by five gene families containing many immunogenic epitopes. Within these families, α-gliadins on chromosomes 6 trigger CD strongly, followed by γ-gliadins, ω-gliadins, and LMW glutenins on chromosomes 1. Secondly, bread wheat is allohexaploid, with three sets of chromosomes referred to as genome A, B, and D. Each of these genomes contains all gluten gene families. As a result, a single bread wheat variety has a combination of gliadins and glutenins, some without any CD epitopes, others with one or more immunogenic epitopes (Van Herpen et al., 2006; Tye-Din et al., 2010; Salentijn et al., 2013; Ozuna et al., 2015). No cultivated wheat or wild relative has been identified that contains only CD safe gluten epitopes (Van den Broeck et al., 2010a,b). Consequently, conventional breeding alone cannot produce hypoimmunogenic varieties.

Gil-Humanes et al. (2010) used RNA interference to reduce the expression of the gliadin gene families by 97%, abolishing stimulation of T cells from CD patients while no major issues were reported regarding seed germination or dough quality (Gil-Humanes et al., 2014). Becker et al. (2012) reduced the expression of up to 20 α-gliadins, but other storage proteins became more abundant. As the transgenic RNAi construct remains in the wheat genome to silence the genes, these plants are subject to GM regulation, which in the EU is expensive, takes a long time, and has an uncertain outcome (Laursen, 2016). In practice this precludes investments in what initially will be a niche product.

Another approach is mutation breeding. Exposure to γ-irradiation has been used to randomly remove large regions of chromosomes in wheat, among which the gluten genes on chromosomes 1 and 6 (Van den Broeck et al., 2009). Selected mutations in separate plants can be combined by crossing and selecting, as was done for "ultra-low gluten" barley (Tanner et al., 2016). We screened a γ-irradiated population of variety Paragon (JIC, Norwich, United Kingdom) to identify relevant deletions in hexaploid bread wheat. Paradoxically, mutation breeding is regulated as conventional breeding based on a history of safe use, although it randomly alters or removes many other genes besides the intended ones.

CRISPR/CAS9 EDITING OF GLIADIN GENES TOWARD HYPOIMMUNOGENIC GLUTEN

Gene editing (Baltes and Voytas, 2015), a prominent New Plant Breeding Technique (NPBT), can be used to develop wheat with hypoimmunogenic gluten (Jouanin et al., 2018; Sánchez-León et al., 2018). A Cas9 nuclease is directed by a guide RNA to a target region within the genome and generates a double strand break. Inaccurate DNA repair by the plant may result in mutations at the target site. As a pilot project, we focussed on mutating epitopes in α- and γ-gliadin genes – which are the most immunogenic – separately and simultaneously using CRISPR/Cas9. We transformed immature embryos of the bread wheat variety Fielder with constructs with Cas9 and multiplex guide RNA constructs, and regenerated plants. Due to the contiguity of the gliadin genes on the chromosome, gene copies located between two DNA breaks may be lost from the genome as well. Sánchez-León et al. (2018) successfully targeted α-gliadins with CRISPR/Cas9, generating small deletions. The Cas9 construct is to be out-crossed in subsequent generations (Schaeffer and Nakata, 2015; Sprink et al., 2015). Alternatively, Cas9 can be delivered through transient expression or as ribonucleoprotein (Zhang et al., 2016; Liang et al., 2017).

First, we tested grains of the plants produced for changes in gluten composition by acid-polyacrylamide gels, and determined the number of mutated or deleted gliadin genes using droplet digital PCR. Some γ-irradiated lines showed identical gliadin profile changes to gene-edited lines (**Figure 1**). Sequencing data enabled determining the type of mutations generated, while proteomics analysis can identify changes in amino acid composition of modified gliadin proteins. These data will enable predicting whether a mutation in an epitope decreases its immunogenicity, as crucial residues have been determined experimentally (Mitea et al., 2010) and the affinity of the human receptors has been fully characterized (Petersen et al., 2014, 2016).

Second, gluten from selected lines should be tested for immunogenicity and dough rheology. These tests are designed in collaboration with gastroenterologists, immunologists, food scientists, and CD patient associations. They comprise *in vitro* studies using epitope-specific T-cell clones isolated from CD patients (Anderson et al., 2000) and bread quality tests. Sánchez-León et al. (2018) made CRISPR/Cas9 mutant wheat lines with altered α-gliadin profiles and a reduction in immunogenicity, which retained acceptable dough quality.

As a third and final step, *in vivo* studies are needed where gluten from mutant grains would be given to voluntary

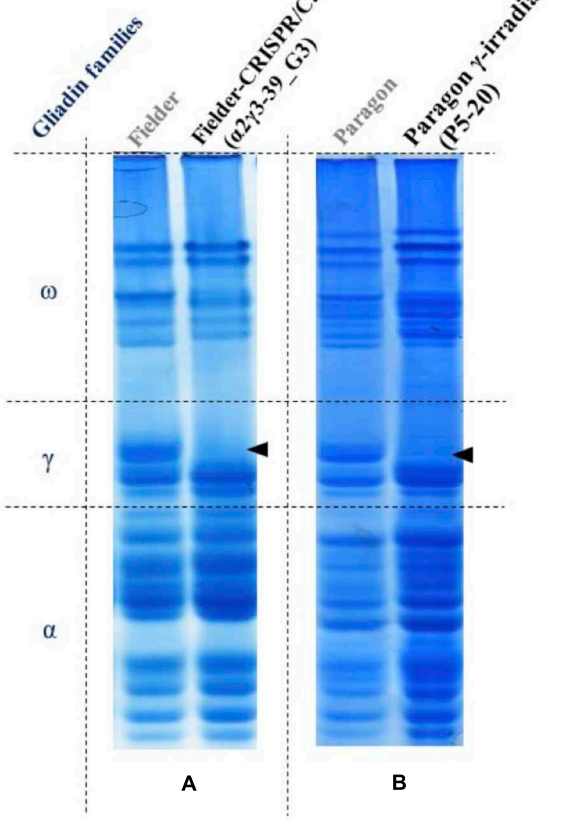

FIGURE 1 | CRISPR/Cas9 gene editing **(A)** and γ-irradiation mutation breeding **(B)** generated similar changes in wheat γ-gliadin protein profiles from Jouanin et al. (2018). Lanes are Acid-PAGE gliadin profiles from control lines (on the left), a T1 Fielder grain, stably transformed with a CRISPR/Cas9 construct, and a M4 Paragon mutant line. Similar amounts of protein extract were loaded.

CD patients to confirm hypoimmunogenicity. Then, hypoimmunogenic wheat will be ready to be cultivated in a separate production chain, carefully controlled from field to packaging to avoid contamination with regular wheat, barely or rye, similar to a GF oat chain (Smulders et al., 2018). It will likely be sold under a specific hypoimmunogenic gluten label.

GENE-EDITED PLANT VARIETIES: REGULATION, SAFETY, ACCEPTANCE AND POLICY IN EUROPE

We describe here, in relation to hypoimmunogenic wheat, the inconsistencies of applying GM regulation for gene-edited plants in Europe while mutation breeding-derived plants are exempted. The EU regulation is based on the process used, not on the product generated, and follows the precautionary principle. Other countries have a product-based system (Canada) or a mixed product/process-based system (United States, Argentina).

The Origin of GM Regulation for Gene Editing Plants in Europe

Competent Authorities of several EU countries, including the Swedish Board of Agriculture, as well as the European Food Safety Authority [EFSA], 2015) are in favor of adopting gene-edited products (Sprink et al., 2016a) with conventional breeding regulations or adapted regulations (Whelan and Lema, 2015). EFSA found a very low level of intended or unintended risks associated with site-directed mutated products (European Food Safety Authority [EFSA], 2012). Furthermore, the former Chief Scientific Advisor to the President of the European Commission (Simon, 2013) and the European Academies Science Advisory Council [EASAC], 2015) supported the regulation of gene editing plants as non-GM. However, the EC postponed a decision, mainly due to pressure from NGOs (Lawler, 2015). Recently, the European Court of Justice ruled that according to the text of the Directive 2001/18/EC, 2001. such products should be regulated as GM (European Court of Justice [ECJ], 2018a,b).

Inconsistent Regulation of Mutated Plants in Europe

Random Versus Targeted Mutations

Mutation breeding deploys chemical mutagens or radiation. Because mutations occur randomly, large mutant populations must be screened to find a plant that contains the desired mutation, and each plant will contain many other mutations. These plants and products are considered as GM but exempted from GM regulation in most countries, including the EU (Directive 2001/18/EC, 2001. Annex 1B), due to a history of safe use and consumption since the 1930's. Over 3200 commercial crop varieties have been produced using mutation breeding (Ahloowalia et al., 2004; Bado et al., 2015).

Gene editing uses a nuclease to generate a double-strand break at a desired target site in the genome, and plants are selected in which a mistake during repair led to a mutation of the target site. Off-targets may occur at a low frequency, much lower than in mutation breeding. In a product-based approach, the fact that plants obtained via gene editing are similar to those obtained using mutation breeding, means that they will follow the regulation of conventionally bred plants due to history of safe use (**Figure 1**). In contrast, in a process-based approach, as used by the EU, it has to go through the process of GM risk assessment.

Detrimental Effects on Costs and Opportunities

GM regulation of gene edited plants in Europe implies time-consuming (6 years) and costly ($35M) GM safety tests and administrative processes (McDougall, 2011), with uncertain outcome as the final permission is still a political decision. GM regulation erases the core advantages of gene editing as a quick, precise, and cheap method to develop high added-value plants to meet the needs of consumers and society.

In the United States, were both mutation breeding and gene editing are exempted from GM regulation, the latter will be preferred since it is more precise, faster, and versatile as it can produce homozygous mutations in several gene families simultaneously targeted (**Figure 2**). Consequently, European

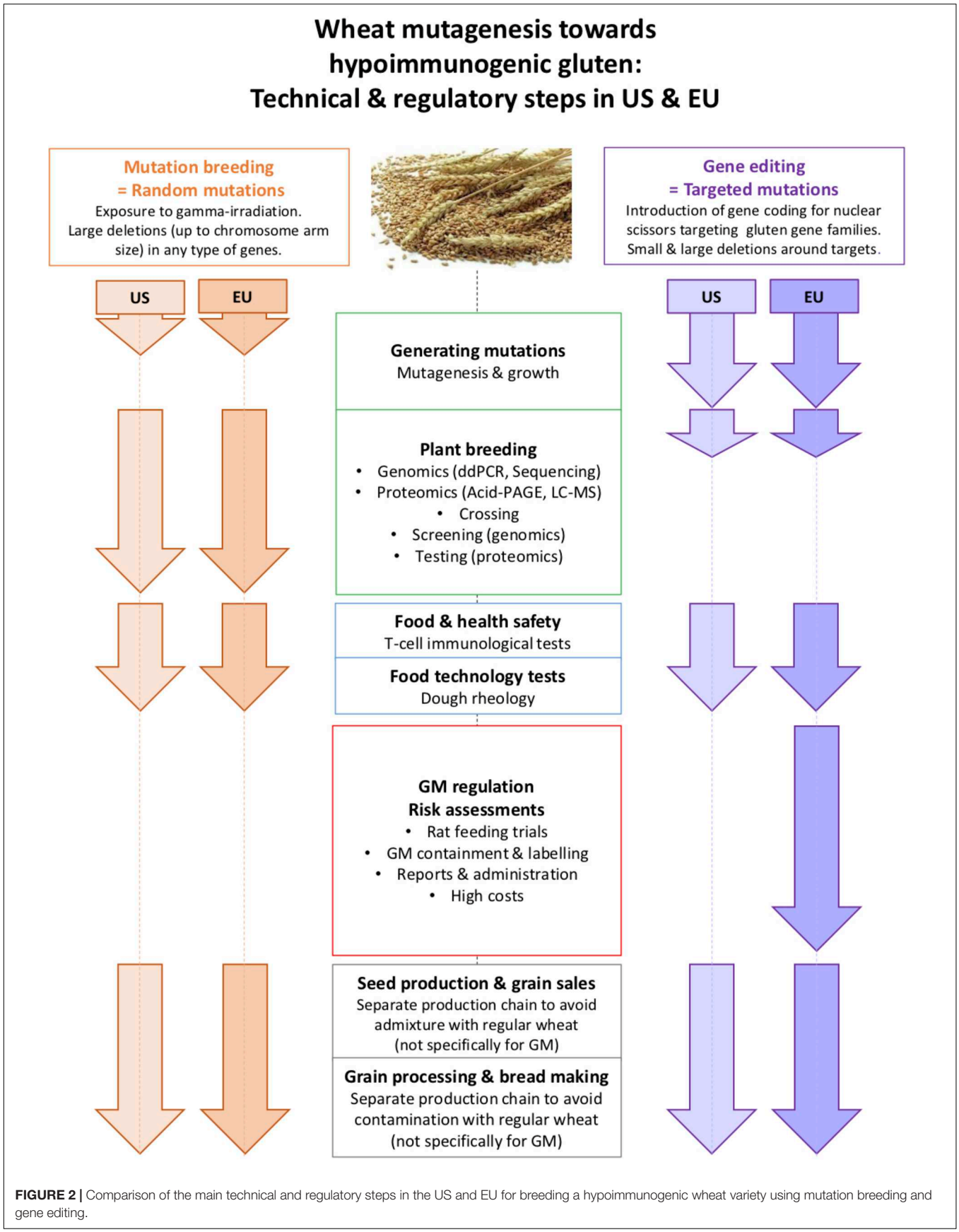

FIGURE 2 | Comparison of the main technical and regulatory steps in the US and EU for breeding a hypoimmunogenic wheat variety using mutation breeding and gene editing.

companies move their research facilities to the United States (Burger and Evans, 2018), and European researchers move to United States start-ups focusing on gene editing, such as Calyxt, which develops reduced-gluten wheat for the United States market. As hypoimmunogenic wheat will initially be a niche product, the costs of GM regulation will be too high for small and medium-sized companies in Europe. Thus, regulation of gene editing as GM will impede innovation, competitiveness, and access to healthier food in Europe.

Detection, Labeling and Effects on Trade

It is often impossible to distinguish products obtained using gene editing from those with mutation breeding or from 'natural', spontaneous mutations (Sprink et al., 2016b). The absence of distinctness will hamper control and labeling of gene editing-derived products, especially when it concerns material from outside Europe where gene-edited varieties are exempted from GM regulation. It represents a major issue. If Europe does not accept gene-edited products due to their lack of compliance with GM regulation applied in EU, this would block the import of any product that is not GM-labeled and tested. Indeed, any non-GM labeled product could potentially be produced with gene-editing, since there is no obligation of labeling gene-edited products in the United States. As a consequence, world trade could be disrupted (Cheyne, 2012).

Some gene-edited plants had similar targeted mutations as plants produced with γ-irradiation (**Figure 1**) and they cannot be distinguished by their gluten profile. In case of hypoimmunogenic wheat, a separate production chain is always required to avoid contamination with regular immunogenic wheat. The traceability is guaranteed, and products will be labeled as hypoimmunogenic, so it would be relatively easy to label them as derived from gene-edited wheat, even in the United States. For other products this will not be the case, as no separate production chain is necessary.

Food Safety, Environmental Safety and Food Security Tests Under GM Regulation

For each new technology one undertakes a cost, benefit, and risk analysis. According to the (European Food Safety Authority [EFSA], 2012), the scientific facts gathered so far show no higher food safety risks of gene-edited plants than mutation breeding-derived plants that have a history of safe consumption. Furthermore, gene editing leads to plants with fewer off-targets modifications, making them at least as safe as conventionally bred ones (Lucht, 2015). This implies, from a risk assessment perspective, that gene-edited plants should be regulated as conventionally bred ones (European Plant Science Organisation [EPSO], 2015).

Food Safety Testing

The GM food safety risk assessment tests are related to the presence of foreign genes in the plants. These tests have not uncovered issues for over two decades (Swiss, 2012) and are not adapted for gene editing due to the absence of foreign genes introduced. In case of hypoimmunogenic wheat varieties, food safety issues will already thoroughly have been tested for coeliac patients, to ensure that epitope content is sufficiently low. However, to comply with the GM regulation for food safety, a rat feeding study has to be performed to test whether animals (that do not have CD) would develop unknown symptoms from eating hypoimmunogenic compared to regular wheat. On top of time, costs, and animal welfare issues, there is no relevance for these tests.

Environmental Safety Testing

Regarding environmental risks, under GM regulation, gene-edited plants have to follow strict containment rules. With regard to outcrossing, bread wheat is a self-pollinated species, and there are no wild populations. Outcrossing to other varieties would introduce hypoimmunogenic gluten, which is safer for human health, while bread quality would barely be affected. Gluten proteins are storage proteins in the grain and loss of gluten storage proteins did not lead to decreased fitness in ultra-low gluten barley (G.J. Tanner, CSIRO, Australia, Personal Communication).

Food Security

Considering food security, regulating gene editing as GM in Europe impedes the goals of increasing food production with fewer inputs (Ishii and Araki, 2016) for all types of agriculture, including integrated and organic farming (Andersen et al., 2015). As the economy of many developing countries relies on food exports to the EU, regulating gene editing as GM in the EU consequently has a negative impact on the availability of the technology for local markets in these countries, affecting their food security (Heap, 2013).

Public Acceptance and Responsible Research and Innovation

The public needs to be better informed about new food technologies, to enable educated choices about food consumption. Scientists should contribute to this knowledge transfer and creation of awareness. However, the complexity of science often confuses people's risk perception, decreasing their trust in scientific facts and increasing their fears, that they base on inaccurate information or wrong concepts from non-scientific sources (Lucht, 2015). This contributes to empower NGOs that influence the regulation-making process by claiming to protect consumers' safety on no scientific grounds.

In a context where scientific communication has proven to be insufficient, the Responsible Research and Innovation initiative (RRI) (Owen et al., 2012) should be implemented as complementary approach. Targeted consumers should be asked for their interest in a potential product benefiting them and their trust in the methods used, in order to assess product acceptance prior its development, and they should remain involved during the whole process.

CD patients are the prime consumers for gene-edited hypoimmunogenic wheat. Following this RRI initiative, the idea of developing such a product has been discussed with CD patient associations early on. They understand the complexity of the challenge and appreciate the effort of scientists to develop a

solution, even if the initial results are not perfect, as often when developing products concerning health issues (Schenk et al., 2011).

Gene-edited hypoimmunogenic wheat fits into a strongly growing market of GF food for coeliacs and other consumers (Sapone et al., 2012). In addition, it may contribute to preventing genetically predisposed children of developing CD, as quantity of exposure matters (Koning, 2012). Thus, there is a clear prospective gain in health which is held back in Europe by the GM regulation of gene editing. CD patients, relatives and others benefiting from gene-edited products should stand up and help the scientific community to convince politicians to adopt a science-based regulation of gene-edited plants and derived products.

Policy Making: "Innovation Principle" Instead of "Precautionary Principle"

Considering the incoherence of applying GM regulation in EU to gene-edited products that may be identical to conventional varieties and anticipating its consequences in terms of food and environmental safety, food security, as well as associated economic issues, we strongly urge the EC to review its position on the matter. So far, "the precautionary principle" (European Commission [EC], 2000) is being applied solely, although technically this principle, meant as a provisional measure to avoid discernible risks based on scientific evidence, is not valid anymore considering the history of safe use of GM [no evidence of hazards for 20 years (Swiss, 2012)]. We argue that the "innovation principle" (European Political Strategy Centre [EPSC], 2016) should be used instead where relevant risk assessment would be designed on a case-per-case base, to enable benefiting of gene-edited products while complying with relevant risks management. This would constitute an appropriate regulation for the future of food security, healthy food, as well as protection of the environment and economy.

CONCLUSION AND RECOMMENDATIONS

Gene editing has made it possible to remove CD epitopes from wheat gluten. It is expected that in America, derived-products from such wheat will be on the market soon. Due to their absence of compliance with GM regulation, these products will remain illegal in the EU, as long as gene-edited products will be regulated as GM, following a process-based approach.

In addition, these niche products would not be developed in EU either due to the lack of profitability associated with expensive GM regulation tests and labeling. These GM tests, based on an precautionary principle, are required to detect unintended effects associated to transgenes, which are not present in the product.

We argue that, instead, gene-edited plants should be regulated as plants made with mutation breeding, on a product-based approach, and follow the innovation principle. This principle values benefits associated with the product while scientifically complying with trait-specific risk management. It should be part of a RRI involving targeted consumers as stakeholders, to ensure their acceptance throughout the gene-edited product development process.

Food safety, environmental safety, and food security in Europe will directly be affected by the regulation of gene editing as GM, and we expect politico-economic issues related to non-GM regulation of gene editing in other countries. Therefore, we strongly advise the EC to review its position on NPBT regulation by considering the present case and the regulatory advices provided.

AUTHOR CONTRIBUTIONS

AJ conceived this perspective and wrote the first version. MS adapted the manuscript. LB and RV introduced arguments and edited the text. AJ and MS edited the final version. All authors approved the final version.

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Policy and Governance Perspectives for Regulation of Genome Edited Crops in the United States

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Genome editing for crop improvement lies at the leading edge of disruptive bioengineering technologies that will challenge existing regulatory paradigms for products of biotechnology and which will elicit widespread public interest. Regulation of products of biotechnology through the US Coordinated Framework for Biotechnology is predicated on requiring burden of proof that regulation is warranted. Although driven by considerations of newly emerging processes for product development, regulation has, for the most part, focused on characteristics of the biotechnology product itself and not the process used for its development *per se*. This standard of evidence and product focus has been maintained to date in regulatory considerations of genome edited crops. Those genome edited crops lacking recombinant DNA (rDNA) in the product intended for environmental release, lacking plant pest or pesticidal activity, or showing no food safety attributes different from those of traditionally bred crops are not deemed subject to regulatory evaluation. Regardless, societal uncertainties regarding genome editing are leading regulators to seek ways whereby these uncertainties may be addressed through redefinition of those products of biotechnology that may be subject to regulatory assessments. Within US law prior statutory history, language and regulatory action have significant influence on decision making; therefore, the administrative law and jurisprudence underlying the current Coordinated Framework strongly inform policy and governance when considering new plant breeding technologies such as genome editing.

Keywords: jurisprudence, administrative law, CRISPR, Coordinated Framework, GMO

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Abbreviations: DNA, deoxyribonucleic acid; EA, environmental assessment; EIA, environmental impact assessment; EIS, environmental impact statement; EPA, United States Environmental Protection Agency; ESA, Endangered Species Act; EOP, United States Executive Office of the President; FDA, United States Food and Drug Administration; FWS, United States Fish and Wildlife Service; GE, genetically engineered; NASEM, United States National Academies of Science, Engineering and Medicine; NEPA, National Environmental Protection Act; NIH, United States National Institutes of Health; NMFS, United States National Marine Fisheries Service; OSTP, United States Office of Science and Technology Policy; PIP, plant incorporated protectant; RAC, Recombinant DNA Advisory Committee; rDNA, recombinant DNA; synDNA, synthetic DNA; USDA, United States Department of Agriculture.

INTRODUCTION

Society now faces a wave of disruptive biotechnology innovation extending from uses of DNA as an information storage medium to applications of human genome editing and synthetic biology (NASEM, 2017). The use of genome editing for crop improvement is at the crest of this wave. Public uncertainty surrounds genome editing and its uses (O'Keefe et al., 2015), even though the scientific underpinnings for the genome editing of plants extend to the last century (Songstad et al., 2017) and regulators have been evaluating plants modified through genome editing since at least 2004 (Camacho et al., 2014).

Declining societal trust in emerging technology predates applications of modern biotechnology, but public resistance to genetic modification as tampering with nature stands as a particularly strong example of how public attitudes toward new technologies have been at odds with scientific institutions, regulatory authorities and traditional information providers (Frewer, 1999). Increasing skepticism of plant biotechnology is evident in the US. The first commercial uses in 1994 were met perhaps with more public curiosity than concern (Bruening and Lyons, 2000), but there has been a steady decline in public support to the point where in 2015, only 37% of the public viewed genetically engineered (GE) foods as safe as compared to 88% of scientists from a wide range of disciplines (Funk and Rainie, 2015).

Against this backdrop, regulators in the US have found no basis in existing regulation to encumber potential entry of genome edited crops into commercial use when the intended product shows no evidence for presence of recombinant DNA (rDNA) (Wolt et al., 2016). But in recognition of the massive amount of product innovation that may arise from emerging genome engineering technologies, including genome editing, there is increasing focus on the role of scientific and public governance mechanisms for decision making regarding future products of biotechnology (NASEM, 2017). Here we focus on bioengineering of plants and consider the historical interactions of regulatory policy and extra-regulatory governance mechanisms as they relate to decision making regarding GE crops. Further, we consider the implications of policy and governance for the emergence of genome edited crops and their derived products. While governance may be considered in a wide variety of contexts, we focus on concepts of jurisprudence applied to rule of law which reflects the administrative governmental structure of the United States (Stack, 2015).

In this paper, we begin with a review of the existing regulatory regime covering biotechnology-derived plants in the United States. Since the regulatory environment changes over time, we include mention of some of the important events that have shaped the present regulatory environment. Existing regulations are vague and ambiguous in their application to new technology, especially genome edited crops. While it might seem obvious that genome editing is biotechnology, genome edited crops need not contain genetic material from other organisms, and might contain no new DNA material at all – in some cases, editing simply involves removing or disabling a bounded genetic sequence or set of sequences. Regulators and others who wish to interpret existing and pending statutes and to understand its implications are therefore faced with a quandary. It is not obvious, *a priori*, to include genome edited organisms under existing regulations covering GE products, or as "products of biotechnology," a term with shifting meaning as applied in law (Executive Office of the President [EOP], 2016). The problem is one of legal interpretation in the context of regulatory decision making. Accordingly, the second part of the paper addresses the problem as a question of jurisprudence, considering alternative theories of legal interpretation from the perspective of administrative law in the effort to evaluate their implications for genome edited foods and crops. The review and analysis in this paper particularly addresses regulation in the United States. Our goal is to explain and evaluate aspects of the *status quo* in US regulatory law as it impinges on accommodating genome edited crops within the Coordinated Framework for Biotechnology.

EMERGENCE OF THE US GOVERNANCE FRAMEWORKS FOR BIOTECHNOLOGY

Scientific realization of the potential and implications of rDNA methods led in the 1970s and 1980s to widespread discourse within the scientific community and federal agencies as to the need for oversight specific to both the processes and products of biotechnology (National Research Council [NRC], 1989). Early discussions focused on the science were broadened to encompass ethical issues and legal liabilities. This culminated in the call from the Asilomar Conference for stringent scientific self-governance until the broader safety implications of rDNA technology could be understood (Berg et al., 1975).

The National Institutes of Health (NIH) formalized, through the Recombinant DNA Advisory Committee (RAC), the statement of principles coming from the Asilomar Conference in the form of binding guidance for contained use in NIH-funded research (US National Institutes of Health [NIH], 1976). This guidance was subsequently relaxed in light of improved understanding of the risks associated with the technology (US National Institutes of Health [NIH], 1978). Throughout the late 1970s and the early 1980s, NIH guidelines evolved to decentralize administration, reduce duplicative review processes, exempt certain types of experiments from review and to broaden scope of the guidance for considerations of human gene therapy and environmental releases (National Research Council [NRC], 1989). The NIH guidelines were adopted throughout federal agencies, and they influenced thinking and actions surrounding rDNA research and development in the private sector.

Influenced in part by *Diamond v. Chakrabarty,* a court challenge that upheld patentability of life forms (United States and Supreme Court, 1980), and thus encouraged commercial development in biotechnology, Congressional hearings considered the adequacy of oversight mechanisms for GE

organisms. These hearings concluded that existing statutory mechanisms were adequate to govern the technology but could benefit from clarification. Further, because there was, at the time, no way to quantify the risks posed by GE organisms to the environment, federal agencies were not able to assess risks to the environment for purposes of regulation (National Research Council [NRC], 1989). Concurrent with these Congressional oversight hearings, the White House Cabinet Council on Natural Resources and the Environment formed an interagency working group which initiated the process leading to formal coordination of biotechnology oversight activities among federal agencies (National Research Council [NRC], 1989).

THE US COORDINATED FRAMEWORK FOR BIOTECHNOLOGY

Beginning in 1984, a series of interagency working groups began in-depth evaluation of applicable laws for oversight of biotechnology, and the agencies most active in addressing biotechnology began formalizing their regulatory roles and policies. Following a shift in biotechnology coordination to the Office of Science and Technology Policy (OSTP), that office released the Coordinated Framework for Regulation of Biotechnology establishing regulatory responsibilities, lead agencies and jurisdictions relying on existing laws for oversight of biotechnology (Office of Science and Technology Policy [OSTP], 1986). As reflected in the Coordinated Framework, "the overall thrust of the regulatory response to biotechnology may be termed a minimalist, cost-effective, priority-driven approach requiring burden of proof that regulation is warranted," (Krimsky and Wrubel, 1996).

The most consequential regulatory approach to emerge from the Coordinated Framework was a shift away from earlier oversight considerations based on the biotechnology process used and toward the product of bioengineering. A critical consideration at the time was whether classical mutagenesis would be caught in the snare of product-focused assessments to force regulatory oversight of products which had not traditionally been subject to regulation (National Research Council [NRC], 1989). This concern stands in juxtaposition to current-day considerations of products developed through site-directed mutagenesis via genome editing where there are questions as to whether these products are analogous to products of classical mutagenesis, which remain outside of regulatory purview, or whether they are uniquely products of biotechnology that are to be regarded within the existing regulatory frameworks in the US and elsewhere (Sprink et al., 2016; Wolt et al., 2016).

The Coordinated Framework has been mutable over time, changing in response to advances in biotechnology innovation, knowledge gain, improved understanding of risks and uncertainties, and changing appreciation of the technology. This has been accomplished without new or revised legal statutes, but rather through the less onerous process of regulatory rulemaking and changes in regulatory guidelines.

In 1992, the Coordinated Framework was updated to clarify how regulatory authority should be exercised where there is latitude as to the discretion that may be taken by the implementing agency (Office of Science and Technology Policy [OSTP], 1992). The very specific language of this update to the framework emphasized that regulations should address only those risks that are "real and significant rather than hypothetical or remote" and show evidence risk is unreasonable. The policy's emphasis on health and safety has been construed by some as excluding considerations of societal impacts leading to "few meaningful opportunities for citizens to consider either the nature of the risks or their acceptability in the larger social context of the potential harms," (Kelso, 2003), however, obligations under the National Environmental Policy Act (NEPA) (Congress, 1969) mandate actions taken under the Coordinated Framework consider the effect on the human environment when "economic or social and natural or physical environmental effects are interrelated" (Code of Federal Regulations [CFR], 2003). Further, Congress has specified that policy, regulations and laws "utilize a systematic, interdisciplinary approach which will insure the integrated use of the natural and social sciences and the environmental design arts in planning and in decision making which may have an impact on man's environment" (42 USC part 4332, United States Code [USC], 2008).

Agency Regulatory Guidance and Rulemaking Actions

From the time of the 1992 update until later efforts to update the regulatory system for biotechnology products, beginning in 2015 (Executive Office of the President [EOP], 2015), there were no major changes brought forward to alter overarching goals and approaches outlined in the Coordinated Framework. In the intervening years, however, changes or attempts to change regulation of biotechnology were witnessed within regulatory guidance or rulemaking.

Food and Drug Administration

In 1992 the Food and Drug Administration (FDA) issued a policy statement on foods derived from plants developed by rDNA techniques. Further, FDA clarified their product-focused position that these foods were substantially equivalent to foods already in commerce and with the exception of "those cases when the objective characteristics of the substance raise questions of safety sufficient to warrant formal premarket review," no explicit regulatory action was needed within FDA (US Food and Drug Administration [FDA], 1992). In 2001 the FDA issued a proposed rule to require that developers submit a scientific and regulatory assessment of a bioengineered food before it is marketed (US Food and Drug Administration [FDA], 2001). Action on this proposed rule has not been taken and FDA continues to adhere to its voluntary consultation process for foods developed with rDNA. Over the years FDA has provided guidance to industry regarding their consultation procedures, early food safety evaluation and voluntary labeling standards for foods derived from GE plants (US Food and Drug Administration [FDA], 2018a).

Environmental Protection Agency

The US Environmental Protection Agency (EPA) has wide-ranging authority of direct or indirect bearing on products of biotechnology. The principle statutes under which EPA considers environmental safety and human health with respect to GE crops are the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA), the Toxic Substances Control Act (TSCA), and the Federal Food, Drug and Cosmetics Act (FFDCA). Crops which are GE to express plant incorporated protectants (PIPs) are considered with regard to the pesticidal protein and not the modified plant *per se*. Examples of PIPs include proteins from *Bacillus thuringiensis* (Bt), which confer insect resistance, and viral coat proteins which confer disease resistance. In addition to PIPs, the EPA indirectly considers crops GE to confer herbicide resistance (e.g., glyphosate resistance) by evaluating exposure to the herbicide (e.g., glyphosate) used to manage the crop. Over time the EPA has defined and refined their processes through specific regulatory actions (US Environmental Protection Agency [EPA], 2018).

Department of Agriculture

The United States Department of Agriculture (USDA), under provisions of the Plant Protection Act (PPA), provides the regulatory oversight of GE organisms to protect plant health. It does so by regulating the introduction of those GE organisms that may pose a pest risk to plants (CFR 7 part 340, Code of Federal Regulations [CFR], 1987). Beginning 2008, USDA undertook an effort to institute new rulemaking for GE organisms to encompass provisions of the Noxious Weed Act of 1972 in addition to the PPA with the intent to broaden the basis for regulation and to streamline the process for determinations of regulatory status of certain GE organisms (United States Department of Agriculture [USDA], 2008). Following extensive public comment, this proposal was withdrawn in 2015 allowing USDA to engage in new stakeholder engagement on APHIS biotechnology regulations and to initiate, for purposes of rulemaking, a programmatic environmental impact statement (EIS) (United States Department of Agriculture [USDA], 2016a,b).

Overarching Regulatory Authority

Agencies working within the Coordinated Framework must also address regulatory processes and determinations for products of biotechnology through federal statutes which have overarching authority. A feature of these overarching authorities is the ability for broader public involvement than is typically experienced under the Coordinated Framework.

The National Environmental Protection Act (NEPA) of 1969, as amended, requires that federal agencies take a "hard look" at how a regulatory action may affect the human environment (Department of the Interior, 2004). Under NEPA, significant environmental impacts of an action must be disclosed to the public prior to the action being taken; but the act does not dictate the nature of action to be taken based on the analysis that is performed (Bean, 2009). The agency prepares an environmental assessment (EA) and if the threshold determination is a Finding of No Significant Impact (FONSI) there is no need for further analysis. If, however, the provisional determination by the agency is that the proposed action may significantly affect the human environment, an EIS is necessary. The EIS outlines the proposed action and alternatives, and evaluates the environmental impact of each in arriving at a final action. The Council on Environmental Quality (CEQ) determines the process and need for NEPA to be applied within federal agencies. For instance, decision-making activities undertaken by EPA are considered "functionally equivalent" to those of NEPA and, therefore, there is no need to undertake an EIS. Legal challenges to how NEPA is applied to USDA petitions for nonregulated status have influenced decision making within USDA and are partially responsible for strengthening of assessments for GE crops (Cowan and Alexander, 2012).

The Endangered Species Act (ESA) (16 USC part 35, United States Code [USC], 2012) requires that federal agencies consider both direct and indirect effects of actions they take on endangered species and their critical habitat. The ESA is administered by the Fish and Wildlife Service (FWS) and the National Marine Fisheries Service (NMFS). Agencies conduct their own internal assessments regarding endangered species and if they determine "no effect," no further action is needed. In cases where there may be an effect the agency must consult with FWS and/or NMFS to determine if the effect is "likely." If a determination of a "likely" effect is made, then assessment responsibilities shift to FWS/NMFS where a determination is made whether the organism or habitat will be placed in jeopardy. Federal statutes applied under the Coordinated Framework have not led to any findings of likely effect for GE crops and, therefore, formal interagency consultation has never taken place (NASEM, 2017). The FDA has been sued over their obligations under the ESA with respect to transgenic AquAdvantage salmon, even though FWS/NMFS were informed of, and concurred with, FDA's finding of no effect (Center for Veterinary Medicine [CVM], 2012). A problematic aspect of the ESA process for products of biotechnology is that assessments for GE organisms under the Coordinated Framework allow some reasonable degree of risk (see for example, Peterson et al., 2006), whereas ESA determinations are concerned with loss of a single individual. Efforts to bridge the endangered species assessment approaches used across agencies have been made (National Research Council [NRC], 2013), but have not yet been applied to bioengineered organisms.

GOVERNANCE AND THE COORDINATED FRAMEWORK FOR BIOTECHNOLOGY

Beyond direct regulatory oversight for products of biotechnology, the broader governance of biotechnology in the United States is evidenced through public comment with respect to proposed regulatory actions, formally constituted advisory committees to federal agencies, legal challenges of regulatory actions and wide-ranging civil discourse.

When new administrative regulations (rules) are proposed or when these rules are subject to change, US government

agencies undertake public comment periods as stipulated under the Administrative Procedures Act of 1946 (5 CFR 553, 2012). Following an advise and consent procedure, any proposed regulation is published in the *Federal Register* and public input is solicited for a minimum of 30 days as written submissions, frequently as digital electronic submissions, and occasionally through public meetings. Before a rule is finalized the responsible agency must respond to the public record which may consist of public comment, expert opinion, scientific data and other factual evidence. Under the Coordinated Framework, rulemaking and other broad policy decisions largely encompass environmental issues and therefore public comment is addressed through programmatic reviews (a NEPA EIS) as a means to determine if the responsible agency has established the relevant baseline for the assessment (Council on Environmental Quality [CEQ], 2014). Public comment impacts the pace and nature of regulatory decisions, as for instance the determination of USDA to withdraw and rewrite the proposed rule of 2008 in response to more than 88,300 comments that addressed the scope and meaning of the rule (United States Department of Agriculture [USDA], 2016a).

When USDA assesses a GE crop through petitions for determination of nonregulated status, the EA is a more commonly used mechanism than is an EIS. The EA has a lower standard for transparency and public engagement than does the EIS, and USDA has been legally challenged to undertake NEPA EIS before granting nonregulated status (Cowan and Alexander, 2012). From 2007 through 2011, the USDA EA process and regulatory determination for nonregulated status of glyphosate resistant alfalfa, as well as conditions for the conduct and determinations arising from a court-mandated EIS, was argued through to the Supreme Court on the basis of economic considerations to growers and to export markets. As a consequence, USDA conducted an EIS, which received 135,000 public comments; the product was fully deregulated in 2011. Somewhat similar arguments, court actions and USDA responses were taken with respect to glyphosate resistant sugarbeet. Following a court-mandated EIS, the product was partially deregulated and full deregulation was undertaken under a subsequent EA. These and other challenges have led USDA to take a more formal and transparent approach to its assessments and to show a greater willingness to conduct comprehensive EIS for determinations of deregulated status.

Agencies working within the Coordinated Framework utilize advisory groups for advice and direction. As previously described, the NIH RAC is a long-standing advisory group providing direction as to procedures for federally-supported biotechnology research activity. The EPA Science Advisory Panel (SAP) meets publicly and solicits public comment in their deliberations. The SAP has undertaken numerous risk assessments and resistance management plans for Bt crops as well as considered appropriate problem formulation and testing for PIPs (NASEM, 2017). The SAP had pivotal roles in considering highly contentious issues relating to Bt maize impact on monarch butterfly and the allergenicity of food derived from Cry9C maize (Science Advisory Panel [SAP], 2000a,b, 2001). Agencies also convene expert advisory panels on an *ad hoc* basis to address issues relevant to assessing on-going programs and to providing direction as to regulation of emerging biotechnology. Oftentimes committees convened through the National Academies of Science, Engineering and Medicine (NASEM) are empaneled to consider issues relevant to biotechnology and its regulation, as for instance, recent activities to consider GE crops, gene drive technology and the future regulatory landscape for products of biotechnology (NASEM, 2016a,b, 2017).

Along with formal processes of deliberation and regulatory decision making, wide-ranging social discourse on GE crops contributes to the broader governance of the technology, but may hinder effective governance as well. Increased regulatory scrutiny over time as evidenced in increased study requirements, higher development costs and longer decision-making timelines can be ascribed in part to pressure brought about through public groups questioning and challenging the regulatory process (Smyth et al., 2014). This increased scrutiny of GE crops has also engendered extra-regulatory governance activity meant to inform regulatory process through transparent public engagement. An example is the Pew Initiative on Food and Biotechnology which from 2000 to 2007, examined and reported far-ranging issues of genetic modification of foods and the ability of the federal government to assess GE-derived food risks and benefits (Pew Trust, 2007). The commercial advent of GE crops is contemporaneous to the development of the internet, and the internet has been pivotal in dissemination of views on GE crops (Wunderlich and Gatto, 2015), however, rather than strengthening technology governance, internet communication has served to polarize positions, especially with the rise of social media where like-minded opinions become reinforced (Smith et al., 2013). Unsurprisingly, evidence is accruing that social media has been used to purposely sow dissenting positions concerning GE crops in the United States (Dorius and Lawrence-Dill, 2018).

CURRENT REGULATION AND GOVERNANCE OF GENOME EDITED CROPS

Although regulation under the Coordinated Framework is frequently described as product focused, the regulatory approach to GE organisms in fact reflects a *de facto* process-based trigger in many instances (Wolt, 2017). Regulation by USDA, for example, has in the past been considered mostly when using *Agrobacterium tumefaciens*, a plant pest for the introduction of rDNA. Genetic engineering to produce the same product with rDNA introduced using biolisitcs, does not meet this standard; thus, it is possible for identical products to be evaluated differently because of the process involved. Similarly, herbicide tolerance arising naturally through spontaneous mutation (e.g., Kidwell et al., 2015) is not subject to regulation, but using genetic engineering to accomplish the same would be of regulatory concern. The EPA follows a more product-focused approach because it restricts its considerations to pesticidal products, and FDA has held firm to the idea that characteristics of the food product are the relevant regulatory concern.

The regulatory conundrum regarding process versus product poses increasing uncertainty with the advent of genome editing. Genome editing can result in a host of outcomes extending from point mutations to safe harbor transgene insertions (Wolt et al., 2016). While products comprising transgene insertions clearly fall within the regulatory realm of GE crops, the products of simple point mutations may be absent of rDNA and may represent genotypes and phenotypes that are indistinguishable from plant variation which may arise in nature. A case in point is sulfonylurea tolerant canola developed by oligonucleotide mediated mutation (Sauer et al., 2016), a form of genome editing; this was first developed in the late 1990s and has been subject to regulatory considerations worldwide since 2004 (Camacho et al., 2014; Wolt et al., 2016). The same trait has been achieved using conventional mutagenesis (Tonnemaker et al., 1992), which is not subject to regulation throughout most of the world. In Canada and the United States, the genome edited product has entered the marketplace, but its regulatory fate remains uncertain in the EU (Sprink et al., 2016).

The USDA has been the most active US agency in dealing with genome edited crops and responded to the first inquiries regarding these crops as early as 2004 (Camacho et al., 2014). The process that enables inquiries is the "Am I Regulated?" portal where USDA accepts Regulated Articles Letters of Inquiry regarding the potential for proposed products of biotechnology to be subject to regulation (United States Department of Agriculture [USDA], 2017c). In responding to these inquires, USDA has not viewed genome edited crops as subject to regulation when the edit involves simple insertion/deletions of limited numbers of bases and the absence of rDNA in the finished product (Wolt et al., 2016). Thus, for instance, herbicide resistance developed through single nucleotide substitutions, which can arise as spontaneous mutation or through conventional mutagenesis (Kidwell et al., 2015; Rizwan et al., 2015), is not subject to regulation by USDA. Similarly, the aforementioned use of genome editing to develop herbicide resistant canola is not subject to regulation by USDA. However, in situations where small native template or directed transgene insertions occur, there remains regulatory interest (Camacho et al., 2014). Thus, a case-by-case paradigm drives regulatory considerations of genome edited crops, but consistency in actions by USDA provides developers with an operational roadmap.

Beginning 2015, the Executive Office of the President (EOP) initiated an activity to update the Coordinated Framework for Biotechnology to clarify regulatory responsibilities and to assure the ability to deal with future products of biotechnology (Executive Office of the President [EOP], 2015). As defined by the EOP, biotechnology products "refers to products developed through genetic engineering or the targeted or *in vitro* manipulation of genetic information of organisms including. . . some of the products produced" by these organisms (Executive Office of the President [EOP], 2016). Concurrently, efforts were initiated by USDA to broaden their remit for assessing products of biotechnology and by FDA to better understand the ways that foods derived from genome edited plants may differ from conventionally derived foods in terms of food safety.

The 2016 USDA programmatic EIS announced the intent of USDA to undertake new rulemaking for GE organisms and the various options under consideration (United States Department of Agriculture [USDA], 2016b). And in early 2017, the agency announced proposed actions to update regulatory oversight for biotechnology (United States Department of Agriculture [USDA], 2017a). Exceptions were made to explicitly exclude conventionally- and mutagenically-derived organisms. Further, the distinction of product versus process as a regulatory trigger was complicated through a redefinition of genetic engineering to "mean techniques that use recombinant or synthetic nucleic acids with the intent to create or alter a genome," thus signaling the focus on *use* of a defined process as the trigger for regulatory considerations. Exceptions to this definition involved processes of directed genome altering (i.e., genome editing) resulting in deletion of any size DNA segment, or occurrence of a single base pair substitution that could otherwise result from the use of chemical- or radiation-based mutagenesis, or genome editing-enabled insertion of DNA segments that could have been achieved through traditional breeding with a sexually compatible species. Further, null segregant progeny of a GE organism could be excluded from regulation when the rDNA or synDNA inserted into the recipient genome was not passed to the recipient progeny and there was no alteration of the DNA sequence of the progeny (United States Department of Agriculture [USDA], 2017a). These exclusions and further language in the proposed rule would make distinction amongst the means of genome editing similar to that currently reflected in USDA actions in response to Regulated Article Letters of Inquiry for genome edited crops. That is, a determination as to whether the product would be subject to regulatory consideration would be based on whether the modification within the progeny's genome involved deletions, point insertions, or native template insertions, and whether rDNA or synDNA remained in the modified organism (see for instance, Wolt et al., 2016). In addition to these process/product definitions, the proposed rule invoked both plant pest and noxious weed considerations to provide greater statutory support for USDA's regulations, an approach which proved problematic in USDA's earlier attempt at rulemaking (United States Department of Agriculture [USDA], 2008, 2016a). Based on public comments expressing a wide range of concerns regarding the new proposed rule, USDA has withdrawn the rule in order to explore alternative policy actions through re-engagement with stakeholders (United States Department of Agriculture [USDA], 2017b). Uncertainties with rulemaking has led USDA to clarify is current position with respect to genome editing for plant improvement as consistent with the planned updates in regulatory oversight (United States Department of Agriculture [USDA], 2018b).

In concert with the EOP effort to rethink the Coordinated Framework, the FDA requested public comment as to genome edited plant varieties used for food and feed (United States Food and Drug Administration [FDA], 2017). At the time of the request, FDA had "not completed a voluntary food safety consultation on food derived from a plant produced using genome editing" (US Food and Drug Administration [FDA], 2018b). In requesting comments, the FDA is seeking to

determine if foods derived from genome edited plants represent "categories of plant varieties" different from plants developed using traditional plant breeding, and if these differences are likely to change food safety risks for human and animal foods.

Governance outside the bounds of the Coordinated Framework is evidenced in local initiatives by Institutional Biosafety Committees (IBC), which have lead researchers at some institutions to self-regulate their design of genome editing research to avoid inadvertent gene drive development (Wolt, 2017). Proactive efforts at the local level have preceded more formalized efforts by NIH to evaluate "the current biosafety oversight framework, and discuss the future direction of biosafety oversight in light of the emergence of new technologies in the life sciences and the evolution in our understanding of risk and safety"[1]. In addition, recent NASEM guidance on gene drive research (a special application of genome editing) has outlined a stepwise approach toward development and deployment of the technology which engages the wider public in the decision-making process at each stage of activity (NASEM, 2016a).

ADMINISTRATIVE JURISPRUDENCE AND REGULATORY RULEMAKING

Administrative Law and Jurisprudence

The Coordinated Framework for Biotechnology draws on statutes and statutory language that predates and does not anticipate the emergence of bioengineering processes for crop improvement. As such it stands as a particularly strong example of "lawmaking by administrative institutions" (Stack, 2015), which reflects the ascendance of bureaucracy as the center for regulatory policy making within the federal government (Strauss, 1984). Within this context, rule of law considerations are especially important to the appropriate conduct of administrative law (Waldron, 2011; Stack, 2015). Proceeding from the work of Strauss (1984) defining the administrative structure of government, Stack (2015) identifies five central standards that must be met for administrative decisions to be legitimate: such decisions must be properly *authorized*, must meet requirements of public *notice*, must be *justifiable* to those to whom they apply, must be *coherent* with settled law, and must meet standards of *procedural fairness* that involve recognition of extant rights and duties.

Given that until recently administrative law doctrines have not been extensively considered, traditional jurisprudence provides a bridge for understanding administrative law rules of governance in terms of how policy making for genome edited crops has emerged in the United States.

Discretion in Traditional Jurisprudence

Regulatory agencies are created by, defined by, and circumscribed by the statutes they are empowered to administer (Office of the Federal Register, 2011). But because statutes typically require clarification and interpretation, they cannot be administered directly. Administration of statutory mandates often requires the creation of rules and guidelines that facilitate the implementation of statutory directives. The agencies that administer the US Coordinated Framework for Biotechnology have significant discretion in the articulation of rules and guidelines. This discretion is circumscribed by statute, by precedent, and by requirements in the Administrative Procedures Act which mandate transparency, public disclosure, and opportunities for public comment and which specify judicial overview for all regulatory actions (Code of Federal Regulations [CFR], 2010). In spite of these restrictions, regulatory discretion gives agencies considerable power to structure the regulatory environment. This power can be exercised in ways that promote a variety of different goals: regulatory decisions might facilitate the adoption of new technologies, respond to the preferences or the interests of constituents, protect the environment, and protect against human, animal or environmental harms. These various goals are not always coincident. For instance, consumers might prefer strict protections, but regulation designed to conform to consumer preferences might retard adoption of new technology. Regulations designed to protect human health or the environment are sometimes perceived by producers as inappropriate or excessive restrictions of their freedom to operate. Regulatory rulemaking, therefore, involves the evaluation of trade-offs among stakeholders whose interests are frequently not aligned with one another. Therefore, the ways agencies of government exercise discretion in rulemaking has significant implications for governance.

Traditional scholarship in jurisprudence has focused primarily on issues of legal interpretation that face judges, and has given comparatively less consideration to the very similar problems faced by regulatory rule and decision making. This is a significant oversight, in part because regulatory decision making is enormously important with practical consequences for policy, but also because similar interpretive issues arise in the contexts of regulatory rulemaking and judicial decision making. In both contexts, interpretive decisions are constrained by statute and precedent, but decision making involves a significant degree of discretion on the part of judges and administrators. Just as judges must give weight, via the principle of *stare decisis,* to the decisions of prior courts, regulatory agencies typically give significant weight to the status-quo rules that were put in place by prior administrators. In both contexts, there is an ultimate authority, with the legal power to specify which interpretations are appropriate and legitimate, and which should be set aside. In the courts, the final legal authority is the United States Supreme Court, which is charged to interpret the law and to give authoritative statements defining what the law *is* on any particular matter. In the case of regulatory agencies, the President of the United States, as head of the executive branch of government, has final authority to review and approve proposed administrative rules, often with the help and advice of the Office of Information and Regulatory Affairs (OIRA). Challenges to the interpretation and execution of administrative rules, however, take place within the judicial system.

[1]https://osp.od.nih.gov/event/nih-guidelines-honoring-the-past-charting-the-future/?instance_id=39

The rulemaking discretion exercised by regulatory agencies is similar, in important respects, to interpretive discretion exercised by judges. Judges have significant discretion when deciding cases, but like regulatory agencies, their discretion is constrained by statute and by precedent. Positivist, naturalist and pragmatist jurisprudential theories may be understood as different accounts of the way discretion should be exercised by judges. These theories also have important implications, *mutatis mutandis,* for the way discretion should be exercised by regulatory agencies implementing the Coordinated Framework.

Legal Positivism

Legal positivists hold that judges may only refer to valid legal rules when deciding cases. Contemporary positivists (Raz, 1970; Hart et al., 2012) hold more generally that a rule's status as a valid law depends on its institutional pedigree. Any rule that passes through the proper validating process becomes a valid law, and only valid laws may be referred to by judges. Defenders of positivism often note that the theory tightly restricts the range of judicial discretion, increasing the significance of legislative action. Positivists hold that the content of law should properly be the province of democratically elected legislators, not unelected judges or bureaucrats. Accordingly, positivist jurisprudence offers a tightly constrained view of decision making and discretion on the part of the decision maker.

Positivists need not be *originalists*. Originalism is a theory of constitutional interpretation that holds that the constitution – and by extension, laws – should be interpreted in light of the original meanings of the words and concepts employed (Scalia and Gardiner, 2012). One might characterize this view as a claim that in the interpretation of legal texts, judges and administrators should exercise their discretion by searching for the interpretation that best fits with the meanings the words employed had at the time when the law or legal instrument was validated as law. There may be important implications of this view for determining whether genome edited crops and derived foods count as "genetically engineered" or "bioengineered" for the purposes of regulation under the Coordinated Framework (see discussion, following).

Regulators administering the Coordinated Framework for Biotechnology are tasked to decide whether genome edited crops count, under regulatory rules, as GE even when they do not contain rDNA. Their decision is constrained by law and by institutional guidelines, but within these constraints there is considerable discretion to evaluate alternative reasons and to exercise judgment in selecting among them. Vagueness and ambiguity in statutory language challenge regulators who must decide how agencies should treat genome edited crops and derived foods.

According to Hart et al. (2012), positivist jurisprudence suggests vague and ambiguous legal concepts have a core area of application, as well as more marginal or questionable areas of application. The concepts we use to describe GE and genome edited organisms present such a problem. Depending on how they are conceptualized and defined, genome edited organisms could lie in the core or the penumbra of the legal concepts that would make these organisms available for regulatory oversight, or they could be outside the bounds for such oversight. The basis for making such a decision lies outside the realm of legal positivism. While Hart et al. (2012) eloquently described the structure of vague legal concepts, and provided an articulate account of the scope of interpretive analysis, Hart did not develop a positivist theory of interpretive meaning that would be of practical value to regulatory rulemakers.

Consider, for example, the very practical question whether the National Bioengineered Food Disclosure Standard of 2016 (Public Law 114-216; United States Department of Agriculture [USDA], 2018a) applies to labeling foods that are created using genome editing technologies. Section 291 of the standard defines "bioengineering" as follows:

> SEC. 291. DEFINITIONS. "In this subtitle: "(1) BIOENGINEERING.—The term 'bioengineering,' and any similar term as determined by the Secretary, with respect to a food, refers to a food–" (A) that contains genetic material that has been modified through *in vitro* deoxyribonucleic acid (DNA) techniques; and "(B) for which the modification could not otherwise be obtained through conventional breeding or found in nature.

This standard is separate from the statutes under which the Coordinated Framework operates, but the definition of bioengineering critically intersects with "products of biotechnology" as defined under the revision of the Coordinated Framework for Biotechnology (Executive Office of the President [EOP], 2017) and its proposed implementation in revised USDA rulemaking (United States Department of Agriculture [USDA], 2017b).

While positivism as such does not provide an answer to this quandary, neither does a positivist theory of jurisprudence rule out all reasonable standards that might provide an answer. But some positivists, including Hart, have argued that where available legal materials run out, those tasked to interpret the law do not have discretion to decide based on reasons that positivists consider to be *external* to law. Where legislative rules *expressly* assign discretion to regulatory agencies, the powers created by statutory authority flow directly from a valid law. In this case, the standard expressly assigns the Secretary of Agriculture the power to extend the statutory definition of "bioengineering" to "any similar term," but does not expressly grant broad discretion concerning the interpretation of statutory language. While its authors obviously tried to be clear, the National Bioengineered Food Disclosure Standard of 2016 does not interpret itself, and its language does not expressly answer whether foods derived through genome editing are products of bioengineering, under the given statutory definition. Furthermore, as a matter of administrative law, it is not clear whether there is coherence in the language and intent of this labeling standard and the bioengineering products addressed through the revised Coordinated Framework.

Naturalism and Pragmatism in Traditional Jurisprudence and in Regulatory Rulemaking

Naturalist and pragmatist theories of jurisprudence and statutory interpretation offer a slightly more expansive view of judicial and regulatory discretion. Advocates of jurisprudential naturalism (Dworkin, 1977, 1986, 1996, 2011; Barber and Fleming, 2007) argue, in the context of decision making, for discretion in ways that make the law *best.* Given alternative available interpretations of statutory language, argues Dworkin (1986), decision makers should ask which interpretation most effectively protects rights, promotes well-being, and advances public values embodied in the law and the constitution. According to Dworkin, judges have a degree of discretion in selecting among alternative interpretations, but their discretion is not absolute since they could make better or worse interpretive decisions. While naturalists urge that there are strict boundaries that limit the discretion of judges and others who are tasked to interpret the law, some legal pragmatists (Posner, 1999, 2008, 2013) have argued that such limits are mere rhetoric. According to Posner, judges and other decision makers should appeal broadly to diverse sources of information, from social sciences to economics to the "hard" sciences, to ensure that their decisions will be informed by the best possible understanding of issues surrounding the legal decision in question. As we have discussed, this is consistent with the broad stated intent of Congress with regard to statutes and regulations, but has conflicted with interpretation of statutes such as NEPA and the Endangered Species Act by regulators working under the Coordinated Framework.

Naturalist jurisprudence

So-called "naturalism" in jurisprudence is most strongly associated with the work of Dworkin (1977, 1986, 2011) and others (notably Barber and Fleming, 2007). It might seem obvious to say that judges and administrators should select the interpretation that makes the law *best,* but this naturalist view has sometimes been viewed to be at odds with the positivist insistence that the interpreters of law may only appeal to sources *within law* in support of their judgments. The appeal to "what is best" has sometimes been seen as a way to substitute private value judgments for what would otherwise be a more objective legal standard.

Dworkin is careful to note the limits of interpretive discretion and identifies various types of discretion. A decision maker may have "ultimate" discretion when there is no higher decision-making authority who can overrule the decision made and where there is no further appeal. A different kind of discretion applies when application or execution of rules or orders requires the exercise of judgment. In that case, officials who interpret and apply rules may have a range of alternatives available, and within that range will not be bound by standards set by some higher legal authority (Dworkin, 1967). Interpretative and rulemaking discretion that is not *ultimate* may be stronger or weaker, depending on context and institutional circumstances. In administrative law, the *authorization* of regulatory rules will depend, in part, on whether rulemakers act within the bounds of discretion permitted by governing rules and institutional powers. Regulatory officials are vested with the authority to use limited discretionary judgment in the interpretation and execution of statutory law (Stack, 2015).

Within US law, the US Supreme Court has ultimate interpretive discretion, since there is no higher appeal within the structure or extant law. In regulatory rulemaking, it is the President who has ultimate authority over the processes by which regulatory agencies make and apply rules, but this does not represent ultimate discretion because the courts determine whether the processes are conducted consistent with existing law (Code of Federal Regulations [CFR], 2012). In these cases, the legislature can provide a check on the power of the courts or the President by passing legislation that rebuts an unwelcome decision. The kind of discretion can we apply to regulatory agencies tasked to administer the Coordinated Framework for Biotechnology, when they articulate rules that interpret extant regulatory law, is not so clearly defined. These regulators do not have *strong* discretion: their decisions can be better or worse in a variety of different ways. Nor do they have *ultimate* discretion, since there is a higher authority – the President or courts – who may overrule their decisions. Arguably, the discretion of regulatory agencies is similar to that of lower-court judges. Regulatory agencies are responsible to interpret and execute regulatory law. The process requires oversight, public input and appropriate consultation with subject-matter experts. Regulatory rulemaking is not a democratic process, but administrative rulemakers are required to hold public hearings so that the public can exercise its right to influence the process. Presumably this requirement of public input is intended to take public input into account, but the regulators are not bound to do what the public wants. Other legislative and institutional constraints that apply to the rulemaking process are similar: they provide a significant range of choice, within specified constraints. Legal naturalism recommends that regulators should consider the scope of discretion and select among the options that lie within that scope. Among the available options, they should select the one that is best.

Critics of natural law jurisprudence worry that natural law theory may be undemocratic, and that it may give decision makers license to substitute their own personal moral values for the legal rules that should more appropriately constrain their choices. Its defenders, however, urge that naturalism incorporates the best features of positivism without adopting its excessive constraints.

Legal pragmatism

Legal pragmatism is a family of loosely related legal theories. For the purposes of this discussion, the term will be associated with the work of Posner where "legal pragmatism" is the idea that legal interpretation is a practical human activity where interpretive practice should not be bound by absolute principles such as "moral, legal, and political theory when offered to guide legal and other official decision making," (Posner, 2003, p. 3).

Pragmatism as a method leads judges and regulatory rulemakers to recognize decisions may have unexpected consequences which inform later decisions with recognition of prior error and success. Whereas judicial commitment to

principles of interpretation may lead decision makers to ignore important data that should properly influence the actions, pragmatism in principle applies no limitation on what kinds of considerations may appropriately provide insight in making decisions, such as recent findings of social and physical sciences or the effect different rulings would have on public opinion. Pragmatists place no in-principle restrictions on the scope of the discretion available to decision makers charged to interpret law and come to a ruling in hard cases. Critics of this view worry, predictably, that it grants too much discretion and too much power to judges and other interpreters of law.

Challenges of Governance for Products of Biotechnology

The emergence of genome editing as a promising tool for crop improvement has wide ranging implications not only for regulatory consideration of genome edited crops themselves but also to future innovations from the rapidly advancing field of bioengineering. The US Coordinated Framework for Biotechnology has undergone considerable change through time in an attempt to be responsive to the changing nature and understanding of bioengineered plants. While the overall conduct of these regulatory changes show adherence to administrative standards of authority, notice and justification, they may be faulted in terms of procedural fairness (where judicial decisions have compelled a more widely directed consideration of impacts of biotechnology) and, in particular, coherence.

Interpretive rulemaking requires rules that are coherent – that is, they should be consistent with and supported by the underlying legal materials, and should be appropriately linked to other relevantly similar policies. Coherence is not mere consistency; unrelated statements are consistent with one another, but do not form a coherent whole. The coherence of regulatory policy requires in addition that there should be inferential relationships among the different elements of regulatory law – that rulings should be derivable from underlying materials, or (more minimally) that they should constitute a reasonable interpretation of underlying and surrounding elements of the legal framework. Overtime the Coordinated Framework has exhibited a shifting definition of what is the subject of regulatory concern – rDNA, GE organisms, or products of biotechnology. Such a lack of coherence is unavoidable as long as the focus remains on technological processes rather than on the products themselves. A lack of coherence through time elicits uncertainty on the part of scientists, developers and the public as well as for regulators themselves. The pending implementation of labeling under the National Bioengineered Food Disclosure Standard portends further problems with administrative coherence, since labeling of "bioengineered" foods and its alignment with the Coordinated Framework leaves uncertainty as to which "products of biotechnology" (actually a process consideration as defined under the Coordinated Framework) will be labeled as bioengineered.

As an instance of administrative law, the administrative jurisprudence of the Coordinated Framework – as informed by traditional theories of jurisprudence – will face challenges for its continuance from both inside and outside of government given the accelerating novelty in approaches whereby bioengineering of organisms may be accomplished. In the sequence of views considered here, positivists and originalists would be the least amenable to interpretive discretion in rulemaking under the Coordinated Framework, since they would rely only on existing legal material which is lacking in cases where new technology may have no precedent in law. Legal naturalists, however, argue that in cases involving public dispute, failure to decide serves as a legal precedent, and urge that the discretion available must include the ability to make decisions consistent with law, but also sanctioned by legal principles that guide law. Those who interpret the existing state of policy with respect to genome edited crops as a nondecision may fall into this category. The work of legal pragmatists, like that of positivists and naturalists, has mostly focused on the interpretive role of judges, but the view has natural application to the problems of legal interpretation faced by regulatory rulemakers, including the implications of extant regulation of GE crops to proposed regulation of genome edited crops and derived foods. Legal pragmatism would argue for regulatory principles that are better gauged toward current day scientific and societal understanding of the risk and benefits of genome edited crops.

SUMMARY

Our purpose in this discussion has been to elaborate how governance within the US legal framework is influencing decisions regarding the regulation of genome edited crops. We do not defend or justify the US regulatory system or suggest any given theory of jurisprudence which is preferable for administration of the Coordinated Framework for Biotechnology. Such considerations would require much more serious examination of the norms that constitute the basis of the US regulatory system. However, this analysis of the regulatory framework for biotechnology in the US should provide an explanation of the circumstances in law that have led US regulatory agencies, including the USDA, to their current positions for imposing new rules for crops and derived foods developed through genome editing.

AUTHOR CONTRIBUTIONS

The authors contributed equally to ideation and development of this contribution. JW developed the historical review of the Coordinated Framework and discussion of its implications toward genome edited crops. CW developed the jurisprudence content and its use for interpreting regulatory decisions with respect to genome edited crops.

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Genome Editing for Crop Improvement – Applications in Clonally Propagated Polyploids With a Focus on Potato (*Solanum tuberosum* L.)

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Genome-editing has revolutionized biology. When coupled with a recently streamlined regulatory process by the U.S. Department of Agriculture and the potential to generate transgene-free varieties, genome-editing provides a new avenue for crop improvement. For heterozygous, polyploid and vegetatively propagated crops such as cultivated potato, *Solanum tuberosum* Group Tuberosum L., genome-editing presents tremendous opportunities for trait improvement. In potato, traits such as improved resistance to cold-induced sweetening, processing efficiency, herbicide tolerance, modified starch quality and self-incompatibility have been targeted utilizing CRISPR/Cas9 and TALEN reagents in diploid and tetraploid clones. However, limited progress has been made in other such crops including sweetpotato, strawberry, grapes, citrus, banana etc., In this review we summarize the developments in genome-editing platforms, delivery mechanisms applicable to plants and then discuss the recent developments in regulation of genome-edited crops in the United States and The European Union. Next, we provide insight into the challenges of genome-editing in clonally propagated polyploid crops, their current status for trait improvement with future prospects focused on potato, a global food security crop.

Keywords: genome-editing, clonal propagation, polyploidy, potato (*Solanum tuberosum*), CRISPR/Cas system, TALENs, *Agrobacterium*-mediated transformation, protoplast transformation

INTRODUCTION

Genome-editing technologies such as TALENs (Transcription Activator Like Effector Nucleases), CRISPR/Cas9 (Clustered Regularly Interspaced Short Palindromic Repeats/CRISPR-associated systems), CRISPR/Cas12a (Cpf1, CRISPR from *Prevotella* and *Francisella* 1), and Cas9-derived DNA base editors, provide an unprecedented advancement in genome engineering due to precise DNA manipulation. Genome-editing is being widely applied in plants and has revolutionized crop improvement. Polyploidy and vegetative reproduction are unique to plants, frequently found in a large number of important food crops including root and tuber crops, several

perennial fruit crops as well as forage crops (McKey et al., 2010; Gemenet and Khan, 2017). Several cultivated polyploids have vegetative mode of reproduction (Herben et al., 2017) and with allopolyploidy combined with heterozygosity makes breeding challenging in these crops. In order to introduce genetic diversity by crossing two heterozygous parents, multiple alleles segregate at a given locus. Backcrossing techniques to add traits cannot be used because it will destroy the unique gene combination within a preferred variety.

Potato, (*Solanum tuberosum* Group Tuberosum L.) ($2n = 4x = 48$) represents one such heterozygous, polyploid crop that is clonally propagated by tubers. Potato is a global food security crop and is the third most important food crop after rice and wheat (Devaux et al., 2014). While conventional breeding and genetic analysis are challenging in cultivated potato due to the above mentioned features, majority of diploid potatoes possess gametophytic self-incompatibility (SI). Historically, conventional breeding has been used to create improved potato cultivars. Yet due to its unique challenges, breeding is inefficient when a large number of agronomic, market quality and resistance traits need to be combined or if novel traits not present in the germplasm bank are wanted. Insertion and expression or silencing of economically important genes is being used to improve potato production and quality traits without impacting optimal allele combinations in current varieties (Diretto et al., 2006, 2007; Rommens et al., 2006; Chi et al., 2014; Clasen et al., 2016; Sun et al., 2016; Andersson et al., 2017; McCue et al., 2018). Genome sequence information coupled with established genetic transformation and regeneration procedures make potato a strong candidate for genetic engineering. In 2017, the U.S. Department of Agriculture's (USDA) Animal and Plant Health Inspection Service (APHIS), the Environmental Protection Agency (EPA) and the Food and Drug Administration (FDA) approved Simplot Plant Sciences to commercially release genetically engineered potatoes with reduced bruising and acrylamide content in tubers (Innate potatoes[1]).

In this review, we describe various genome-editing platforms available for plants, their delivery mechanisms and discuss the recent USDA and the European Union clarifications regarding regulatory aspects of gene-edited crops. Next, we discuss the challenges of genome-editing in clonally propagated polyploid crops and summarize the insights gained from case studies along with future prospects focused on enhancement of potato breeding using this technology.

GENOME-EDITING – EMERGING TECHNOLOGIES FOR GENETIC MANIPULATION IN PLANTS

Genome-editing by sequence-specific nucleases (SSNs) such as CRISPR/Cas9 and TALENs facilitate targeted insertion, replacement, or disruption of genes in plants. SSNs create double stranded breaks (DSBs) at the target locus and rely on cellular repair mechanisms to correct these breaks (**Figure 1A**).

[1]http://www.innatepotatoes.com/newsroom/press-releases

The CRISPR/Cas9 system has demonstrated great potential in various crop species due to simplicity of use and versatility of the reagents (Jiang W. et al., 2013; Sun et al., 2015; Svitashev et al., 2016; Zhang et al., 2016; Shimatani et al., 2017; Soyk et al., 2017). Engineered CRISPR/Cas9 nucleases target DNA adjacent to the 5′-NGG-3′, protospacer adjacent motif (PAM), in a single guide RNA (sgRNA) specific manner (Jinek et al., 2012; **Figure 1A**). CRISPR/Cas12a that has PAM requirement of "TTTN", allowing targeting of AT rich regions, is emerging as equally effective alternative, implemented in various plants (Kim et al., 2017; Tang et al., 2017). For multiplexing, to target more than one gene at a time, Cas12a requires only a single RNA PolIII promoter to drive several crRNAs, whereas Cas9 requires relatively large constructs (Zetsche et al., 2017). In TALENs, the TALE protein is engineered for sequence-specific DNA binding and is fused to a non-sequence-specific *Fok*I nuclease to create a targeted DSB (Bogdanove and Voytas, 2011; Voytas, 2013).

Base-editing technology, based on CRISPR/Cas9 system generates base substitutions without requiring dsDNA cleavage. Cas9 is engineered to retain DNA-binding ability in a sgRNA programmed manner without the nuclease activity such as catalytically inactive Cas9 (dCas9) or a nickase (nCas9) (Jinek et al., 2012). If either dCas9 or nCas9 is fused with a cytidine deaminase that mediates the conversion of cytidine to uridine, the result is a base editor that results in a C→T (or G→A) substitution (Komor et al., 2016; **Figure 1A**). More recently, adenine base editors have been developed that convert A→G (or T→C) (Gaudelli et al., 2017). Base editing has been successfully applied in plants to confer both gain of function by incorporating correct mutations and loss of function by generating knock-out mutations (Chen et al., 2017; Li et al., 2017; Lu and Zhu, 2017; Shimatani et al., 2017; Zong et al., 2017; Kang et al., 2018).

DELIVERY OF GENOME-EDITING NUCLEASES INTO PLANT CELLS

The three major methods of genetic transformation in plants are: *Agrobacterium*-mediated transformation, biolistics and protoplast transfection. By far the most commonly used method to introduce genome-editing reagents in potato is by *Agrobacterium*-mediated transformation (**Figure 1B**). A binary T-DNA vector is used to deliver and express the reagents in plant cells. Once inside the nucleus, the T-DNA randomly integrates into the plant/host genome leading to stable transformation resulting in persistent activity of reagents. However, there is a possibility that it remains extra-chromosomal leading to transient gene expression.

The other common method is polyethylene glycol (PEG)-mediated protoplast transfection. Protoplasts facilitate direct delivery of DNA into cells with gene-editing reagents expressed as plasmid DNA for transient transformation. Protoplasts have greater transformation efficiency compared to other methods (Jiang F. et al., 2013; Dlugosz et al., 2016; Baltes et al., 2017). They retain their cell identity and differentiated state and, for some plant species, have the capability to regenerate into an entire plant.

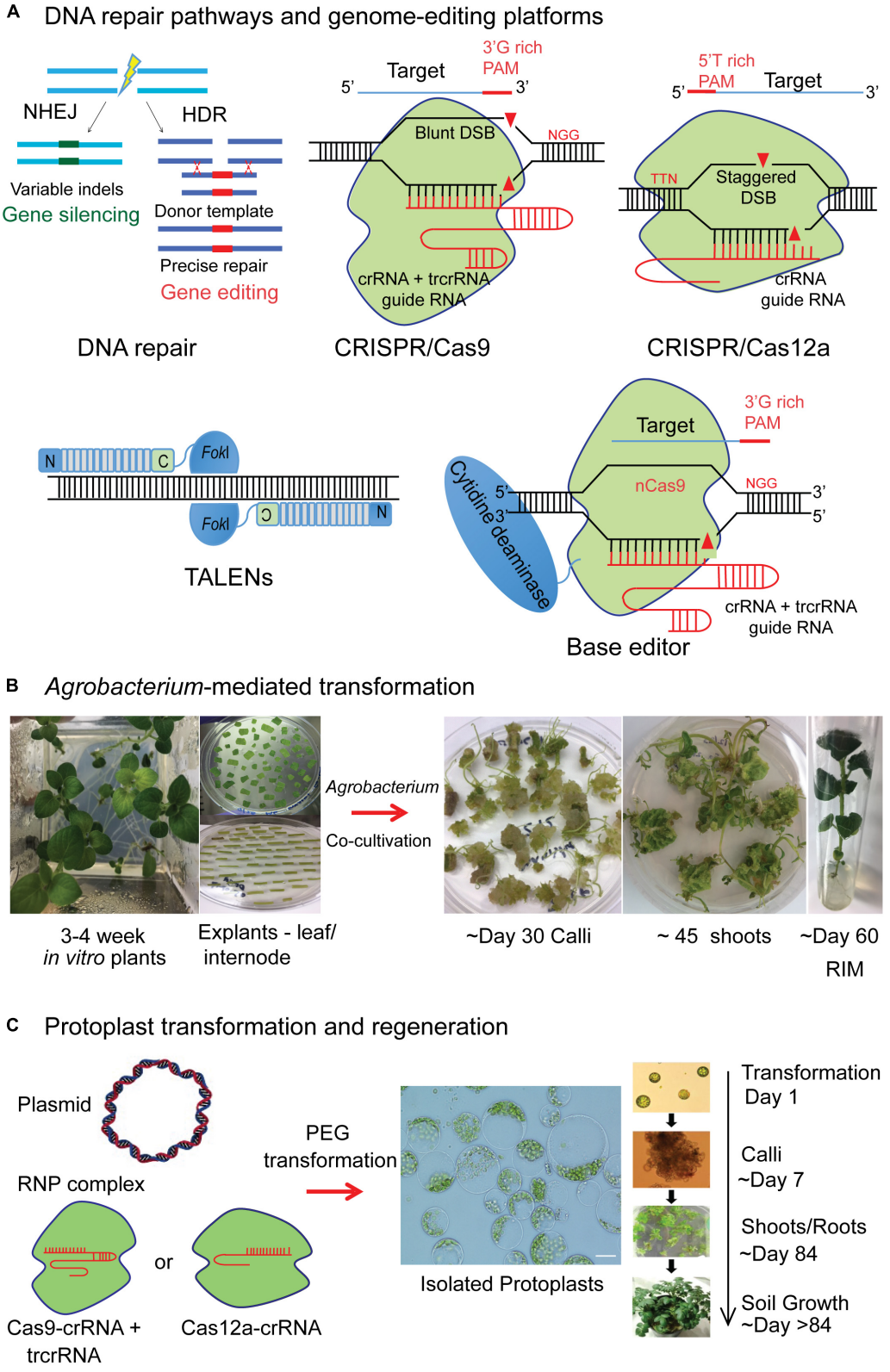

FIGURE 1 | Illustration of genome-editing platforms and genetic transformation procedures in potato. **(A)** Double stranded DNA (dsDNA) break repair in a cell occurs either by non-homologous end joining (NHEJ), where the cleaved DNA molecule is simply rejoined, often with indels in coding regions (green) that result in gene

(Continued)

FIGURE 1 | Continued
knock-out or by homologous recombination (HR), where a donor repair template (red) can be used for targeted knock-in experiments, where a single or few nucleotides alterations, insertion of an entire transgene or suites of transgenes can be made. CRISPR/Cas9 nuclease engineered to have a Cas9 protein and a guide RNA (gRNA) that is a fusion of CRISPR RNA (crRNA) and *trans*-activating crRNA (tracrRNA). Cas9 and gRNA complex can recognize and cleave target dsDNA that is complementary to 5′ end of target spacer sequence that is next to protospacer adjacent motif (PAM) of 5′-NGG-3′. CRISPR/Cas12a is a single CRISPR RNA guided nuclease lacking tracrRNA. Cas12a has PAM requirement of "TTTN" allowing targeting of AT rich regions and expanding the target range of RNA-guided genome-editing nucleases. Cas12a cleaves DNA at sites distal to PAM and introduces a staggered DSB with a 4–5-nt 5′ overhang, unlike blunt DSB by Cas9. Transcription activator-like effector nucleases (TALENs) bound to their target site are shown. The TALE array contains repeat variable di-residues that make sequence-specific contact with the target DNA. TALE repeats are fused to *Fok*I, a non-specific nuclease that can cleave the dsDNA upon dimerization. Base editor constitutes fusion of nickase Cas9 (nCas9) with cytidine deaminase enabling the editing of single bases by C→T conversion of single-stranded target.
(B) *Agrobacterium*-mediated plant transformation and regeneration in potato. 3–4-week-old *in vitro* propagated potato plants in a Magenta box are shown. Ex-plants are prepared from leaf and stem internodes and placed on callus induction media after *Agrobacterium* inoculation and co-cultivation. Callus growth observed from the ex-plants. After 6–8 weeks, shoots emerge and are grown on shoot induction media. 1–2 cm shoots are excised and transferred to root induction media. The lines that develop roots and have growth on selection media are chosen as candidates for molecular screening to confirm the gene editing events.
(C) Delivery of the gene editing reagents as plasmid DNA or as preassembled Cas9 or Cas12a protein-gRNA ribonucleoproteins (RNPs) into protoplasts by polyethylene glycol (PEG) mediated transformation. The timeline from protoplast transformation to regeneration of mutagenized plants in potato is reproduced from Clasen et al. (2016) with the permission of the copyright holder (John Wiley & Sons, Inc.).

To improve specificity and to reduce the duration of activity of SSNs in the cell, purified recombinant Cas9 or Cas12a protein with an *in vitro* transcribed or synthetically produced sgRNA resulting in a ribonucleoprotein complex (RNP) is delivered into protoplasts (**Figure 1C**). The Cas9 protein continues to be expressed in the cell for several days when delivered as a plasmid, whereas it is degraded within 24 h when delivered as RNPs, improving the specificity of the reagent (Zetsche et al., 2015). Preassembled CRISPR/Cas9 or Cas12a RNP complexes were successfully delivered into protoplasts of Arabidospsis, tobacco, lettuce, rice, wheat, soybean and potato and plants were regenerated with heritable targeted mutagenesis (Woo et al., 2015; Kim et al., 2017; Liang et al., 2017; Andersson et al., 2018). Using RNPs, possibility of integration of plasmid-derived DNA sequences or foreign DNA into the host genome can be eliminated. Plants regenerated from protoplast cells without the integration of any foreign DNA would likely avoid the regulatory process (Haun et al., 2014; Clasen et al., 2016).

REGULATORY ASPECTS ON GENOME-EDITED CROPS – IMPACT ON ADVANCING CROP IMPROVEMENT

Genome-editing has been successfully implemented in several plant species, and some cases, the regulatory status of the edited plants has been considered by USDA/APHIS ("Am I Regulated?"[2] (Waltz, 2018). USDA considers genome-editing as a novel breeding tool and released a definitive statement that if plant varieties developed through genome-editing do not possess any foreign genetic material and they are indistinguishable from those developed by conventional breeding or mutagenesis approaches, then they will not be regulated (USDA press release[3]). The edits made in edited varieties can include deletions of any length, single base substitutions or genetic variation from any species or variety that is sexually compatible. In the case of *Agrobacterium*-mediated delivery of SSNs, any stably integrated T-DNA sequences can be segregated away by meiotic recombination. Null segregants – progeny of the transgenic, edited parent that still retain the germline edit but lack the integrated T-DNA or other foreign sequence – are excempt from regulation. In clonally propagated plants like potato, null segregants are difficult or impossible to obtain. However, transient expression in protoplasts, for example, can achieve gene edits, and regeneration of the edited protoplasts can create edited plants without any foreign DNA and hence are exempt from regulation by USDA/APHIS (Clasen et al., 2016) ("Am I Regulated?"[2]). In Japan, a government panel recently recommended following a regulatory policy similar to that of USDA/APHIS, that gene edited plants in Japan should not be regulated (TheScientist news[4]).

Clarity on guidelines for regulating gene-edited crops will undoubtedly promote wider use of this technology in the United States. In contrast, the European Union recently declared that plants generated by genome-editing are not exempt from regulation; rather, they must be treated just like transgenic plant lines (Court of Justice of the European Union verdict[5]). The EU's argument is that gene editing alters the genetic material in a way that is not natural, and edited plants might have adverse effects on human health and the environment. Unlike the United States, Europe chose a "process-based" approach to regulation, rather than a "product-based" approach. Gene editing could be used to create genetic variation that is identical to that already present in crop varieties grown in Europe; however, it would nonetheless be regulated due to this process-based approach. A "product-based" regulatory policy allows multiple levels of checks and balances. For example, in the United States, the FDA can weigh in on health benefits or concerns of a given crop, and the EPA can weigh in on potential environmental effects of an edited plant variety. The conservative, process-based approach adopted by Europe will likely both slow the development of the technology in European

[2]https://www.aphis.usda.gov/aphis/ourfocus/biotechnology/am-i-regulated
[3]https://www.usda.gov/media/press-releases/2018/03/28/secretary-perdue-issues-usda-statement-plant-breeding-innovation
[4]https://www.the-scientist.com/news-opinion/japanese-authorities-recommend-not-regulating-gene-editing-64675
[5]http://curia.europa.eu/juris/celex.jsf?celex=62016CJ0528&lang1=en&type=TXT&ancre=

TABLE 1 | Genome editing case studies in clonally propagated crops.

Potato	Ploidy	Genome editing	Translormation method	Target gene	Trait associated with the gene	Purpose of the study	Reference
S.tuberosum cv. Sassy	4x	TALENs	Agrobacterium	*Sterol side chain reductase 2 (StSSR2)*	Steroidal glycoalkaloids reduction in tuber	Identify key enzyme in the biosynthesis of cholesterol and related steroidal glycoalkaloids	Sawai et al., 2014
S.tuberosum cv. Desiree	4x	TALENs	Protoplasts	*Acetolactate synthase1 (StALS1)*	Herbicide resistance	Transient expression of TALENs in potato protoplasts for targeted mutagenesis and regeneration	Nicolia et al., 2015
S.tuberosum cv. Ranger Russet	4x	TALENs	Protoplasts	*Vacuolar invertase (StVInv)*	cold induced sweetening, acrylamide content in tubers	Tuber improvement for cold storage	Clasen et al., 2016
S.tuberosum cv. Ranger Russet	4x	TALENs	Agrobacterium	*StALS1*	Herbicide resistance	Use of TALENs for targeted T-DNA integration	Forsyth et al., 2016
S.tuberosum cvs. Russet Burbank, Shepody	4x	TALENs	Agroinfiltration	*1,4-alpha-glucan branching enzyme gene (SBE1), StVInv*	Degree of starch branching, cold induced sweetening	Rapid testing and effective delivery of TALENs	Ma et al., 2017
S.tuberosum cv. Kuras	4x	CRISPR/Cas9	Protoplasts	*Granule-bound starch synthase (StGBSS)*	Tuber starch quality	Transient expression of CRISPR/Cas9 in potato protoplasts for targeted mutagenesis and regeneration. Potato tuber with altered starch content developed	Andersson et al., 2017
S.tuberosum cv. Kuras	4x	CRISPR/Cas9 RNPs	Protoplasts	*StGBSS*	Tuber starch quality	Use of RNPs for genome-editing in potato protoplasts and regeneration of mutant lines with knock-out of all four alleles	Andersson et al., 2018
S.tuberosum cv. Desiree	4x	CRISPR/Cas9	Agrobacterium	*Transcription factor gene StMYB44*	Phosphate transport via roots	Understand the molecular basis of phosphate stress responses in potato	Zhou et al., 2017
MSX914-10, *S. tuberosum*	2x, 4x	CRISPR/Cas9	Agrobacterium GVR	*StALS1*	Herbicide resistance	Targeted mutagenesis in potato and germline inheritance	Butler et al., 2015
MSX914-10, *S. tuberosum* cv. Desiree	2x, 4x	CRISPR/Cas9, TALENs	Agrobacterium GVR	*StALS1*	Herbicide resistance	Gene targeting via homologous recombination using donor template and geminivirus replicons	Butler et al., 2016
S. tuberosum Group Phureja double monoploid	2x	CRISPR/Cas9	Agrobacterium	*StIAA2*	Petiole hyponasty and shoot morphogenesis	Targeted mutagenesis using native StU6 promoter driving the sgRNA	Wang et al., 2015
S. tuberosum Group Phureja S15-65	2x	CRISPR/Cas9	Agrobacterium	*Stylar ribonuclease gene (S-Rnase)*	Self Incompatibility	Knock-out of self-incompatibility gene *S-RNase* in diploid potato line resulted in self compatibility	Ye et al., 2018

(Continued)

TABLE 1 | Continued

Potato	Ploidy	Genome editing	Translormation method	Target gene	Trait associated with the gene	Purpose of the study	Reference
No mention	N/A	TALENs	Agrobacterium	*StGBSS*	Tuber starch quality	Development of a Gateway system for rapid assembly of TALENs in a binary vector	Kusano et al., 2016
Cassava							
Manihot esculenta cvs. 60444 and TME204	2x	CRISPR/Cas9	Agrobacterium	*Phytoene desaturase* (*MePDS*)	Albino devoid of green tissue	Targeted mutagensis in Cassava	Odipio et al., 2017
Manihot esculenta cv. 60444	2x	CRISPR/Cas9	Agrobacterium	*novel cap-binding proteins (nCBP-1.nCBP-2)*	Cassava brown streak disease (CBSD)	Delayed viral disease incidence and reduced severity of storage root necrosis	Gomez et al., 2018
Apple							
Malus domestica cv. Golden delicious	2x	CRISPR/Cas9 RNPs	Protoplasts	*DspE-interacting proteins of Malus(DIPM 1-4)*	Fire blight resistance	Use of RNPs for genome-editing in apple protoplasts	Malnoy et al., 2016
Malus domestica cv. JM2	2x	CRISPR/Cas9	Agrobacterium	*MdPDS*	Albino devoid of green tissue	Targeted mutagensis in apple	Nishitani et al., 2016
Grape							
Vitis vinifera cv. Chardonnay	2x	CRISPR/Cas9 RNPs	Protoplasts	*Mildew Locus* 0 *(VvMLO-7)*	Powdery mildew resistance	Use of RNPs for genome-editing in grape protoplasts	Malnoy et al., 2016
Vitis vinifera cv. Chardonnay	2x	CRISPR/Cas9	Agrobacterium	L-*idonate dehydrogenase gene (IdnDH)*	Biosynthesis of tartaric acid	Use of grape suspension cells for genome-editing resulting in reduced tartaric acid production	Ren et al., 2016
Vitis vinifera cv. Thompson Seedless	2x	CRISPR/Cas9	Agrobacterium	*VvWRKY52*	*Botrytis cinerea* resistance	Use of grape suspension cells for genome-editing resulting in Botrytis resistance	Wang et al., 2018
Vitis vinifera cv. Neo Muscat	2x	CRISPR/Cas9	Agrobacterium	*VvPDS*	Albino devoid of green tissue	Genome-editing using grape embryonic callus	Nakajima et al., 2017
Banana							
Musa spp cv. Williams (AAA Cavendish), Rasthali (AAB Silk) (AAB Silk)	3x	CRISPR/Cas9	Agrobacterium	*MaPDS*	Albino devoid of green tissue	Genome-editing using banana embryonic callus achieving tri-allelic mutations	Kaur et al., 2018; Naim et al., 2018
Sweet orange							
Citrus sinensis Osbeck *Citrus paradisi* (Duncan grape fruit)	2x	CRISPR/Cas9	Agrobacterium	*Lateral organ boundaries 1 (CsLOB1)*	Citru canker (*Xanthomonas citri)* resistance	Demonstrated that editing promoter or coding region of *CsLOB1* resulted in canker-resistant citrus cultivars	Peng et al., 2017; Jia et al., 2016, 2017
Citrus sinensis cv. Valencia Carrizo Citrange *[Poncirus trifoliata L. Raf.*x *Citrus sinensis* L Osb)	2x	CRISPR/Cas9	Agroinfiltration Agrobacterium	*CsPDS*	Albino devoid of green tissue	Demonstrated that *Xanthomonas* citri pretreatent before Agroinfiltration targeting *PDS* resulted in albino phenotype and Cas9 driven by *A.thaliana* YAO promoter has enhanced targeteged mutagenesis compared to CaMV35S promoter	Jia and Nian, 2014; Zhou et al., 2018

(Continued)

TABLE 1 | Continued

Potato	Ploidy	Genome editing	Translormation method	Target gene	Trait associated with the gene	Purpose of the study	Reference
Sugarcane							
Saccharum spp cv. CP88-1762	10-13x	TALENs	Agrobacterium Biolistics	*caffeic acid O-methyltransferase (SoCOMT)*	Reduction in lignin content	Produce lignocellulosic ethanol from sugacane by altering the cell wall properties	Jung and Altpeter, 2016
Strawberry							
Fragaria vesca	2x	CRISPR/Cas9	Agrobacterium	*Tryptophan aminotransferase 1* (*TAA1*), *Auxin response factor (FveARFB)*	Auxin biosynthesis and response	Targeted mutagenesis and germ-line inheritance in strawberry as a proof of concept	Zhou et al., 2018
*RNP = Ribonucleo protein	GVR = geminivirus replicon						

research labs and will also have global ramifications in terms of trade of gene edited commodities.

GENOME-EDITING CHALLENGES IN CLONALLY PROPAGATED POLYPLOID CROPS – CASE STUDIES IN POTATO

Genome manipulation in polyploid heterozygous crops include the task of simultaneously targeting multiple alleles and screening large number of transformants to recover multiallelic mutagenic lines. Moreover, unlike the seed producing species where Cas9 can be segregated out, it is not feasible in clonally propagated plants. Nevertheless, genome-editing using TALENs and CRISPR/Cas9 has been successfully demonstrated in a number of clonally propagated crops presented in **Table 1**. Potato is chosen for case studies, since it has been subjected to more genome-editing, even though it is a tetraploid, compared to other crops.

The first successful demonstration of the use of TALENs in a tetraploid potato cultivar was by knocking out all four alleles of *Sterol side chain reductase 2* (*StSSR2*) (Sawai et al., 2014) involved in anti-nutritional sterol glycoalkaloid (SGA) synthesis (Itkin et al., 2011, 2013). Similarly, using CRISPR/Cas9 and TALENs, geminivirus replicon-mediated gene targeting (by HR) was successfully demonstrated in diploid and tetraploid varieties. The endogenous *Acetolactate synthase1* (*StALS1*) gene was modified to incorporate mutations using a donor repair template leading to herbicide tolerance and mutations were shown to be heritable (Butler et al., 2015, 2016). *StALS1* was also targeted by TALENs via protoplast transfection and successful regeneration of *StALS1* knock-out lines from transformed protoplasts was demonstrated in tetraploid potato (Nicolia et al., 2015). Initial studies in potato mainly constituted proof-of-concept demonstrations of the genome-editing technology.

However, improvement in tuber cold storage quality of a commercial tetraploid cultivar, Ranger Russet, was achieved by targeting *Vacuolar invertase* (*StVlnv*) using TALENs via protoplast transformation and regeneration (Clasen et al., 2016). Vlnv enzyme breaks down sucrose to the reducing sugars glucose and fructose in cold-stored potato tubers which form dark-pigmented bitter tasting products when processed at high temperatures (Sowokinos, 2001; Kumar et al., 2004; Matsuura-Endo et al., 2006). In addition, the reducing sugars react with free amino acids via the nonenzymatic Maillard reaction to form acrylamide, a carcinogen (Tareke et al., 2002). Tubers from *StVlnv* knock-out lines had undetectable levels of reducing sugars, low acrylamide, and made light colored chips along with no foreign DNA in their genome (Clasen et al., 2016). Recently, a waxy potato with altered tuber starch quality was developed by knocking out all four alleles of *Granule-bound starch synthase* (*GBSS*) in a tetraploid potato cultivar via CRISPR/Cas9. By transient expression of reagents as plasmid DNA or via RNPs in potato protoplasts, mutagenized lines in all four alleles were regenerated with tubers that had the desired high amylopectin starch (Andersson et al., 2017, 2018).

Furthermore, studies related to technological advances in genome-editing in potato have been reported such as utilizing a native StU6 promoter to drive sgRNA expression, targeted insertion of transgenes, a Gateway system for rapid assembly of TALENs, and delivery of TALENs via agroinfiltration for rapid mutagenesis detection (Wang et al., 2015; Forsyth et al., 2016; Kusano et al., 2016; Ma et al., 2017).

FUTURE PROSPECTS TO ENHANCE POTATO BREEDING USING GENOME-EDITING

Genome-editing has tremendous potential for crop improvement, and although implemented in many crops, it has yet to be fully realized in clonally propagated polyploids like potato. Only certain cultivars of potato are amenable to transformation and others need to be tested for transformation and regeneration in tissue culture. Protoplast transformation and regeneration of plants from leaf protoplasts also can lead to somaclonal variation, which may have negative impact(s) on plant development.

In potato, Late blight, caused by fungus *Phytophthora infestans,* is the most critical problem and threat to global potato production (Fry, 2008; Fisher et al., 2012). Two approaches currently used to combat this disease are fungicide spraying and breeding for disease resistance. Canonical disease resistance genes, *R*-genes, belong to nucleotide-binding, leucine-rich repeat (NLR) class of intracellular immune receptor proteins that recognize pathogen effectors to initiate defense responses in the plant (El Kasmi and Nishimura, 2016; Jones et al., 2016). Due to continued high rates of evolution of effector proteins, pathogens overcome recognition, thereby limiting the durability of resistance (Raffaele et al., 2010; Dong et al., 2014). Genome-editing by base editors could potentially be applied to engineer potato for late blight resistance by editing the codons encoding specific amino acids in *R*-genes essential for effector recognition.

Loss of susceptibility is considered as an alternative breeding strategy for durable broad spectrum resistance (Pavan et al., 2009). Silencing of multiple susceptibility genes (*S*-genes) by RNAi resulted in late blight resistance in potato (Sun et al., 2016). Since RNAi does not always result in a complete knockout, genome-editing could potentially be used to simultaneously knockout genes belonging to the *S*-locus. Recently, an extracellular surface protein called receptor-like protein ELR (elicitin response) from the wild potato species, *S. microdontum,* has been reported to recognize an elicitin that is highly conserved in *Phytophthora* species offering a broad spectrum durable resistance to this pathogen (Du et al., 2015). Introducing both extracellular and intracellular receptors in potato cultivars by genome-editing can aid in attaining durable broad-spectrum resistance for late blight.

Tuber quality traits, such as reduced SGAs or potatoes with reduced bruising, are some of the traits that could be improved using genome-editing. Previously, RNAi silencing of *Polyphenol oxidase* (*PPO*) was shown to reduce the browning in tubers due to mechanical damage (Bachem et al., 1994; Coetzer et al., 2001; Arican and Gozukirmizi, 2003). Recently, anti-browning genetically modified apples have been successfully introduced into market developed by RNAi silencing of *PPO* (Waltz, 2015). Anti-browning mushrooms, developed by targeting *PPO* using CRISPR/Cas9, are not regulated by the USDA, suggesting that traits created by knocking out genes may have an accelerated path to market (Waltz, 2016, 2018).

Reduction of SGA levels in the tuber is another important breeding objective in potato previously achieved by targeting different genes in SGA biosynthetic pathway (Sawai et al., 2014; Cárdenas et al., 2016; Umemoto et al., 2016; McCue et al., 2018). As per industry standards, total glycoalkaloid content must be less than 20 mg/100 g tuber fresh weight to be released for commercial tuber production. However, SGAs also have positive impact as defensive allelochemicals deterring insect herbivores (Sinden et al., 1980; Sanford et al., 1990, 1997). Therefore, reduction of SGAs in aboveground tissues may deteriorate pathogen resistance (Ginzberg et al., 2009). Studies have shown differential levels of SGA accumulation among plant organs and developmental stages (Valkonen et al., 1996; Eltayeb et al., 1997; Friedman and McDonald, 1997). Although α-chaconine and α-solanine, which constitute >90% of SGAs, are the predominant SGAs found in cultivated potato (Moehs et al., 1997; McCue et al., 2005, 2006, 2007), other novel SGAs are found in various *Solanum* species (Shakya and Navarre, 2008; Itkin et al., 2013; Cárdenas et al., 2016). For example, in *S. chacoense,* leptines and leptinines accumulate only in aerial plant organs and are correlated with plant resistance to Colarado potato beetle (Sinden et al., 1980, 1986; Sanford et al., 1990; Mweetwa et al., 2012). Such qualitative differences in SGAs in terms of organ specificity and composition provide opportunities to select specific targets for potato improvement via genome engineering. For example, silencing exclusively tuber expressed members of the SGA biosynthetic pathway or editing specific gene targets expressed in aerial organs can be achieved. The ultimate goal would be to develop new potato cultivars with low SGA levels in tubers while still maintaining high levels in above ground tissues for crop protection.

Breeders are currently working toward re-inventing potato as a diploid crop in order to accelerate progress toward understanding the genetics of complex traits such as yield, quality and drought resistance (Jansky et al., 2016). Genome-editing combined with inbred diploid line development would be a monumental shift in the potential for genetic improvement and opens up possibilities for creating a better potato breeding pipeline. Moving to diploid potatoes enables us to develop hybrids based on selected inbred lines by which we can improve various agronomic traits such as disease resistance and remove compatibility barriers. Genome-editing was successfully applied in diploid potato to overcome gametophytic SI by knocking-out the *Stylar ribonuclease* gene (*S-RNase*) (Ye et al., 2018). Self-compatibility allows fixing of gene edits and segregating out any insertions of foreign DNA from the process of transformation by selection in the progeny. Genome-editing will best be applied to potato improvement using diploid F1 hybrids. There is a need for a set of germplasm that is diploid, inbred and self-compatible forming tubers with commercial shape and appearance and high regeneration capability in plant transformation. Although, some existing diploid lines have some of the characteristics, more work is needed to produce germplasm that meets these requirements.

AUTHOR CONTRIBUTIONS

SN and CB conceived the idea, SN wrote most of the manuscript. DV and SN wrote the regulatory aspects. CB, DD, CS, and DV contributed to part of writing and overall improvement of the manuscript. All authors read and approved the manuscript.

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Beyond Risk Considerations: Where and How Can a Debate About Non-safety Related Issues of Genome Editing in Agriculture Take Place?

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INTRODUCTION

Having the potential to realize breeding objectives that were out of reach so far, genome editing (GE) surely constitutes a major advancement in the field of plant research, especially for the agricultural sector. Only recently has the debate about GE and its possible use in food and feed production transcended the scientific circle toward a political discussion. Considering the discussions about genetically modified organisms (GMOs) in the past, it is very likely that the public debate about genome edited food and feed products will be highly controversial. This article will show that the debate about genome editing is already risk-focused and that the resulting confinement structurally hampers a sound discussion of the values that are at stake. In contrast, to this development I argue that a comprehensive deliberation of values is needed in the context of genome editing in agriculture. Moreover, those deliberations should be separated from risk analysis and allow for individual decisions within our value system. Finally, I will discuss food labeling and consumer choice as an institution to support communication about values and to broaden the perspective on the agricultural use of genome editing and its products.

THE DEBATE ABOUT GENOME EDITING IS RISK FOCUSED—CONTENTWISE AND STRUCTURAL

Every human action, but especially actions with a wide range of effects that have not yet been tested, such as the use of new genome editing technologies, are inevitably linked to uncertainty and ignorance. Therefore, it should not come as a surprise that risk issues are prominent in the debate about genome editing. However, they are addressed very differently in various settings of the discussion. These differences can partly be traced back to different notions of risks. Within the scientific discussion, the risk of an action is defined as the product of the extent of damage and the probability of its occurrence (Knight, 1921). This notion leads to a gradual risk concept that allows for empirical assessment and the balancing of risks and opportunities for action. However, it tends to cover only those risks that are accessible from the scientific perspective (Jasanoff et al., 2015). Accordingly, scientific investigation has a focus on measures to reduce foreseeable risks arising, for example, from off-target effects of the technique (Kadam et al., 2018). In contrast, within the public debate the term risk is used and understood in

a much broader sense, to include unknown and unforeseeable risks. Moreover, the very same problems of the technology, such as off-target effects, are perceived as a black box that scientists are principally unable to penetrate and therefore become principally unpredictable hazards (Testbiotech Background, 2018). For this wide notion of risk the balancing approach of risk management, favored by scientists, is not applicable. Instead many public actors promote a strong precautionary strategy[1] to avoid, in extreme cases, any possible risk regardless of possible benefits.

This wide, colloquial notion of risks as hazards is highly problematic, but very efficient in terms of opinion formation. Thought through to the end this notion—in combination with the strong interpretation of the precautionary principle—leads toward the acceptance of any existing grievance, because unknown and unforeseeable risks of actions to improve bad situations could always exceed the existing problem. By raising apprehensions and fears, especially when human health is at stake, safety-related arguments become very compelling and are often used as discussion-terminating arguments without further need of proof. However, besides human health, other goods, such as ecological issues, autonomy, and matters of social justice are also prominent in the public debate about genome editing. The extent to which those issues are relevant for the appraisal of the technology and the way in which they are addressed not only depend on the technology itself, but also on the historical and socio-cultural setting of the debate in question (Torgersen, 2009; Sassatelli and Scott, 2010). In the case of genome editing, the initial scientific framing of the GMO debate (Jasanoff et al., 2015) prompted a risk perspective on the whole spectrum of issues and arguments concerning genome editing in agriculture. This means that the focus lies on the difference between a status quo, which is postulated a neutral point of reference, and potential deterioration of the status quo due to the technological innovation. The predominance of the risk perspective has several negative impacts: First of all, it narrows the scope of the discussions toward safety-related issues. Second, questions of personal preferences and lifestyle choices are marginalized. Thirdly, the value-nature of the goods at stake–the wellbeing of humans, human societies and ecological societies–and the conflicts that can emerge between those goods fall out of focus within the prevalent discussion structure. These effects strongly suggest an improvement of the debate by preventing that risks are perceived as hazards without further ado. At the institute Technic-Theology-Nature science (TTN) we investigate how ethics can contribute to that improvement[2]. While Jasanoff et al. endorse to open up the risk debate for societal apprehensions in order to overcome the constraints of a purely scientific perspective (Jasanoff et al., 2015), we argue in favor of a risk-independent value discussion.

[1]A comprehensive analysis of the strong and the weak interpretation of the precautionary principle was done by Rippe (2001).

[2]For more information visit the TTN website: http://www.ttn-institut.de

WHY DO WE NEED A RISK-INDEPENDENT DEBATE ABOUT VALUES IN THE CONTEXT OF GENOME EDITING IN AGRICULTURE?

Many voices–involving proponents and critics of agricultural application of genome editing–already claim that value considerations should be acknowledged in the admission process of genome edited products for various reasons (Myhr and Myskja, 2018; Röcklingsberg and Gjerris, 2018). For one, scientists working with genome editing techniques often argue that those techniques could help us to realize higher-level values, such as human health, protection of the environment or sustainable agriculture, which were not achievable by other breeding methods at all or within a certain time frame. In other words: social-political goals may be achieved by cultivating genome edited plants and livestock. Genome edited plants, like a mildew-resistant wheat, could, for example, contribute to reducing the use of pesticides. However, many breeding goals are relevant only to specific societal groups, like allergen-free peanuts, or can even hinder the achievement of societal goals, if they for instance promote herbicide resistance thereby increasing the use of those chemicals. In addition, not only the nature of genome edited products, but also the practices and circumstances of their cultivation and distribution will shape the impact of the new technologies. Therefore, also these aspects should be questioned for possible threats to social values, such as justice, autonomy and respectful interaction with nature. Finally, giving full consideration to ethical, social and sustainability related aspects of genome editing is crucial for the acceptability of the technology. Moreover, neglecting ethical, social and sustainability related aspects could be interpreted as a political failure, especially by people approving a reasonable employment of the new technologies.

For these reasons, value-based arguments should not be discredited as mere expressions of irrational attitudes (Pirscher and Theesfeld, 2018). However, the discussion of value arguments requires a different procedure and different solution strategies than a scientific risk discussion. Disagreement about scientific knowledge is at least theoretically easy to overcome because findings become wrong and irrelevant when contradictory evidence has proven to be right. In contrast, discord about values is much more durable and leads to continuing conflicts because conflicting values can exist side by side. Moreover, they only become significant within the context of a value system including other, potentially conflicting values. Although values and their fundamental relations are commonly shared within a society and thereby have normative potential, individual members of a society frequently differ in their point of view when it comes to indissoluble value conflicts. Because value decisions can differ within the scope of ethically acceptable choices, consensus solutions cannot be considered as the ultimate objective for societal value conflicts (Bogner, 2015). This particularly holds true, if the conflict at stake touches on the lifestyle of individual persons including their food choices. Instead societies need tolerance to enable people with different attitudes, interests and preferences to live together. This is an important difference between the appropriate handling of safety

issues and value conflicts about genome editing in agriculture: If someone, by her choice of production or consumption, exposes people or the environment to unreasonable risks, I will legitimately reject that behavior. In contrast, if someone takes a decision in accordance with values that do not parallel my own, or opts to pursue goals that we may share through different means, it remains necessary that I defer to her specific value orientations to a certain extent. Tolerance here means that I attempt to understand and respect how and why her thought processes do and need not mirror my own. Hence, societal value debates do not come to a single result that defines the universally applicable action, but should allow for multiple options that can persist in parallel. But how and where can that plurality be implemented?

FOOD LABELING AS AN INSTITUTION FOR VALUE DELIBERATIONS CONCERNING GENOME EDITED PRODUCTS

The proposal of integrating value considerations into the admission process of genome editing and its agricultural products will face a number of problems. A classical objection is that value criteria are vague and subjective (Zetterberg and Björnberg, 2017) and therefore not easy to justify. Value-sensitive regulations must also be defended against the reproach of nudging the public in a paternalistic way when they are state-imposed. With respect to the claim of providing a plurality of value attitudes within a society, one of the greatest drawbacks of implementing value considerations in the regulation of the production of genome edited foods is that it would reduce the spectrum of available products and thereby impose a concrete constraint on consumer decisions. This procedure not only elides how and why positive freedom, i.e., the prerequisite of having substantial options, is ultimately crucial for instituting freedom of choice (Taylor, 1979), but is particularly problematic in relation to a concept of social freedom (Honneth, 2014) which is claimed fundamental to communities based on liberality and solidarity and implies that decisions–especially governmental decisions–should be judged by the extent to which they foster the freedom of others. These restraints of freedom can be sufficiently justified for immediate safety reasons regarding human health or the environment. However, it is not in accordance with value decisions, derived from prioritizing one value over another, yet creditable value. While closing down the debate is necessary in the first case, opening up would be adequate in the latter (Stirling, 2008). One way to open up the debate about food and its production not mentioned by Stirling is to allow for value decisions on the level of consumption. For example, by labeling genome edited products and/or foods produced without that technology in a way that allows for communication about associated values. Furthermore, in the light of rapid technological development time pressure constitutes a serious problem for comprehensive and well-considered regulatory decisions regarding the agricultural use of genome editing. Labeling, instead, could stagger the processes of deliberation allowing for cautious governance of the new breeding technologies. In other words, allowing for case-by-case decisions in the supermarket could lessen the freedom-reducing effect of national governance decisions and render them adaptable to development in the public attitude, because also consumers who did or could not engage in the public debate are continuously able to make or change their decisions. However, labeling and consumer decision does not render scientific expertise and governmental institutions unnecessary. As with every new technology, genome editing for agriculture requires safety precautions concerning human health, society and ecosystems, which rely on scientific justification and governmental implementation and cannot be passed on to the consumers. It is particularly the decisions according to personal lifestyle, values, and beliefs that ought to be transferred to the consumer, because no institution can competently decide on them in behalf of the individual. To that end an adequate and accurate division between risk and value considerations needs to be performed. In fact, here the question touched to what extent food is and should be a matter of privacy (and self-responsibility)–a topic that has extensive potential for further research and social discussion.

As part of the research consortium "Ethical, legal and socioeconomic aspects of genome editing in agriculture" (ELSA-GEA)[3], funded by the German Federal Ministry of Education and Research (BMBF), we analyze the requirements a food label has to meet in order to function as an institution for value deliberations. We assume that labels do not only inform consumers about the qualities of available products, but function as a means to communicate preferences by purchasing or rejecting specifically labeled products. Understood that way, food labeling can also serve as an institution or platform for the negotiation of values. To that end, it should, for one thing, not intermingle risk and value aspects. In our view, this stipulation is not answered satisfactorily by current German mandatory and positive GMO labeling practice[4]. Although Kolodinsky and Lusk found that, in the case of Vermont (US), the mandatory label led to an improvement of the public attitude toward genetically engineered food (Kolodinsky and Lusk, 2018), a trust-improving effect (Slovic et al., 1986; Lusk et al., 2014; Kolodinsky, 2018) has not been reported for Germany (Christoph et al., 2008; BMU–Bundesministerium für Umwelt, 2018). Hiding the information about the use of genetic engineering techniques in the fine print and on the backside of product packaging and the exclusion of the majority of those products from the market is likely to contribute to the assumption that–although the product was proven to be safe–there is still something wrong with it. Along with other studies, claiming that a mandatory GMO label increases the apprehensions of consumers (Carter and Gruère, 2003; Zepeda et al., 2003; Sunstein, 2017), we doubt that extending the German GMO label to genome edited products will foster value-based consumer decisions. Secondly, labeling of GE products should not be biased toward a concrete value or value decision. That means, that consumers, who disagree with the use of the technology in agriculture, should be able

[3]For more information visit the ELSA-GEA website: www.dialog-gea.de

[4]For detailed analysis with different types of labels and their effects on markets, view for example: (Gruere and Rao, 2007; Bonroy and Constantatos, 2014).

to identify and purchase products that were not produced with genome editing. However, people who endorse the use of genome editing for specific reasons, should be given information that allows them to actively support the realization of their values. Therefore, the information content of a potential GE label has to exceed the fact of the mere use of the technology in the production process. It should indicate to which end the technique was used and supplementary information concerning practices and challenges in agriculture and food production should be easily available. However, increasing the information content of a label is always associated with the risk of subverting its orientation function due to information overload (Verbeke, 2005; Kronberger et al., 2012). As a third aspect, comprehensibility and clarity have therefore to be taken into account. To a certain degree, new technical solutions such as QR codes[5]could help to mediate between the demands for information supply and clarity. But ultimately, a label that assigns priority to being comprehensive rather than informative also runs the risk of forsaking the orientation function by simplifying too much and encouraging misinterpretations, such as the assumption that GMO labeling is a safety warning. In our opinion such a label does not meet the requirements for mandatory labeling, which should provide absolutely necessary information and therefore must not be ambiguous. In other words, we argue in favor of GE and non-GE labels designed to communicate relevant information for value decisions by the consumer. Reflecting the current situation in Germany, this cannot be achieved by using the existing GMO label. Instead new meaningful GE labels should be designed and issued. To that end producers and governmental institutions have to engage with societal values in the context of agriculture, thereby probably already improving the use of the technology or even the technology itself (Nowotny, 2006).

[5]QR codes are product specific labels which do not provide information immediately, but need to be scanned by electronic devices such as smartphones. When used to provide product information for consumers they usually lead him or her to a website offering detailed information.

CONCLUDING REMARKS

When new technologies are invented not only do we have to consider whether they are safe to use, but also wherefore and how we want to use said technologies. Therefore, not only scientific knowledge is relevant, but also practical aspects of the application of the technology and societal goals that may be realized or threatened by the technology. However, those situations always confront us with the problem of dealing with divergent but nevertheless legitimate goals within a society. While safety issues regarding the technology itself may be sufficiently examined by scientific means and can be subjected to regulatory policies accordingly, dealing with values requires tolerance, continuing communication and the possibility of coexistence. Consumer communication via labeling offers a good means to govern the desirable variety of legitimate preferences within a society. But only if those divergent preferences are not communicated as mutual threats. The foreseeable necessity to label genome edited products should be seen as an opportunity to institutionalize a comprehensive debate about values relevant in the agricultural context by connecting technological knowledge, societal goals, and individual consumption decisions.

AUTHOR CONTRIBUTIONS

The author confirms being the sole contributor of this work and has approved it for publication.

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Precaution, Responsible Innovation and Beyond – In Search of a Sustainable Agricultural Biotechnology Policy

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The recent ruling by the European Court of Justice on gene edited plants highlighted regulatory inadequacy as well as a decades-old political problem, namely how to reconcile diverging expectations regarding agricultural biotechnology in Europe. Over time, regulators had tried out various tools to address concerns and overcome implementation obstacles. While initially focussing on risk (with the Precautionary Principle), they later tried to better embed technology in society (e.g., through Responsible Research and Innovation). The PP got criticized early-on; meanwhile, it seems to have lost much of its salience. Responsible Research and Innovation (RRI) is associated with problems of participation and political impact, often rendering it a public awareness tool only. We discuss problems with both approaches and conclude that also RRI falls short of facilitating technology implementation in the way regulators might have had in mind. Rather than leaving political decisions to technical risk assessment or ethics and public awareness, we argue for re-establishing a broad yet sober process of opinion formation and informed decision-making in agricultural policy.

Keywords: biotechnology policy, European Union, GMO regulation, gene editing, Precautionary Principle, Responsible Research and Innovation

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INTRODUCTION

The European Court of Justice's ruling (Court of Justice of the European Union [ECJ], 2018) that gene edited crops should be assessed like traditional genetically modified organisms (GMOs) elicited split reactions. While some scientists criticized that it jeopardized the future of plant breeding in Europe (Stokstad, 2018), others lamented other scientists' hypocrisy (Stirling, 2018). NGOs greeted it in the name of consumer rights (Friends of the Earth [FoE], 2018). The comments not only suggest regulatory inadequacy but also show how deeply split stakeholders are over the future of agricultural biotechnology in Europe. They seem to agree, though, that gene editing is a game changer, offering unprecedented opportunities for achieving new traits without introducing foreign DNA. Since distinguishing gene edited from 'naturally' bred varieties will be difficult, the technology might be a vehicle for bringing crops with targeted genetic alterations onto the field – a relief for some and a nightmare for others. However, it remains unclear how the ruling can be implemented.

The European Court could have followed the more relaxed proposal by the advocate-general, who argued that gene editing should be exempted because there is no new DNA in the plant

(Abbott, 2018). However, the Court focussed on the 'lack of experience' with targeted alterations of the genome, in contrast to the results from older methods of mutagenesis that had proven safe. Thus, the Court used the same argument as applied for regulating recombinant DNA long ago. Today however, in the light of 30 years of safe use, the latter might be considered safe as well. Since it is not considered as such, it is unclear which amount of experience will be held sufficient to exempt a technology from additional scrutiny in the future. After all, gene editing is one of the latest innovation to challenge European regulation but probably not the last.

Whilst regulatory inadequacy is a problem in itself it highlights a bigger political problem: how to reconcile diverging demands and expectations regarding agricultural innovation among the European Union's stakeholders, institutions and member states. It is by no means a new problem as regulators had to learn early that agricultural biotechnology would not proceed in a business-as-usual way. For decades they strived to 'make biotechnology happen' (Torgersen et al., 2002), promoting innovation by generously supporting research and development toward economic applicability together with ensuring safety by providing restrictive risk regulation (Jasanoff, 1995). Thus they tried to meet widespread concerns that impeded the implementation of biotechnology.

This double strategy was not without problems: "Obviously, governments thought that biotechnology was something worth developing and they supported it with alacrity. Yet they also styled themselves as impartial regulators of what many perceived to be a risky endeavor. This ambiguity later proved to be one of the sources of public distrust" (Torgersen et al., 2002, p. 23). Despite all research support and risk regulation, the European public could never be convinced of the advantages of agricultural GMOs[1]. Efforts spent on understanding the background for public skepticism (e.g., Gaskell et al., 2004, 2010) made it clear that the underlying reasons are complex and often prone to misinterpretations[2].

Over time, regulators came up with a variety of innovative policy tools to address the conundrum, in their view, of public concerns and thus to overcome the obstacles to technology implementation. The initial focus on risk mitigation ran into difficulties as it proved to be too narrow. Later, it was supplemented by a broader approach aimed at anchoring technology in society. However, both attempts had their particular problems and eventually failed. We claim that analyzing the role of these tools provides a fruitful analytic perspective to distinguish different attempts at 'making biotechnology happen' that may also influence future endeavors in this respect. From such a perspective, we argue for shifting the emphasis from regulating the technology to pursuing comprehensive agricultural policy goals.

Following this rationale, the article will focus on the Precautionary Principle (PP) as a tool to mitigate uncertain risks and, more contemporary, on Responsible Research and Innovation (RRI) as a value oriented concept to anchor technological development in society. Although the PP and RRI have little in common content-wise, we think they shared a political function, albeit using different strategies: they both should prevent or bring down controversies over particular applications among stakeholders and the public. These controversies were seen as the major obstacles to the implementation of biotechnology (i.e., to 'make biotechnology happen'). In the context of the political and regulatory efforts to overcome controversies, the PP' rationale appeared as that of an 'emergency brake' in (rare) cases of unclear but potentially severe risks. While it was intended to reassure critics it fostered, in practice, a rhetoric of scientific risk arguments and their dismissal. We will address how this narrow focus proved insufficient to address the underlying concerns and how the PP eventually became the target of criticism itself.

When the attention turned to new areas like nanotechnology that seemed prone to elicit similar controversies, a broader approach appeared necessary that transgressed the boundaries of technological risk assessment and addressed societal issues as well. Over time, attempts concretised under the umbrella of 'Responsible Research and Innovation' (RRI)[3]. It catered to shortcomings of previous attempts to foster cooperation rather than conflict in various ways: (i) Since the authorisation process proved to be too late a step for leverage, activity sets in much earlier. (ii) The concept of mission orientation appeared handy to align innovation with 'grand challenges' addressing major contemporary problems. (iii) Societal preferences as they emerged from public debate are taken into account, together with, and framed by, established ethical principles and normative frameworks. (iv) Rather than in a top-down way, technology development is reconciled with societal values and expectations through participatory procedures. The rhetoric exceeded the narrow focus on risk; however, and despite considerable efforts at defining, fleshing out and implementing RRI through big EU funded projects,[4] it remained a framework providing orientation at best rather than becoming a policy principle.

Since both tools with their respective reference to risk or ethical principles and societal values could not sustainably cope with the recalcitrant problems of 'making biotechnology happen,' the question now is how to proceed in the light of technologies like gene editing. Since business as usual does not seem feasible, we will finally ask how a solution could look like. In our view, the regulatory orientation at the technology must be revised in favor of a goal-oriented comprehensive agricultural policy emerging from an open political process of EU-wide opinion-formation among stakeholders and society at large, difficult as it probably will be.

[1]Skepticism seems even to have spread to the United States, see International Food Information Council foundation [IFIC], 2018.

[2]For example, the hypothesis of a general 'resistance to new technology' out of a lack of knowledge among lay people was dismissed in the 1990s already (Bauer, 1997) but remained popular among scientists and regulators (Rip, 2006).

[3]The official website (https://ec.europa.eu/programmes/horizon2020/en/h2020-section/responsible-research-innovation) states: "Responsible Research and Innovation (RRI) implies that societal actors (researchers, citizens, policy makers, business, third sector organizations, etc.) work together during the whole research and innovation process in order to better align both the process and its outcomes with the values, needs and expectations of society."

[4]for example: https://www.rri-tools.eu/de

PRECAUTION OR THE TRANSFORMATION OF POLITICAL DISPUTES INTO RISK ISSUES

The Precautionary Principle and Its Double Role in Risk Controversies

While most new agricultural technologies did not raise much concern, the genetic modification of crops triggered questions of safety and risks, benefits and their equitable distribution long before the technology was put into practice. In the late 1980s, risk claims might not have been surprising: with little experience, it was still unclear whether the new breeds would behave as predictably as traditional ones. Scientifically determined health and environmental risks, if evident, usually entail regulatory action, so technology critics tried to prove such risks, though largely in vain. Technology supporters considered speculations about risks as unscientific and demanded sticking to positive evidence as the only legitimate basis for regulation (Miller and Conko, 2001). Nevertheless, in the absence of conclusive evidence any remaining uncertainty perpetuated risk claims (Bourrier and Baram, 2011). Mitigation tools failed to solve the conflict because a variety of other fears looming behind took the shape of risk arguments (Gaskell et al., 2004).

During the 1990s, the European Commission took up previously existing ideas of precaution and reformulated them. The PP in its then new form became the hallmark of European risk regulation. It addressed a pressing problem: if there are strong hints at a risk but experts disagree about its presence, magnitude or cause, long legal battles and an unacceptable delay in regulatory action might ensue. In some cases such as tobacco and asbestos, this had caused unnecessary uncertainty and a high death toll (Harremoes et al., 2001). Here, it would eventually prevent particular risk-prone applications of the new technology from being implemented. The PP might provide a regulatory shortcut in those (rare) cases where there are strong indications but no full evidence of a severe risk (Von Schomberg, 2013), provided that there are cost-effective ways to reach the desired aim of risk reduction[5].Thus, the principle of uncontested scientific evidence as a precondition for case-specific regulatory action became questioned. To allow sorting out the few cases where the PP might apply from the vast majority of others the notion of uncertainty got further specified, integrating risk assessment into a 'precautionary process' (Stirling, 2007). Despite such attempts at sophistication, the temptation to apply the PP as a last resort in cases where a product was unwanted remained: in a number of trade-related conflicts, the EU, referring to the PP, tried to prevent the import of food products with the argument of health risks (e.g., Millstone et al., 2004). These cases highlighted the propensity to political misuse that critics always had feared.

In contrast, the political intention might well have been that the PP should facilitate technology implementation by reassuring critics that no risks had to be feared as preventive action would be taken even if full evidence was lacking. For example, the European Directive 90/220 on the Deliberate Release of genetically modified organisms (European Council, 1990) made precaution mandatory, emphasizing the safe use of the technology. When the then new Gene Technology Law was debated in 1992, the Austrian Parliament demanded that any application should be made subject to the PP; it therefore went into the preamble (Österreichischer Nationalrat, 1994). It was a concession to the critics to ensure a safe and smooth introduction of the technology. However, the PP was often understood as reversing the burden of proof, which manifested in preventing any deliberate release or marketing of GM products. The political basis for such an understanding was a widespread public aversion against GM crops and food, effectively orchestrated by environmental groups, some farmers and big retail companies (Lassen et al., 2002)[6].

The lesson learned was that referring to the PP in a political way proved to be effective to halt a technology. Among innovation conscious policy makers (especially in the United States) the PP therefore became anathema[7]. Everybody thought over twice before invoking the PP in a concrete case because this could have unpredictable consequences. Intended as a pragmatic means to evade long and futile legal battles, the PP had been turned first into a policy tool to reassure critics that risk would not be tolerated so that the implementation of a contested technology could proceed. In a second step, it resulted in severe obstacles to technology implementation and innovation – even if not invoked. Regarding GMOs, namely, its impact was symbolic and political rather than contributing to mitigate risks in practice.

The attempted policy function in managing the controversy – precautionary action to calm critics – had a perverse effect as disputes over the appropriate interpretation and application of the PP itself became part of the debate (Van den Daele et al., 1996). Rather than providing a solution to the on-going conflict, the interpretation of the PP opened up a novel turf that mirrored local idiosyncrasies in member countries (Levidow and Carr, 2005), where preferences on how to deal with agricultural biotechnology differed[8]. On the EU level, the incongruent assessment manifested in conflicts over the market approval of GM crops and, consequently, in diverging voting behaviors of the competent ministers in the European Council. Analyses showed that voting mostly depended on political factors such as public opinion or the government party

[5]See the formulation in the respective EU Directive (European Commission, 2000), not to be understood as shifting of the burden of evidence. Proving the absence of risk would be intellectual nonsense.

[6]In retrospect, political action to prevent agricultural biotechnology in some countries might be considered as an early form of contemporary populism.

[7]The United States Administration formulated their own 'precautionary approach' based on existing legal instruments, arriving at less restrictive but similar precautionary measures without much resistance.

[8]A strong driver of the conflict in the 1990s were various concomitant food scandals such as over BSE. Unrelated from a technical point of view, BSE influenced the GMO case as it "turned 'mad cow' into a potent metaphor mobilizing public distrust in regulatory arrangements by linking several policy issues." (Levidow and Carr, 2010, p. 20).

line (Mühlböck and Tosun, 2018). Attempts to solve the issue on an EU level therefore ended up in a limbo as member countries could not agree on a common policy (Hampel et al., 2006). Eventually, a revision of the Directive allowed national governments to ban GM crops temporally (European Parliament and European Council, 2015). Thus, the EU regulation including the PP had (almost pathetically) failed to mitigate risks while ensuring harmonized innovation in a functioning common market.

As a result, and in contrast to other technologies having become less controversial over time, the conflict over GMOs petrified. Official debates over alleged or uncertain risks together with public mobilization and the reluctance of European food retailers to offer GM products efficiently halted the technology. This stalemate has not changed despite an ever more sophisticated regulation.

One reason was that a variety of concerns built on different framings of the issue (Bogner and Torgersen, 2015), to the effect that opponents and proponents lacked a common basis of understanding. The perpetuated administrative focus on risk did not help much as it prevented politics from developing a broader political perspective to reconcile different interests and world views, which might have addressed underlying problems better (Levidow and Carr, 2010). In the meantime, the battle over GMOs in agriculture and food became paradigmatic for controversies mixing risk and non-risk arguments that were expected to arise over other novel technologies. Even if they never manifested, technology developers came to fear them (Rip, 2006; Torgersen and Schmidt, 2013). Since the PP had clearly impeded technology implementation, another way to address concerns in the absence of evidence of risk was deeply needed – not only to solve the GMO conflict but for innovation in general.

Transgressing the Narrow Focus of Technological Risk

Over recent years, the PP seemed to have lost salience as a risk management tool[9]. Yet the problem of uncertain risks from novel developments remained. For example, experts from three risk evaluation panels of the European Commission identified considerable uncertainty over safety and security from Synthetic Biology (SCENHIR/SCHER/SCCS, 2015). Accordingly, gene drive experiments could pose particular risks to the environment. Radically novel traits or modifications of animals and human beings might bring deep-rooted dreams and fears nearer to realization. 'Xenobiology' – unpredictable foreign forms of life incorporating new chemical components – appear possible. Synthetic biology might also render itself to do-it-yourself activities raising serious security and safety issues. One could have expected the PP to play a certain role in their conclusions; rather, they laid emphasis on not foregoing potential benefits from overestimating risks while taking up concerns among the public. Their advice becomes somewhat understandable in the light of the debate on the PP itself.

Not only social scientists had long suspected that risk and its perception is a political issue. Early on, critics of the PP had found the principle to be socially biased as it is said to be sensitive to risks associated with technological change or ecological interventions while being blind for risks from regulation (Sunstein, 2003). Accordingly, this is due to the 'selectivity of precautions': the publics (and eventually politics) in different countries are sensitive for particular risks and not for others, subject to national patterns of cultural value preferences[10]. As a result, precaution fosters regulation only if the risk addressed is politically relevant. Therefore, the PP fails to reduce overall risks as it ignores some of them. For example, avoiding potential environmental or health risks by prohibiting a technology does not away with risks from older competing technologies and, in addition, may entail new risks from regulation, if only indirectly[11].

As an answer to frequent criticism, the European Commission proclaimed an 'innovation principle' as a counterweight to the PP, intended to repair its (political) shortcomings (European Commission, 2016). While the PP emphasizes risk, the innovation principle focusses on the opportunities of a new technology, to which any risk should be compared. If a technology would not be implemented due to potential risks, this should be weighed against the benefits forgone, such as avoiding known risks from technologies replaced. Together, it was argued, both principles would adequately represent the double face of technological innovation, balancing the risks when implemented with those when not. If in doubt, the benefits from innovation may weigh heavier as risks are speculative. In practice, however, putting up risks and benefits from old and new technologies against each other is rarely done, so the impression prevails that the innovation principle's role, too, is mostly symbolic.

Taken together, the focus on risk is subject to political and cultural preferences while seemingly promising objectivity. Reports on the social psychology behind the debate over GM food have shown that the rejection mostly originated from a fear of the 'unnatural' and hybrid as a result of the technological tinkering with food (Gaskell et al., 2004; Wagner et al., 2006). In this light, it becomes understandable that the rejection of GM crops and food remained a social fact irrespective of arguments. As a consequence, Sunstein (2003) demanded that technology governance should aim at a better policy to address a broad range of societal concerns as well as benefits including, but exceeding, risk aspects.

[9]A recent call for proposals under H 2020 addressed an obvious lack of empirical data regarding the salience of the PP vi-á-vis the Innovation Principle. It asks to take stock of the implementation of PP since 2000 in various contexts, analyze the effects of the PP and propose several scenarios for the future of the PP and IP (see https://ec.europa.eu/research/participants/portal/desktop/en/opportunities/h2020/topics/swafs-18-2018.html#fn1).

[10]Accordingly, the United States took a highly precautionary approach to risks associated with terrorism, tobacco smoking and universal health care, but not to global warming, poverty and, until recently, obesity. Germany, in contrast, was especially concerned with global warming, nuclear energy, gun possession and the genetic modification of food (Sunstein, 2003).

[11]For example, the effort for complying with the regulation of transgenic corps might render the development of regionally adapted varieties unrewarding, promoting seed uniformity and the risk for pest resistance with a global impact.

THE 'PARTICIPATORY TURN' IN TECHNOLOGY POLICY – GOVERNING INNOVATION RESPONSIBLY

The New Mission Orientation in (Bio)technology Policy: From ELSI to RRI

While the EU tried to overcome the GMO conflict by developing and refining a precautionary handling of potential risks, attempts to develop new technologies in line with social values and expectations gained salience and prevail today. Such an orientation at a mission aims at addressing societally and/or economically relevant benefits from technology application and finding ways to realize them (Mazzucato, 2017). It sees a genuinely political task in determining which benefits should be addressed in whose interest. Thus, political action not only pursues the classical task of protecting people from risks. Rather, it aims at the conscious or planned design of innovation and at political impulses for the development of marketable technologies through, i.a., research funding. Rather than being realized top-down, technology will be implemented through governance approaches that build on a network of actors including politics and business, science and civil society.

Dedicated mission orientation emerged after World War II with the era of 'Big Science,' leading to success through collaborative work and large resources. The resulting technologies might not have been developed via private initiative or normal scientific progress (Gassler et al., 2006), such as nuclear power, space exploration, semiconductors and, later, ICT or bio- and nanotechnologies. Classical mission orientation contributed to making innovation paramount: "Governments have made of technological innovation an instrument of industrial competitiveness, world leadership, and national wealth." (Godin, 2016, p. 548).

With a focus on innovation, a new mission orientation was developed that not only focuses on profitable products but also on pressing societal problems ('Grand Challenges'), with sustainable development as a cross-cutting issue. Value questions such as the responsibility for consequences and non-technical, especially ethical criteria for decision-making are taken into account. Advisory bodies such as the National Ethics Council or the Council for Sustainable Development in Germany illustrate their (symbolic) salience[12]. Rather than eliminating risks, the aim is to implement innovations by reconciling technological development with societal values and expectations.

This approach is condensed in the principle of Responsible Research and Innovation (RRI). It has become a reference point in the debate on governance through a number of EU research projects and policy initiatives[13]. The term had been coined during the 2000s when the controversy over nanotechnology was prevalent. In their 'European Strategy for Nanotechnology' the European Commission (2004) defined responsible development as a deliberative process based on the idea that nanotechnology could be guided by ethical principles and solutions, whenever appropriate, should be enforced through regulation. Since then, the European Commission, EU Member States and associated countries have launched various initiatives and activities under the header of RRI. It has been institutionalized as a cross-cutting issue under Horizon 2020, the EU research framework program 2014–2020.

More than 250 articles covered RRI from a social sciences perspective and provided numerous definitions (Burget et al., 2016). Since 2012, René von Schomberg's influential take appeared in several EU calls on 'Science with and for Society':

"Responsible Research and Innovation is a transparent, interactive process by which societal actors and innovators become mutually responsive to each other with a view to the (ethical) acceptability, sustainability and societal desirability of the innovation process and its marketable products (in order to allow a proper embedding of scientific and technological advances in our society)" (Von Schomberg, 2013, p. 63).

What ethically acceptable, sustainable or socially desirable means remains contested, though. In a pluralistic society, normative criteria cannot be defined *a priori* in a technocratic manner, rather, they have to be deliberated by a broad range of societal actors (Stilgoe et al., 2013). As a stopgap, Von Schomberg (2013) referred to normative anchors as stated in Article 3 of the European Treaties. Furthermore, a set of common denominators cut across all the different understandings. According to the extensive review by Burget et al. (2016), three aspects are to the fore:

- A focus on values: Most definitions and frameworks affirmatively refer to moral and ethics in technology issues. Even though more precise definitions are lacking, the call for ethics implies that technology development and innovation should be aligned with the values, needs and expectations of society in order to be acceptable, sustainable and societally desirable.
- Ethics as a design element: In previous technology controversies, the call for ethics was associated with the idea of taming or restricting innovation. Ethics was practiced reactively; it was intended to prohibit unwanted consequences after the innovation had been developed or products had entered the market. With RRI, ethics is referred to as a design element shaping innovation responsibly and proactively rather than an ex post evaluation tool as before. Hence, RRI deliberately uses ethics and ethical arguments to shape technology rather than clinging to a particular ethical tradition itself.
- Public participation: With a more prominent role of value question, the innovation agenda is opened up for all kinds of expertise and experience. In technology controversies focusing on risk, primarily expert knowledge

[12]Even if in the reality of research funding the focus on value questions remains symbolic rather than having a real impact, actors have to deal with them, which makes them explicit and opens up new lines of argumentation.

[13]The European Commission explained RRI as follows: "Responsible Research and Innovation means that societal actors work together during the whole research and innovation process in order to better align both the process and its outcomes, with the values, needs and expectations of European society. RRI is an ambitious challenge for the creation of a Research and Innovation policy driven by the needs of society and engaging all societal actors via inclusive participatory approaches" (European Commission, 2012, p. 2).

is deemed legitimate. While the focus on risk privileges expert authority, taking other aspects (justice, exclusion, inequality, marginalization, privacy) into account gives stakeholder and lay knowledge a greater role. Therefore, the focus on value questions goes along with an invitation to a variety of actors to participate in the innovation process.

Participatory governance had its precursor in debates around the Human Genome Program on ethical, legal and societal implications (ELSI) that were expected to materialize as soon as the genome sequence would have been established. It served as a blueprint for similar programs on other emerging technologies. From 1994 on, the European Union provided research funding for ethical, legal and social aspects (ELSA) of emerging technologies only to abandon the term two decades later in favor of RRI (Zwart et al., 2014). The main difference laid in the emphasis on socioeconomic impacts such as valorisation, employment and competitiveness. Nevertheless, RRI became charged over time with aspirations at a more democratic and social responsive technology development (Stilgoe et al., 2013).

All this was not intended as a replacement for the PP, although RRI also stipulated that potential risks should be identified early and dealt with in a 'responsible' way. Rather than hindering a potentially risky product from being marketed, the process should prevent such a product from being developed at all or ensure that potential risks were catered to during development. The political function was to pre-emptively address potentially disruptive issues in a public debate over newly emerging technologies, be they concerns over risk, ethical implications or societal misfit. Aligning innovation with societal goals and making it 'responsible,' so the hope, would take the steam out of a pending controversy and foster technology implementation.

The Grand Challenge of Public Participation

The focus of RRI on values and ethics immediately suggests a focus on public engagement, because value conflicts and ethical questions cannot be decided on the basis of expert knowledge alone. Vice versa, the emphasis on public engagement for designing innovation indicates that distributed intelligence, pluralism and dissent may have a constitutive role. With research, the tendency toward inclusion is reflected in the concepts of Citizen Science (Irwin, 1995), transdisciplinarity or Mode 2 science (Gibbons et al., 1994). With regard to technology, it manifests in various forms of technology assessment (TA) such as participatory, constructive (Schot and Rip, 1997) or 'real-time' TA (Guston and Sarewitz, 2002).

In the context of RRI, however, the status of public participation goes beyond that in TA. In participatory or constructive TA, citizens, consumers and stakeholders participate in isolated events conceived as participation projects (Bogner, 2012). They are non-binding and provide complementary information about citizen values rather than being part of the innovation process itself. RRI, in contrast, promotes formats that enable the continuous involvement of relevant actors. The objective is to institutionalize and routinise public participation in research and innovation (Owen et al., 2013). Attention is paid to heterogeneity, taking into account a large number of divergent perspectives and actors such as stakeholders (NGOs, industry, trade unions, science communication), policy and administration (parliamentary commissions, research funding) and academia (universities, non-university research). In addition, the broader public (as constituted topic-specifically for a participatory event) must be involved. Added value, so the hope, comes from a multiplication of perspectives, a consideration of alternative rationalities and knowledge forms as well as an opening of decision-making processes.

However, there are severe challenges to participation, especially with respect to emerging technologies, along several dimensions: with regard to (1) social aspects, (2) the 'issue framing,' i.e., how to discuss what, (3) the timing of an event and (4) the definition of the problem to be addressed.

(1) Regarding social aspects, public engagement requires a panel with a balanced composition of participants, taking into account gender as well as representing various societal perspectives (Rask et al., 2016). At least, particular interests or perspectives must not dominate the deliberation process. To ensure balance, the actors invited should represent a diversity of values and forms of knowledge. RRI also requires a comprehensive, objective ('balanced') view upon the issues. The assumption is that participants (especially stakeholders) enter the process holding preconceived interests and views and reproduce the usual conflicts. As a remedy, Von Schomberg (2013) demanded that stakeholders should transcend their stereotypical arguments and strategies – industry representatives should not only highlight economic benefits, NGOs not only risks; rather, they should see the world through the eyes of the other, respectively. However, it is unclear why a stakeholder should forego a short-term benefit from pursuing his or her self-interest in exchange for fostering public welfare-oriented responsible innovation. Institutionalizing public participation in the spirit of RRI therefore demands changing established power relations and conflict structures. Another practical problem is that participants often experience social difficulties in participatory procedures. For example, they are not accustomed to tolerate opinion pluralism or the obligation to provide reasoned arguments. If discussions turn controversial (e.g., on values or identities), those with extreme positions often feel inadequately represented and may leave the group (Bogner, 2012).

(2) With regard to the issue-framing, participation entails prioritizing value questions over questions of knowledge or interests. RRI therefore tends to change the focus from risk to ethics. Risk discourses privilege expert knowledge because claims for health or environmental hazards must be backed with scientific arguments. Ethics that is not limited to medical issues (see Beauchamp and Childress, 1994) includes aspects like justice, exclusion, privacy, marginalization, etc., which extends the range of issues beyond those of risk. In practice, however, standard arguments are rarely exceeded because members of a 'participation industry' (experts and institutions from science communication, STS and TA) usually initiate and organize processes from outside. In addition, and especially with new and emerging technologies, participants are rarely involved in the issues at stake and lack the necessary knowledge. Therefore,

organizers must define the problems in advance, running the risk of marginalizing alternative problem-solving perspectives (Sykes and Macnaghten, 2013, p. 100). Emerging technologies have not yet found many concrete applications that elicit concerns or hopes. To make due, organizers use analogies, i.e., established problem-solving perspectives from comparable controversies, again marginalizing alternative perspectives. For example with synthetic biology, concrete hopes reared by science and industry prevailed in British participation processes (Bhattachary et al., 2010). In contrast, questions were rather generic: how can synthetic biology contribute to the bio-economy? Can synthetic biology solve the antibiotic crisis? etc. Finally, whether stakeholders are willing to participate depends on the scope of the event. Any seeming indication of lopsided criticism or euphoria jeopardizes a balanced representation (Stilgoe et al., 2013).

(3) Regarding timing, RRI demands early and continuous participation. From 2000 on, nanotechnology triggered a plea for 'upstream involvement' (Kurath and Gisler, 2009). Arguments for early participation were derived from the idea that the path from basic research to application was not linear, since technical feasibility and marketability would influence basic research already. Decisions over the path the technology would take were made early, therefore, so the argument, participation must also set in early to render it effective. Thus, the concept of technoscience (Latour, 1987) also fostered early participation. However, few applications exist at an early stage that cause conflicts or inspire the public's imagination; consequently, few people show interest and people have to be motivated. They are more interested if the subject is relevant to their everyday life or if it is controversially discussed in the media. Then, however, the trajectory usually cannot be changed anymore[14].

(4) Regarding problem definition, the lack of popular perspectives may either lead to the debate remaining very concrete, with the researchers' motivations and problems of laboratory processes as subjects. Alternatively, the discussion turns to the meta-level, where general considerations on technology and democracy or the future of participation dominate[15]. Thus, the deliberation runs the risk of remaining abstract, little committed or expert dominated, with normative dissent often remaining implicit (Felt and Fochler, 2010) – participants debate on the meta-level how to responsibly discuss ethics. With positions remaining largely consensual, their main concern is whether all relevant aspects are getting represented. Rather than being advocates of the common good or of perspectives based on their personal value bases they see themselves as service providers: the task is to contribute to the success of a project.

Regarding gene editing, the current EU framework program supports several projects investigating potential implications under the perspective of RRI. However, it is not always clear what exactly to discuss. Subject to the concrete application this must be decided from case to case. Whether the focus on the technology makes sense is therefore questionable. Another problem is the difference between a discussion under RRI and under the PP. As risks should be considered under RRI as well, a usual subject of the debate is whether there are any risks and what they would entail. In the light of the skepticism in some countries, the results of a participation event may therefore not fundamentally differ from that of many previous exercises on GMOs. The hope that technology implementation would be facilitated thus appears futile.

CONCLUSION: WHERE TO GO FOR A SUSTAINABLE POLICY?

Both the PP and RRI had a role in the (non)implementation of agricultural biotechnology in Europe, although they proceeded from opposite angles, addressing different aspects of the development and operating at various stages in the implementation process. Yet, regarding their common function of 'making technology happen,' both show a disappointing performance.

The PP turned out to prevent not only risky developments but the implementation of the technology in general. Designed as a last resort tool to ensure that ambiguous risks would not lead to endless court trials and block the technology as such, it was applied when political decisions appeared impossible to defend. Referring to the PP allowed actors to use seemingly scientific arguments that nevertheless were politically grounded. The PP may have been intended as a reflexive way of dealing with potential risks; however, the controversy over GM food has never been a risk debate only. Rather, it had many roots like the widespread public unease over current agricultural food production systems. When, in addition, national food idiosyncrasies became jeopardized, organized protest ended up in petrified skepticism[16]. This suggests that risk regulation may be an essential part of the regulatory process but an inappropriate tool to cope with political stakes.

Responsible Research and Innovation was intended to guide research and innovation practice toward societal acceptability while fostering innovation. However in practice, it often ends up in a mere tick-boxing activity filling in research proposals forms or in somewhat futile participatory activities as ends in themselves. Activities to involve stakeholder and the public without real impact on the decisions taken have an unclear remit and mostly serve to introduce bits of new technology to a public that has little to say about it. Referring to ethics and a poorly defined 'responsibility' of stakeholders (or even laypeople) does not solve the political problem of organizing the relevant sector, agriculture, in a way that would find support with stakeholders and critical citizens alike.

Both principles seek to tackle a major problem for innovation policy, namely to remove obstacles to technology implementation

[14]The situation reminds of the Collingridge dilemma (Collingridge, 1980): when a new technology emerges, its trajectory can still be influenced but knowledge is insufficient to steer it. When knowledge would be sufficient, the trajectory is set.

[15]Meta-level ethics throws up questions like: should we refer to ethics when talking about new technologies? Is it useful to discuss ethical aspects or does it fuel the controversy?

[16]However, there are indications that public perceptions slowly change. For example, younger people appear to be less critical (Gaskell et al., 2010) – not only in Europe but also in China (Cui and Shoemaker, 2018).

caused by public skepticism. Both turn out to do so with insufficient means – in an attempt to either dress up politics with scientific arguments or to address political problems with public relation tools fostering awareness among a little interested public. Although both risk regulation or laboratory-like deliberation events having turned out to be the wrong turf, neither the PP nor RRI should be dismissed. Rather, they should be applied to those cases they were intended for, namely the reasonable treatment of uncertain risks and the better alignment of innovation policy with societal values. In addition, the remit of participation must be better defined; current procedures may not yet be adequate to uncover demands and concerns nor cultural value preferences; for the moment, participatory events too often focus on disseminating awareness regarding a new technology.

In our opinion, the most important factor for addressing the underlying problems adequately is comprehensive sectoral policy reaching out to other sectors. After all, issues pertain to agriculture and food production, to research and innovation, trade and various other sectors. Rather than principles in need of interpretation being politically instrumentalized as 'magic bullets,' deliberate scientifically supported and democratically legitimized policy may be more adequate to tackle those problems. A major shortcoming from previous policies, however, is their strictly sectoral scope. Since agriculture reaches out to so many areas, an inter-sectoral and multi-level approach is needed. For example, sustainability in agriculture is not only an issue for biotechnology regulation but also for R&D funding, industry development, trade rules, regional policy and many more fields.

Regarding regulatory principles, biotechnology policy has traditionally focussed on regulating the technology. This is one reason why in case of a problem, it easily succumbed to seeming solutions replacing the political with either science or ethics. As a remedy, the prerogative of serious long-term oriented policy needs to be reinstalled and the focus on technology replaced by a focus on the common understanding of the aims agriculture should pursue. This implies that the different tasks of agriculture should be openly discussed and the properties of crop plants adapted accordingly. In other words, properties should not only reflect agronomic and economic parameters but a variety of demands according to the context, which may differ from one place to the other. Whether the respective seed has been developed by traditional breeding, chemical mutagenesis, recombinant DNA technology, gene editing or any technology to come, however, is hardly relevant in this context (see Davison and Ammann, 2017). This does not preclude applying the PP in appropriate cases or addressing a relevant measure under the umbrella of RRI. However, the PP is a risk management tool, while RRI, as the name suggests, focusses on shaping (technological) innovation – perspectives too narrow to tackle all the questions that need to be addressed.

The future of agricultural biotechnology is not an issue for plant breeders and researchers only. It requires a broad debate among many disciplines and stakeholders. A good example was a recent Summer School organized by the faculty of Theology University of Munich, where pertaining aspects could be discussed. New ideas diffuse into mainstream thinking as well, which seems to focusses less on technology and more on real problems now. The European Commission seems aware that the system is not sustainable (European Commission, 2015). More recently, a report on the 'authorisation processes of plant protection products from a scientific point of view' by the Commission's Scientific Advice Mechanism (Group of Chief Scientific Advisors [EC-SAM], 2018) advocated not only scientific reasoning but also extending the scope of arguments to criteria usually held to be 'political.' Similarly, a report from UN Environment argued for an extension of parameters to take into account when it comes to measuring the performance of agriculture, including the contribution to sustainability goals (TEEB, 2018). These initiatives highlight the need to discuss, define and agree on the many tasks of agriculture. With clear ends we may devise adequate regulatory mechanisms for the means available. The technology used is only one factor, and a less important one, provided it is safe and effective.

As agriculture is one of the hardest bones of contention among the EU policy fields, such a demand might sound unrealistic. Yet in our view, there is no way beyond an open and transparent process of opinion formation that not only involves stakeholders and the European Institutions but includes scientists, politicians from member countries and, preferably, large parts of the European society. Only if we have a clear vision of future agriculture and its various tasks we will be able to decide over GM crops or the products of any other technology to come.

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AB and HT contributed equally to the research and the writing of the article and agree to be accountable for the content of the work.

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Normative Criteria and Their Inclusion in a Regulatory Framework for New Plant Varieties Derived From Genome Editing

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Any legal regulation has to take into account fundamental interests and concerns, whether of private or public nature. This applies in particular to the politically and socially sensitive question of regulating plant biotechnology. With the advent of new breeding techniques, such as genome editing, new challenges are arising for legislators around the world. However, in coping with them not only the technical particularities of the new breeding techniques must be taken into account but also the diverse and sometimes conflicting interests of the various stakeholders. In order to be able to draft a suitable regulatory regime for these new techniques, the different interests and concerns at play are identified. Subsequently, a determination is made on how these interests relate to each other, before regulatory concepts to reconcile the conflicting demands are presented. The examined normative criteria, which can have an impact on regulatory decisions regarding genome edited plants and products derived from them, include: industry interests, farmer interests, public opinion, consumer rights and interests, human health and food safety, food security, environmental protection, consistency, and coherence of the regulatory framework and ethical or religious convictions. Since those interests differ from country to country depending on the respective political, economic, and social circumstances, the respective legislator has the task of identifying these normative criteria and must find a suitable balance between them. To this end, a concept is developed on how the different interests can be related to each other and how to deal with conflicting and irreconcilable demands. Additionally, a legislator may have recourse to a number of further analyzed regulatory measures. An approval or notification procedure can be used for a risk assessment or a socio-economic evaluation. Coexistence measures and labeling provisions are able to reconcile interests that are at odds with each other and the precautionary principle can justify certain safeguard measures. As a result, the individual country-specific regulatory outcomes regarding genome edited plants are likely to be as manifold as the interests and regulatory measures at hand.

Keywords: genome editing, regulation, genetically modified organism (GMO), new breeding techniques (NBTs), CRISPR, genome edited plants, stakeholder interests

INTRODUCTION

A crucial function of the rulemaking process and its end result is the reconciliation of various interests. Only a rule that balances conflicting views and concerns is perceived as fair and just. The perceptibility of such an intrinsic fairness is a corner stone of many regulatory efforts, since the effectiveness of norms and regulations depends in part on their societal acceptance (Davis et al., 1978, p. 75; Allott, 1981, p. 229, 235). However, different concerns are not only taken into account by lawmakers to ensure a just legislation, but also to respond to external demands of their constituency. Especially the rulemaking process of democratic societies is exposed to external influences through lobbying, pressure groups or public opinion (Friedman, 1977, p. 59–60; Kau and Rubin, 1981, p. 141; Friedman, 1986, p.771).

This applies likewise to the highly controversial matter of regulating activities relating to genetically modified organisms. The hardly reconcilable positions of environmental activists and industry lobbyists often resulted in a burdensome legislative process or a de facto stalemate as witnessed in the European Union (Dederer, 2016b, p. 147–50; Davison and Ammann, 2017, p. 13–14). With the advent of new breeding techniques, the question how to regulate biotechnology in a prudent manner arises once again.

To be able to formulate a suitable regulatory regime for these new techniques, it is decisive to identify the various interests and concerns at hand. Subsequently, a determination must be made as to how these interests relate to each other, before regulatory concepts to reconcile the conflicting demands can be applied.

GENOME EDITING AND NEW BREEDING TECHNIQUES

The development and adoption of high-yielded crop varieties, together with the use of agro-chemicals and new methods of cultivation in the 1960s marked the beginning of a new era in agriculture (Farmer, 1986, p. 175–76). Although not undisputed (Shiva, 1991; Tilman, 1998, p. 211–12), this so called "Green Revolution" led to a large increase in productivity, a decline in food prices and an improvement of human welfare in the following decades (Evenson and Gollin, 2003, p. 759–61; Kush, 2005, p. 1).

Against the backdrop of new agricultural challenges in the form of extreme weather events (droughts, floods, heavy rainfall, and storms), decreasing soil fertility, and increasing resistance formation in plant pests, there is an ever-growing call for a "Second Green Revolution" (Wollenweber et al., 2005, p. 337; Lynch, 2007, p. 493–95; Davies et al., 2011; McAllister et al., 2012,p. 1011).

The aim of this envisaged agricultural revolution is the development of plant varieties that are able to counter these adverse effects. With the advent of so-called new breeding technologies (NBTs), a solution to these problems seems now within reach.

NBTs is a collective term for different newly developed plant breeding techniques which allow a faster and more precise development of new plant varieties (Lusser et al., 2011, p. 23–27; European Food Safety Authority, 2012c, p. 6–12). These new methods have all in common that some kind of artificially induced genetic alteration is involved in the creation of a new crop variety.

The most promising of these techniques is the so-called genome editing with engineered site-directed nucleases (SDNs). This method makes it possible to target a specific position in a genome and change the DNA at that position precisely in the way intended. Together with an ever-growing understanding of genetics and a better knowledge of the genes that are responsible for expressing a certain trait, genome editing is a powerful tool for the development of new plant varieties. Four different types of engineered nucleases are currently available: meganucleases, zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and the clustered regularly interspaced short palindromic repeats system (CRISPR/Cas). The newest and since 2012 (Peng et al., 2016, p. 1219) rapidly adopted representative of that subsection of NBTs is the CRISPR/Cas-method. Since it is even easier to handle, less expensive and has more potential than its predecessors, it is at the center of attention when it comes to new developments in plant biotechnology (Kole et al., 2015, p. 10; Travis, 2015, p. 1456; Kamthan et al., 2016, p. 1647–49; Georges and Ray, 2017, p. 2).

In contrast to traditional genetic engineering, genome editing is way faster, more cost efficient and precise which allows for new areas of application (Abdallah et al., 2015, p. 195–97; Osakabe and Osakabe, 2015, p. 395–97; Wolt et al., 2016, p. 511–12). However, these new possibilities are also associated with newly emerging and partly conflicting interests.

DEMAND FOR A REGULATORY OVERHAUL

Before discussing those interests that influence legislative action, it is necessary to clarify why new regulatory issues arise when it comes to genome editing.

The need for a new regulatory framework is usually justified by a comparison of plants derived from traditional genetic engineering or conventional mutagenesis techniques and those derived from genome editing. The traditional recombinant DNA (rDNA) technology makes it possible for a plant breeder to introduce genes from any living organism into a plant, irrespective of their sexual compatibility (Academy of Science of South Africa, 2017, p. 29). The gene is incorporated at a random position into the genome of the organism without any *ex ante* control over the effect this insertion may have. The result is a new transgenic plant variety, which could not have evolved naturally. Conventional mutagenesis via radiation or chemical mutagen causes random undirected mutations in the genome. This leads to a plant that does not cross species boundaries and, at least theoretically, could have evolved naturally as well. Genome editing, on the other hand, enables the plant breeder to cause site-specific genetic changes that are—like mutations caused by conventional mutagenesis—indistinguishable from naturally occurring alterations in plant DNA. Since these changes could

occur in nature or via conventional mutagenesis as well, it is argued that such genetic changes should be subject to a different regulation than transgenic plants.

The difference between traditional genetic engineering and genome editing is, however, not as clear-cut as it seems at first glance. More precisely, the genome editing technique can be used to cause mutations (small insertion or deletion), gene replacement, gene insertion, and site-directed deletions, or inversions (Curtin et al., 2012, p. 42–44). Regarding genome editing using SDNs, a distinction is made between three application methods (European Food Safety Authority, 2012b; Lusser et al., 2012, p. 232; Sprink et al., 2016, p. 1497; Wolt et al., 2016, p. 514; Voigt and Klima, 2017, p. 321): SDN-1, SDN-2, and SDN-3. SDN-1 applications cause a double-strand break without the addition of a repair template. Consequently, the break is repaired solely by the plant's own repair mechanism resulting in a mutation. In the case of SDN-2, a small repair-DNA-template is introduced together with the nuclease to create a site-specific predefined mutation. The cell's repair mechanism uses that template to repair the double-strand break by copying the genetic information of the template into the plant cell. The result is a mutation at the locus of the double-strand break in accordance with the provided template. SDN-3 is used to insert new genetic material into the plant cell. To this end, apart from the double-strand break a larger stretch of donor DNA is introduced into the cell and the plant's natural repair mechanisms incorporates the donor DNA at the locus of the double-strand break.

While plants derived from SDN-1 and SDN-2 are indistinguishable from their conventionally bred counterparts (Lusser et al., 2011, p. 69, 2012, p. 237; Schenkel and Leggewie, 2015, p. 265; Sprink et al., 2016, p. 1497; Townson, 2017, p. 11; Voigt and Klima, 2017, p. 321), SDN-3 can lead to transgenic plants, depending on its specific nature of application. At the same time, the techniques of traditional genetic engineering can also be used for the development of plants which do not cross species boundaries (i.e., cisgenesis) (Holme et al., 2013, p. 395–97; Ribarits et al., 2014, p. 184; Jogdand et al., 2017, p. 691–92). Therefore, the difference between traditional genetic engineering and genome editing is not that the one method creates transgenic plants while the other leads to non-transgenic varieties. Genome editing differs from traditional genetic engineering techniques mainly in its more precise, targeted and less burdensome application and its ability to overcome some of the limitations of traditional genetic engineering (Kamthan et al., 2016, p. 1647–49).

Consequently, the question of whether non-transgenic plants should be excluded from the strict regulation of genetic engineering existed already before the advent of genome editing (Schouten et al., 2006; Conner et al., 2007, 351; Rommens et al., 2007, p. 402; Jacobsen and Schouten, 2008; Waltz, 2011, p. 677; European Food Safety Authority, 2012a). Therefore, genome editing does not only raise exclusively new regulatory questions, but is also used to put regulatory issues, which have existed before, on the agenda again.

This view is confirmed by the fact that the term NBTs seems to be used in some cases to avoid expressions like genetic engineering or genetically modified. The wording "new breeding techniques" gives the impression that it describes methods sui generis with completely distinct regulatory demands. In this way, the pressure on the legislature to take action can be increased without being associated directly with the controversial matter of genetic engineering.

Notwithstanding the fact that the regulatory questions are not entirely new, compared to traditional genetic engineering and conventional mutagenesis, genome editing has special characteristics, which must be considered.

Due to the possibility of specifically targeting a certain gene sequence, unwanted side effects are far less likely. Genome editing may cause so called off-target effects but it is still more precise than the random insertion of genes by traditional genetic engineering (Vogel, 2012, p. 60) and causes far less unwanted changes than conventional mutagenesis (Kahrmann et al., 2017, p. 177). Additionally, over the past years researchers have managed to limit off-target effects associated with CRISPR/Cas9 (Cho et al., 2014, p. 137–38; Peng et al., 2016, p. 1227) or are able to use the underlying mechanism to target multiple sites at once (Hyams et al., 2018, p. 2184).

Moreover, genome editing is frequently only used for minor changes in the genome instead of the insertion of large DNA segments or the generation of numerous random mutations. These factors can have an impact not only on the risk assessment, but also on the applicability of the existing regulations. Therefore, legislators worldwide are asked to take those special attributes of genome editing techniques into account and to give them a suitable legal framework.

NORMATIVE CRITERIA

However, a legislative effort will most likely take into account not only the technical specifics of genome editing, but also the various interests at hand.

This includes (1) industry interests, (2) farmer interests, (3) public opinion, (4) consumer rights and interests, (5) human health and food safety, (6) food security, (7) environmental protection, (8) consistency and coherence of the regulatory framework, and (9) ethical and religious convictions.

The following analysis has the purpose to show how these interests are able to affect legislation in manifold and substantive ways and in what way they assume the status of normative criteria for the legislative undertaking of regulating plants derived from genome editing.

Industry Interests

Biotech Industry

Due to lobbying, economic considerations, and political self-interest, national legislation is usually prone to take into account the demands of the domestic industry. Therefore, the kind of expectations companies invested in biotechnology bear, can have a considerable effect on regulatory decisions.

Legislative and political support for marketing

Historically this interdependency between industry interest and political action can be witnessed by comparing the

approach to genetic engineering in the USA and the EU since the 1970s. The far more extensive public spending on life science in the US compared to the EU encouraged the development of an innovative biotechnology sector in the US. At the same time, stricter rules on the use of pesticides were imposed in the EU and the US since the 1970s. While European companies tried to keep their competitive edge in agrochemicals by developing environmentally friendlier pesticides, the US biotechnology firms tried to meet the higher regulatory requirements by developing new plant varieties (Graff and Zilberman, 2007, p. 245). As a result, American companies have been engaged in biotechnology research from early on and therefore have dominated the development and commercialization of agricultural biotechnology since the beginning (Pan, 2002, p. 230; Owen, 2017, p. 19). European companies, however, whose focus was still on traditional agrochemicals, increasingly fell behind in this area.

As a consequence, European agrochemical companies potentially had an interest in slowing down the adoption of biotechnology, while their American competitors were trying to facilitate its breakthrough (Graff and Zilberman, 2007, p. 245–56; Zilberman et al., 2013, p. 202–03; Graff et al., 2015, p. 681–82). Since the political influence of industry stakeholders is the strongest in their respective home countries, the American biotechnology companies were able to influence the US legislation in their favor while a negative stance was able to become solidified in Europe (Graff and Zilberman, 2004, p. 2–3; Zilberman et al., 2013, p. 206). However, it would be an oversimplification to attribute the EU's (in)action to the lack of industry intervention only. In holding back the adoption of genetic engineering, the EU adopted an effective strategy to protect the competitiveness of the domestic agrochemical sector. In this way, the US biotechnology companies were not only blocked from access to the European market, but also the global adoption of biotechnology was slowed down considerably. Since genetically-modified agricultural products can only be imported into the EU if they have been subject to the approval procedure, the EU's de facto moratorium on GMOs has resulted in a restrained use of genetically modified plants in exporting countries (Pollack and Shaffer, 2009, p. 296; Laursen, 2013, p. 579; Adenle et al., 2017, p. 249–50).

As a consequence, the legislative attitude toward the adoption of NBTs will depend significantly on how much skin in the game the respective domestic industry has. At this early stage of research and development, it is difficult to make reliable predictions in that regard. However, there are first indications that the commitment of the scientific community and the biotech-industry is not as one-sided as it used to be concerning traditional genetic engineering. Figures from the year 2010 (Lusser et al., 2012, p. 233) show that 44% of the publications on NBTs were published by researchers from the EU whereas only 32% could be assigned to North America. This could lead to a shift in European policy toward a more embracing attitude when it comes to genome editing.

Protection of intellectual property rights

To work profitably, biotech companies must generate a steady revenue stream by selling their genetically modified plants. To prevent farmers from paying only once for the seeds by reusing their last crop, developers depend on the protection of their new plant varieties by intellectual property laws or a similar protective mechanism.

The likelihood of lawmakers accepting new plant varieties as intellectual property or protecting it in a comparable way depends mainly on the economic interest the respective country has in having access to such biotech products. As a result of past experience (Bronstein, 2016; Monsanto, 2016; Reuters, 2016), it is to be expected that producers will withhold new products from national markets as long as their effective protection is not ensured. Therefore, as long as the dependency of the domestic agricultural sector is significant enough, the biotech industry will be able to shape the regulatory framework in their interest.

In addition to the mere existence of an effective protection mechanism, the biotech industry needs to be able to determine if, where and by whom its products are used to collect the royalties. Since it is possible to create plants by means of genome editing which are indistinguishable of naturally mutated plants there are additional obstacles to the proof of origin.

This endeavor is less burdensome in legal orders that allow for a prima facie evidence. Even though it is possible that exactly the same mutation caused by genome editing also occurs naturally, it is, however, highly unlikely and utterly implausible on a large-scale. In that case, the farmers would bear the burden of proof and would have to show that the genetic alteration in their harvest originated from a natural process—an evidence that can de facto not be provided.

If such a prima facie evidence is not allowed and lawmakers cannot be pressed to adopt an amendment in that regard, an identity preservation system (IPS) could serve the industry interest as well. Since this would coincide with the interest of organic farmers, an IPS could turn out as a mutually agreeable solution.

Whether an IPS is actually in the interest of biotech producers, depends, however, on who bears the costs and how GM contamination is treated under national legislation.

In Germany, for example, GM farmers have to compensate their conventional or organic counterparts if a contamination of their harvest with GMOs makes it illegal to place their products on the market, requires to label the products as containing GM, or prevents them from using a certain label (e.g., "GM-free") (Kohler, 2005, p. 566; Dederer, 2007, 2016a p. 222, 121). In that case, the biotech industry might have a certain interest in the existence of an IPS since otherwise the liability risk is likely to deter farmers from adopting GM technology. However, if biotech farmers have to bear the costs of an IPS alone, the deterrent effect would be mostly the same.

Streamlined approval or notification procedure

The costs caused by regulatory requirements or delays (Kalaitzandonakes et al., 2007, p. 509–10; Smyth et al., 2016, p. 185–87) can have a detrimental effect on the company profit and discourages new investments in the development of

biotechnology innovations. However, it should be noted that an effective approval procedure is not just a private but a public interest as well. On the one hand, a time-saving procedure attracts investment in the domestic economy. On the other hand, public sector institutions are also engaged in the development of new crop varieties, especially in developing countries (Cohen, 2005, p. 32). Since they depend on public funding, high approval costs might cripple their efforts to provide a public good (Smyth et al., 2016, p. 188).

Therefore, it stands to reason that the biotech industry would welcome it if genome-edited plants fell outside the scope of a strict approval or notification procedure. However, the current market leaders might have a strong self-interest in a costly and burdensome approval procedure. This shields their market share from new competition and discourages smaller but innovative competitors to invest in research and development (Miller, 1997, p. 184). At the same time, the big biotech-companies have the sufficient cash flow, human resources, and past experience to work the system.

In any case, a streamlined regulatory framework has to be balanced against public safety and environmental issues (see below).

Organic Food Industry

The organic food industry not only positions itself as an environmentally friendly alternative to genetic engineering, but also actively combats the adoption of genetically modified plants (Apel, 2010, p. 636). From a purely economic point of view, that approach seems rather non-sensical since the delimitation to genetic engineering and conventional agriculture is an important selling point of organic farming. The abolition of GM plants would deprive organic farming of one of its most prominent distinguishing features.

The lobbying against genetic engineering can be partly explained as an expression of an agricultural idealism and the deeply rooted conviction that tempering with nature is inherently harmful.

However, it should not be left ignored that the opposition to GM techniques is also a very effective—though possibly unintended—marketing strategy. By establishing genetic engineering as an unmanageable risk to human health and the environment, a moral incentive to buy organic products is created. This is reinforced by consumers' fear of the negative consequences of the consumption of GM products. At the same time, the biotech industry as a common enemy serves as a catalyst to create a social movement with the aim to change the future of agriculture. The organic food industry managed to be recognized as the spearhead of that movement providing everyone with the opportunity to rally behind its cause. This way the production and consumption of organic food is not a mere economic process but also part of a political agenda.

In addition to this political motivation, the organic food industry has also a purely economic interest in hampering the widespread adoption of GM plants. The industry relies on the price premium consumers are willing to pay for organic food. A large-scale use of GM varieties would most likely lead to falling prices for non-organic agricultural products (Moschini et al., 2000, p. 48; Qaim and Traxler, 2005, p. 82; Brookes et al., 2010, p. 31–32). As a consequence, the gap between organic and non-organic products would widen. Surveys indicate, however, that consumers are willing to buy GM products when they are offered a significant discount (Lusk et al., 2005, p. 40; Knight et al., 2007, p. 508; Aerni et al., 2011, p. 835). A larger gap between consumer prices of organic and non-organic agricultural products could therefore significantly affect the market share of the organic food industry in a negative manner.

On the basis of these economic and political interests, it can be assumed that the organic food industry will take a negative stand in respect of genome editing and actively lobbying for a strict regulatory framework. The predominantly condemning policy statements regarding genome editing by non-governmental environmental organizations (GMWatch, 2014; Greenpeace, 2015; IFOAM-Organics International eV, 2015; GM. Freeze, 2016; Paul et al., 2017) are the first indicator for this development.

The impact those efforts will have on the law-making process will most likely depend on the degree of correlation between the interest of the organic food industry and public opinion or in other words on the level of correlation which can be suggested to policy-makers. Since legislators have an incentive to act in accordance with the opinion of their constituency (Denzau and Munger, 1986, p. 102), it can be assumed that interest groups are most effective when their policy aim is consistent with public opinion. However, to benefit from this nexus, it should be sufficient for interest groups to make the legislators believe that such a correlation exists.

Farmer Interests

If the new breeding technologies can live up to their promise to increase yield while reducing the nutritional and climatic demands of plants, from the farmers' point of view, everything suggests a large-scale application of the new plant varieties.

This assumption is backed historically by the adoption of the previous generations of genetically engineered plants. Due to the increase in yield, the declined expenses for pesticides and the time-saving manner of application, the farmer's profit increased significantly—even if higher seed prices are taken into account (Qaim, 2009, p. 672; Smale et al., 2009, p. 11–32; Areal et al., 2013, p. 18–27; Carpenter, 2013, p. 251; Brookes and Barfoot, 2016). At the same time, a delayed adoption caused significant foregone income benefits (Kalaitzandonakes et al., 2016, p. 228 with further references).

There are still critical voices that doubt the economic value of genetically modified plants in agriculture (Greenpeace, 2008; Friends of the Earth, 2018). Those critics, however, find it difficult to explain why in countries, where farmers have the free choice between conventional and GM varieties, the adoption rate of the latter supersedes the former by a vast margin (Lucht, 2015, p. 4255). This contradiction could be explained only with the unreasonable assumption that the farmers are fundamentally inclined to act against their own economic interests.

External factors, on the other hand, can undermine those positive economic effects of genome edited new plant varieties.

The premium "GM-free" products are able to obtain in some national markets (Goodwin et al., 2015, p. 25) distorts the economic performance of conventional and GM varieties to a certain degree. Therefore, the group of farmers that benefits from this price premium has an incentive to refrain from adoption of genetically modified plants from a purely economic point of view. Since the farmers are only able to charge the premium if the unintended presence of GMOs can be prevented effectively, they have a strong interest that an identity preservation system is in place to assure the coexistence of GM and GM-free agriculture. To this end, a minimum distance between the different cultivation areas, separate processing facilities or a traceability system can be used to preserve the producers' freedom of choice. However, it must be noted that measures of coexistence result more often than not in a marginalization of genetically altered plant varieties since the rules securing coexistence are usually biased in favor of traditional agriculture. For example, due to possible liability risks, large distance space and a costly traceability system, GMOs are de facto prevented from having a significant share in acreage in Japan and the EU (before the cultivation was banned in many member states) (Varela, 2010, p. 353; Sato, 2015, p. 15–16).

Moreover, not all farmers act solely out of economic interests. Farmers who are not only guided in their activities by economic considerations, but also by their ethical, political or environmental convictions might be inclined to refrain from the adoption of genetically modified plant varieties.

Besides that, (especially European) farmers have an additional incentive to oppose a permissive regulatory framework regarding genome-edited crops. Since a ban, moratorium, or mere regulatory obstacles have the effect of a non-tariff barrier to trade, it is a potent method to shield the domestic market from international competition (Grossman, 2010, p. 125; Graff et al., 2015, p. 682; Phillipson and Smyth, 2016, p. 204).

In case the national agroindustry depends heavily on the export of agricultural products, farmers must also take into account their sales opportunities with their trading partners. If the regulatory framework of their main trading partner does not allow the importation of a certain genetically modified crop variety, farmers have no interest in this plant variety.

Summing up, the degree of interest farmers have to adopt new genome edited plant varieties depends on the economic viability of cultivating such plants. However, the economic benefits cannot be determined solely by comparing the agricultural performance of conventional with genome edited plant varieties. The individual country-specific external factors and the personal convictions of the farmers must be considered as well to draw a convincing conclusion regarding the farmers' interests.

Public Opinion

In order to assess the role public opinion plays when it comes to the formulation of a regulatory framework for genome editing, it is decisive to understand the effect and impact public opinion has on public policy in general.

While it is mainly undisputed that public opinion can have an impact on the legislative process (Monroe, 1983, p. 38–39; Page and Shapiro, 1983, p. 175 with further references; Block, 1987, p. 65; Korpi, 1989, p. 323; Hill and Hinton-Anderson, 1995, p. 924 with further references; Stimson et al., 1995, p. 544; Smith, 1999, p. 860; Dahl, 2006, p. 131–32; Domhoff, 2014, p. 130–31), it is, however, unclear how strong its influence can be.

On the one hand, this depends on the respective political system. It is fair to say that the responsiveness to public opinion is pronounced in democracies (Dahl, 1971, p. 1). Regular elections, freedom of expression and an independent press allows a more direct interaction between the public will and the policy-making process. However, this does not mean that other political systems are completely lacking in dependence on the people's will. Even though dictatorships or authoritarian regimes can take less consideration of the public opinion (Peden, 1984, p. 360), they are not completely independent of it (Mueller, 1999, p. 139; Ojieh, 2015, p. 46–47). This circumstance is based on the fact that a lack of support in the society can be substituted only to a certain degree by the use of compulsory powers.

On the other hand, the way in which public opinion is articulated has a tremendous effect on its level of efficiency. Concentrated minority interests tend to have greater political influence than dispersed majority interests (Olson, 2002, p. 36). This could lead, for example, to a marginalization of the public interest by a contradictory but concentrated industry interest. In such a case, however, it should not be easily assumed that politicians have a reasonable incentive to act against public opinion. It seems more likely that they are simply unaware of the disparity between common and industry interest (Lohmann, 1993, p. 320; Burstein, 2003, p. 31). This danger is, however, mitigated by the fact that there is an increasing number of lobby groups representing consumer interests in a concentrated manner.

Moreover, the issue salience plays a central role for the degree of governmental responsiveness (Haider-Markel, 1999, p. 120). Since issues with a high salience are more likely to be taken into account by the voters on election day (RePass, 1971, p. 400; Jones, 1994, p. 14; Bélanger and Meguid, 2008, p. 479; McGrane et al., 2013, p. 5), politicians are more receptive to the public opinion on those matters. Nevertheless, public opinion on issues with a low salience is unlikely to be ignored completely due to the possibility that the emphasis shifts in the future (Burstein, 2003, p. 30).

Since genome editing is a fairly new technology, a nuanced public opinion on it has not yet emerged. However, it seems to be questionable if there will ever be a public opinion that differentiates between genome editing and traditional genetic engineering. The differences between the various methods of genetic engineering are of such an academic and technical nature that a differentiation by the public cannot reasonably be expected. It is also not more promising to ask for the position on transgenic and non-transgenic genetic modification, as this does not distinguish traditional genetic engineering and genome editing (cf. above). Therefore, it seems safe to assume that the existing public opinion on genetic engineering is going to find its continuation in relation to genome editing (similar Ishii and Araki, 2016, p. 1508).

Since there are significant regional differences concerning the public attitude toward the adoption of genetic engineering, the impact of public opinion on the regulation of genome

editing has to be assessed by a country-specific case-by-case approach.

In general it can be stated that the public opinion on genetically modified food is more positive in developing countries than in the developed world (Li et al., 2002, p. 148; Curtis et al., 2004, p. 70; Pachico and Wolf, 2004, p. 159; Powell, 2013, p. 198 with further references). Looking closer at developed nations perceptions of GMOs are more favorable in North America than in Europe or Japan (Moon and Balasubramanian, 2001, p. 223; Lusk et al., 2005, p. 37; Lusk et al., 2006, p. 10; Vecchione et al., 2015, p. 330).

The existing surveys on consumers' attitude should be treated with caution, though. Due to the social stigma of GM products—especially in Europe—the adverse answers given in questionnaires can deviate significantly from the actual, more accepting behavior of consumers (Mather et al., 2011, p. 506; Desaint and Varbanova, 2013, p. 185; Sleenhoff and Osseweijer, 2013, p. 169–70).

In the end, the impact of public opinion on national legislation will depend mainly on the responsiveness of the political system, the issue salience, concentrated actions of like-minded interest groups and the lack of opposition from opposing societal or industry forces.

Consumer Rights and Interests

When it comes to genetically altered products the interests and rights of the end-consumers also plays a significant role when tailoring a suitable regulatory framework.

One might assume that consumers have the right to have access to conventional and organic as well as to genetically modified food. However, it seems difficult to argue why there should be a legal right to have access to certain product categories. As long as there are no health concerns at play, this is rather a luxury than a necessity and therefore unlikely to be guaranteed by law. Nevertheless, even if there might be no right, there is certainly an interest of consumers in having access to organically or conventionally produced food next to genetically modified ones.

Additionally, there is a widespread assumption (Gruère et al., 2008, p. 1473)—sometimes even presented as fact—that consumers have the right to know if a product contains genetically modified material. A closer examination reveals, however, that while there is a consumer right to know in the EU [*Treaty on the Functioning of the European Union (TFEU)*, Art 169 (1)], other countries are much more reluctant to grant such a right with regard to labeling provisions (Keane, 2006, p. 292–93; Federici, 2010, p. 517).

However, even if there is no consumer right to information, a prevalent and substantial consumer interest in labeling might pressure legislatures to introduce corresponding laws. Against this backdrop, it can already be questioned whether the majority of consumers really wants to know if a product contains genetically altered material. Surveys show that consumers asked, if genetically modified ingredients should be labeled, are strongly in favor of such an obligation (The Mellman Group, 2012; Wunderlich and Gatto, 2015, p. 848; Committee on Genetically Engineered Crops Board on Agriculture Natural Resources Division on Earth Life Studies National Academies of Sciences Engineering Medicine, 2016, p. 303–04). However, the answers given to such a question should be treated with caution due to the inherent bias of that inquiry. When asked whether something should be labeled with regard to food, it is already implied that this information might be of significance for the consumer. It is also not plausible why a consumer would not want to know more about a product he is about to buy. It is therefore likely that a consumer will answer a question concerning the desire for further information in the affirmative, regardless of the content of that information. In a European consumer survey only 54.1% of respondents stated that they always read (or have previously read) the label before deciding to buy a particular food item (Sleenhoff and Osseweijer, 2013, p. 168). This is an indicator that consumers have a far lesser interest in proper food labeling in an actual shopping situation than anticipated. This is confirmed by the fact that consumers in countries, where a negative attitude toward genetically altered products prevails, are willing to buy genetically modified food products as long as they receive a price discount (Moses and Fischer, 2014, p. 67; Lucht, 2015, p. 4258–59). And even if consumers say that they do not buy genetically modified food, they often purchase them regardless (Sleenhoff and Osseweijer, 2013, p. 169). There is therefore a considerable discrepancy between the articulated and actually practiced interests of consumers with regard to the labeling of genetically modified products.

At the core of the interest of many consumers is, furthermore, the ability to purchase high quality products at low prices. The anticipated beneficial impact of genome editing on the nutritional value of food (Abdallah et al., 2015, p. 185; Khatodia et al., 2016, p. 9; Jiang et al., 2017; Karkute et al., 2017, p. 4; Lima et al., 2017, p. 238) combined with the expected market price drop (Voytas and Gao, 2014, p. 4–5; van Erp et al., 2015, p. 87) suggests that the adoption of products derived from genome edited plants would meet the consumer interest in that regard.

Additional indirect consumer interests may also result from considerations concerning health, food safety, food security, the environment, and ethical convictions (see below).

Human Health and Food Safety

Decisive for the regulation of genome edited organisms (GEOs) are their implications for food safety and human health, since safety considerations are ordinarily the cornerstone of the regulatory efforts.

An assessment of these implications can be based on the potential toxicity, allergenicity, nutritional effects, and any unintended effects which could result from the genetic modification (World Health Organiziation, 2005, p. 12). It is, however, more often than not unclear which effects a GEO might have from an ex ante perspective. Therefore, an abstract regulation is only able to manage the general risk potential.

Against this backdrop, potential health risks of GEOs can be divided into four categories: the known knowns, the known unknowns, the unknown unknowns and the unknown knowns (For the origin of these general risk categories see U. S. Department of Defence, 2002; ŽiŽek, 2004; Daase and Kessler, 2016).

"Known knowns" means already clearly identified risks and certain knowledge of specific consequences of genome editing. This refers to such consequences that are already well-understood, like the fact that no different potential adverse effects can be attributed to plants bred via SDN-1/2 compared to plants resulting from conventional mutagenesis since the same genetic alterations can occur by means of both techniques. Furthermore, the lack of traceability/identifiability due to indistinguishability of certain GEOs from naturally occurring or conventionally induced genetic alterations can be mentioned in that context (Ribarits et al., 2014, p. 185–86).

"Known unknowns" describes the situation in which, although one is aware of the possibility of a risk, one does not know about the actual risk itself. This category includes, for example, off-target effects. In advance one does not know where they occur or what effect they might have, but it is clear that they can occur—even though off-target effects using genome editing are less likely compared to traditional techniques of genetic modification and conventional mutagenesis (cf. above). A further example would be the unauthorized use of genome editing with the help of so called "CRISPR home kits" (Sample, 2016) or an unauthorized form of application by using edited viruses and bacteria as biological weapon in a terrorist attack (Rodriguez, 2017, p. 4). Possible adverse long-term effects of artificial genetic modifications can be attributed to this category as well. With regard to genome editing the fairly unpredictable long-term effects of so called gene drives are just one example (Champer et al., 2016, p. 156–57; Chneiweiss et al., 2017, p. 712).

The "unknown unknowns" refer to those risks one does not even know if they exist. By nature of this risk category, it is not possible to give an example for such an unknown unknown. That is why it seems doubtful if an unknown unknown can be regulated at all—even if considering a maximal precautionary approach. With regard to GEOs a protection of unknown unknowns can only be guaranteed by refraining from the use of GEOs entirely. However, this could lead to the manifestation of a different unknown unknown arising from exactly that non-use of the technology. Consequently, an inclusion of unknown unknowns in a legislative effort seems not feasible.

The term "unknown knowns" applies to those risks that one is unaware of, although one actually knows or at least could know them. Since genetic modification is an extremely risk sensitive and risk aware area, an example for this category cannot be identified. It is worth considering, however, whether "unknown knowns" could be interpreted in a different way. Instead as suppressed risk, it seems more appropriate to read "unknown knowns" here as perceived risk even though its very existence has been scientifically disproven. This applies, for instance, to the often denied, but scientifically proved, lack of a specific risk inherent to genetic engineering as such (Dederer, 1998, p. 32–49).

Apart from the risk potential, GEOs can also have a beneficial impact on human health.

For instance, an improvement of the nutritional value of crops is frequently associated with genome editing (Abdallah et al., 2015, p. 185; Khatodia et al., 2016, p. 9; Jiang et al., 2017; Karkute et al., 2017, p. 4; Lima et al., 2017, p. 238). This is of special importance to developing countries since the population is often relying only on a single staple food—especially cereals—to meet their nutritional needs (Christou and Twyman, 2004, p. 35; Bouis, 2007, p. 79). However, the nutritional value of food is of lesser concern in countries where the population has access to a wide variety of food (Key et al., 2008, p. 292).

Positive effects on human health can also be the indirect result of beneficial impacts on food security and the environment (cf. section Food Security and Environmental Protection).

With regard to the legislative impact of effects on human health and food safety, it can be presumed that in developing countries the benefits are more likely to be considered as out-weighing potential risks, while developed countries might be more risk sensitive.

Food Security

"Food security exists when all people, at all times, have physical and economic access to sufficient, safe and nutritious food to meet their dietary needs and food preferences for an active and healthy life" (World Food summit, 1996, Para.1). The global food security is increasingly under pressure due to an ever-growing world population (United Nations Department of Economic Social Affairs, 2017, p. 1), scarcity of arable land, the adverse effects of climate change (Mendelsohn and Dinar, 1999, p. 278; Olesen and Bindi, 2002, p. 246; Schmidhuber and Tubiello, 2007, p. 19703–04; Lobell et al., 2008), a higher per capita consumption (Godfray et al., 2010, p. 812), the vulnerability of monocultures (Altieri and Nicholls, 2004, p. 172; Georges and Ray, 2017, p. 5), and the formation of resistances in plant pests (Tabashnik, 1994, p. 47; Beckie, 2011, p. 1039; Tabashnik et al., 2013).

As a result of the population growth it is estimated that global agricultural production has to double until 2050 (Ray et al., 2013, p. 1). However, current rates of yield increase are not sufficient to meet this goal (Ray et al., 2013, p. 2). It is anticipated that the genome editing technique could close this gap due to its inexpensive, more precise, efficient and less time consuming nature of application (Ma et al., 2017). Against this backdrop, genome editing has shown promise for a more efficient disease control through a targeted mutation of specific disease-resistance genes (Georges and Ray, 2017, p. 5–6). With regard to climate change, it is expected that genome editing could lead to new cold, heat, or drought resistant crops varieties (Khatodia et al., 2016, p. 9; Scheben et al., 2016, p. 7). At the same time, genome editing can be used to increase the nutritional value of a plant product or knockout genes responsible for the production of anti-nutrients or allergens (Kamthan et al., 2016, p. 1649).

The presumed beneficial impact of genome edited crops on food security is more likely to lead to an embracing regulatory approach in those countries which already have to deal with malnutrition or are going to be adversely affected by climate change. Especially developing countries are often afflicted by both (Lobell et al., 2008), whereas Europe and the US might overall benefit from climate change from a purely agricultural perspective (Olesen and Bindi, 2002, p. 257; Reilly et al., 2003, p. 65). However, security interests regarding the countries affected by malnutrition and growing migratory pressure could also convince industrialized countries to rethink their attitude toward genetically modified crops. Since an agricultural surplus

produced in industrialized countries would decrease the world market price, food, and feed would become more accessible to those struggling countries. This improved food supply could in turn lead to the desired stabilization and strengthening of destabilized regions.

Environmental Protection

After decades of widespread environmental pollution and degradation, regulators and the public became more and more sensitive toward environmental protection issues. By now, environmental impact assessments and protective measures are a cornerstone of many regulatory endeavors. Any regulation of GEOs is therefore likely to include environmental considerations as well.

Potential risks for the environment include unintended effects on (non-)target organisms, the ecosystem, or biodiversity (Secretariat of the FAO/WHO Global Forum of Food Safety Regulators, 2005, p. 202). This includes among others off-target effects, the displacement of wild species by their stronger genome edited counterparts and unforeseen consequences of a gene-drive (Rodriguez, 2017, 2). The already established risk categories of known knowns, known unknowns, unknown unknowns and unknown knowns are here applicable as well.

However, the use of genome edited plants might also have a positive impact on the environment. As it has been observed in the case of GMOs (Smyth et al., 2015, p. 25–28), it seems reasonable to assume that the adoption of GEOs could result in less use of fertilizers and pesticides as well.

Furthermore, GEOs might have a positive effect on climate change. Higher yield gains of plants used for bioenergy production in combination with carbon capture and storage could increase the carbon removal rates (Humpenöder et al., 2014, p. 7). In addition, a higher yield could lead to less land use and make reforestation possible or could at least prevent further deforestation.

Consistency and Coherence of the Regulatory Framework

In many societies the principle of the rule of law is deeply rooted in and a cornerstone of their legal system. The rule of law requires that laws comply with certain formal requirements: They should be general in nature, accessible by the public, prospective, coherent, consistent, compliable, and administered orderly (Fuller, 1969, p. 39; Raz, 1979, p. 214–18).

With regard to a regulatory framework for GEOs it is especially the consistency with other legal obligations and the coherence of the regulatory regime as such that might be at odds with the rule of law.

On the one hand, any domestic legislation must be in conformity with the applicable rules of international law. Against this backdrop, obligations originating from World Trade Law (Keane, 2006, p. 314–29; Kahrmann et al., 2017, p. 182) and Free Trade Agreements come to mind. It stands to reason that a different treatment of domestic conventionally bred plants and imported genome edited ones might clash with non-discrimination clauses.

On the other hand, the rule of law requires that the regulatory framework of GEO is coherent with other national laws by the same legislator. This raises the question of whether a different regulation of conventional mutagenesis and mutagenesis via genome editing is compatible with this principle. Since exactly the same outcome can be reproduced theoretically by either technique, it is rather difficult to argue why they should be regulated differently.

Ethical and Religious Convictions

Ethical considerations are often referred to in order to oppose genetic modifications of plants. The main concerns articulated are (1) that humankind should not temper with the natural order (naturalness), (2) that the risk potential of genetic engineering cannot be estimated with sufficient certainty and its application is therefore unjustifiable (uncertainty), (3) the danger of corporate control over the food industry and exploitation of farmers via intellectual property rights, and (4) the failure to live up to the responsibility for further generations (Rollin, 2003, p. 15; Weale, 2010, p. 584–87; Rodriguez, 2017, p. 4).

The naturalness argument (1) is highly contentious (Rollin, 2003, p. 15–16; Weale, 2010, p. 584–85). There is no convincing logic argument why naturalness should be the benchmark for human action or why a natural state should be preferred ethically over an artificial one. Furthermore, it is often not possible to draw a sharp line between a natural and an artificial state (Weale, 2010, p. 584–85). Not even the crossing of species boundaries provides a clear demarcation line since this happens without human intervention as well (Weale, 2010, p. 585) and those boundaries are rather fluid (Rollin, 2003, p. 15; Robert and Baylis, 2005, p. 13–17).

With regard to the uncertainty of the risk potential (2) it seems at least questionable if uncertainty alone gives reason to an ethical imperative not to use genome editing at all. It seems to be more reasonable to demand that the technique is applied in a measured way.

The exploitation of the individual person by corporate or capital supremacy (3) is certainly contrary to generally accepted ethical values. However, agriculture is not more prone to be exposed to exploitation of the individual than any other industrial sector. The particularly pronounced fear of corporate control over the food chain can rather be qualified as an expression of an industry-skepticism instead of an actual ethical conviction.

Furthermore, it is argued that the responsibility for further generations (4) includes the obligation to leave behind a sufficient diversity of species (Rodriguez, 2017, p. 4). In that case, the application of a gene drive, which will eradicate an entire species, might be incompatible with this ethical demand. The same holds true for a release of such an invasive genome edited species that certain wild species become endangered.

On the other hand, there might even be an ethical imperative to use genome editing on plants. Since this is a promising method of combating malnutrition (cf. above), human suffering could be reduced significantly. Furthermore, a sufficient supply with agricultural products fosters peace and social justice within and among societies. It is anticipated that in the near future conflicts over increasingly scarce natural resources like water and arable

land will intensify (United Nations Secretary-General Ban Ki-moon, 2007; Barnaby, 2009; Chellaney, 2013). A more equitable access of the world's population to agricultural products thanks to the adoption of GEOs might help to ease these tensions. A comparable argument can be made with regard to the anticipated mitigating effects of GEOs on climate change.

Religion, on the other hand, is often perceived as being in conflict with and slowing down scientific progress (Russell, 1997, p. 7). A more progressive approach, however, allows to assume that "there can never be a conflict between the broadening of scientific truth and the exercise of religious faith (…) [since] every new discovery reveals more about (…) God" (Grisham, 2012, p. 33) (similar Hathout, 1990, p. 99; Rispler-Chaim, 1998, p. 567; Ratanakul, 2010, p. 139).

From a Christian perspective humanity has a responsibility and a dominion of stewardship for God's creation (Grisham, 2012, p. 36). In a similar way the Qur'an prohibits to change God's creation [Haleem, 2005, p. 62 (4:119)]. Concerning the alteration of plants the mainstream of Islamic and Christian thought adopted the position that genetic engineering does not tamper with God's creation as long as it does not put it at risk and advances human welfare (Rispler-Chaim, 1998, p. 567; Fadel, 2001, p. 904; Conference of European Churches, 2001; Moosa, 2009, p. 142–46; Pope Francis, 2015, Para. 131). The Jewish tradition is even more permissive since it perceives humankind as co-creators with the task to complete God's creation (Green, 2010, p. 125). Therefore, a considerable number of Jewish scholars have no objections in general when it comes to the genetic engineering of animals and plants (Bleich, 2003, p. 67–71 with further references; Wolff, 2005, p. 924–25). Buddhists do not attach any unique or particular value to naturalness (Loy, 2009, p. 184) since they do not believe in a divine creator whose plan could be tempered with (Frazzetto, 2004, p. 554). They are therefore "not inclined to see a man-made creation as something competing with a "good" nature. There is a very positive attitude toward changing nature's course if it enhances the welfare of all living beings, and more so if it allows medical advancements" (Schlieter, 2004). Also in Hinduism there is no religious basis for an outright rejection of genetic modification *per se* (Narayanan, 2009, p. 175). On the contrary, Hindus are open-minded with regard to scientific advances and untroubled by the idea of tempering with a divine creation (Narayanan, 2009, p. 175–76). Even where genetically modified food could be in conflict with certain Hindu dietary rules, this can be neglected as long as there is a health benefit (Narayanan, 2009, p. 175).

In the end, the (mainstream) religious postulations are not at all that different from the already outlined secular factors: Human health, food security, and matters of environmental protection are to be taken into account by a regulatory framework for genome edited plants.

However, the existing opinions with respect to genetic engineering are in religious communities as diverse as in secular ones. Therefore, examples of strong religious opposition against genetic engineering of any kind can be found around the world (Epstein, 2001; Bleich, 2003, p. 67–68; World Council of Churches, 2005, p. 26–27; Moosa, 2009, p. 146–47; Omobowale et al., 2009, at Footnote 40; Loy, 2014, p. 268). As a consequence, in countries where a balanced position has no support and religious leaders have significant influence genetic engineering can face overwhelming obstacles.

It remains to clarify how those ethical and religious considerations can translate into law. Ethical and religious postulations can have a direct impact, if lawmakers are looking for external guidance when it comes to their own action. Religious stakeholders or pressure groups are able to influence lawmakers or public opinion by engaging in the discussion surrounding a legislative process and reaching out to their faith community. This is especially true for developing countries where a purely scientific point of view might be considered as threatening to longstanding traditions and customs (Omobowale et al., 2009, under the section "Discussion"). More often than not, however, ethical considerations are simply used to enforce an existing agenda by serving as an additional argument.

NEXUS OF THESE NORMATIVE CRITERIA

At first glance, it stands to reason that the relation of the described different interests at play can be characterized as either corresponding, reconcilable or irreconcilable. However, the conducted analysis of the different categories of interests revealed that those are not homogeneous enough to make such a determination. For instance, with regard to environmental protection genome edited plants may have both beneficial and detrimental effects. The same holds true for human health considerations. The relationship between these two sets of interests alone is so complex and manifold that it cannot be narrowed down to the categories of "corresponding," "reconcilable," or "irreconcilable." This is all the more true when trying to relate all the interests mentioned above with each other in a logically stringent manner.

Instead, a careful weighing and balancing of the different interests is far more promising. To this end, the significance, value and importance of each single normative criterion must be evaluated. As a result of this assessment, not all interest will turn out to be of such an importance that their inclusion in a legislative process is justified. This means that every criterion must meet a certain threshold of internal significance that makes it worth considering in the first place. The results of such an assessment will vary depending on the internal realities of the respective jurisdiction. For instance, the interest in food security is likely going to be less prominently featured in the regulatory approaches of industrialized countries, whereas public opinion might have a greater impact in democratic organized societies.

However, the criteria which have passed this threshold cannot all be treated alike.

There are the ones that are of such a high value that their weighing or balancing against other interests is not possible. Considerable health risks for a large number of people would fall into this category. However, in case that two or more interests of that kind are not completely aligned, an effort to achieve reconciliation by mutual effectiveness must be made. This can be achieved by finding such

equilibrium between those that every single interest is able to unfold its maximally possible effectiveness under these circumstances.

On the other hand, there are also those criteria that are not absolute and therefore open for a weighing and balancing. This latter category of interests requires a clear assessment of their individual significance, before an appropriate weighing and balancing can take place. In case that an interest of that category is opposed to a normative criterion of absolute validity and it cannot be reconciled, the latter prevails.

A detailed visualization of this abstract concept can be found in **Figure 1**.

REGULATORY CONCEPTS

The aforementioned abstract method to deal with the different normative criteria when considering a new regulatory framework must be embedded in regulatory concepts to make it applicable.

Reconciling Regulatory Concepts

There exist several regulatory approaches that are designed to facilitate a weighing and balancing of different interests or to achieve at least a mutual effectiveness of conflicting normative criteria.

Approval or Notification Procedure

An approval or notification procedure before contained use, field trial, cultivation, or marketing of a GEO provides an opportunity to take into account the different normative factors mentioned above.

Risk assessment

A risk assessment can be used not just to determine possible adverse effects of GEOs but also to identify the importance those risks are going to have in a subsequent process of weighing and balancing.

Pursuant to the Codex Alimentarius (Codex Alimentarius Commission, 2003), a risk assessment of genetically modified food should include an investigation of direct health effects (toxicity), tendency to provoke allergic reactions (allergenicity), stability of the inserted gene, nutritional effects, and any unintended effects which could result from the gene insertion (World Health Organiziation, 2005, p. 12).

However, pursuant to the Codex those principles only apply to genetic modifications "that overcome natural physiological reproductive or recombinant barriers" (Codex Alimentarius Commission, 2003, Para.8). Therefore, those rules are not directly applicable to GEOs that were altered by means of SDN-1 and SDN-2 since they do not cross species boundaries. However, the Codex Alimentarius principles still provide a useful guidance regarding a risk assessment.

However, it should be noted that the more extensive a risk assessment is conducted, the more an approval is delayed and the more costly the market entrance and the final end product get. As a consequence, the desired scope of a risk assessment must be balanced and weighed against these interests, so that a risk assessment has to take place only to the necessary extent.

Socio-economic evaluation

The above-mentioned risk assessment is purely science-based without directly taking into account public opinion, ethical consideration, or societal values. It can therefore be argued that

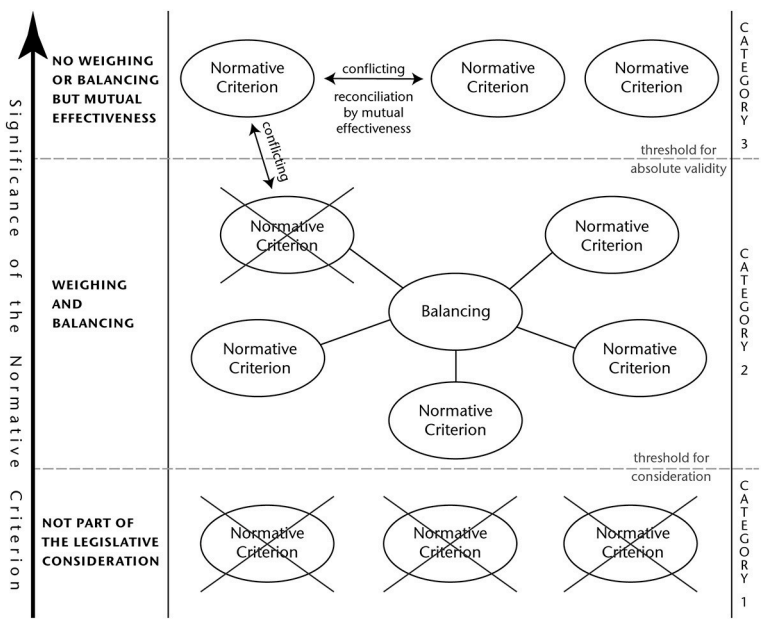

FIGURE 1 | Nexus of the normative criteria and the regulatory implications.

an approval procedure should allow such "soft" criteria to be included as well in the decision-making.

An example for this can be found in the Indian regulatory framework for GMOs which requires that a new genetic event is economically beneficial (Department of Biotechnology, 1998, Sec. 6; Pray and Bengali, 2005, p. 268–69). In the EU a new agricultural plant variety "must be of satisfactory value for cultivation and use" (Council of the European, Union, 2002, Art.4) to be allowed to enter the market. This is the case if "its qualities (…) offer (…) a clear improvement either for cultivation or as regards the uses which can be made of the crops or the products derived therefrom" (Council of the European, Union, 2002, Art.5 Para.4).

However, such an inclusion of non-scientific criteria raises concerns regarding its conformity with non-discrimination and anti-protectionism clauses of international trade regimes. Therefore, a case-by-case analysis has to determine if the introduction of a certain socio-economic approval requirement is legal in the first place.

Coexistence Measures and Identity Preservation Systems

Farmers have only a choice to cultivate either conventional, organic, or genetically modified crops if coexistence measures are adopted (European Commission, 2010, Sec.1.1). At the same time, consumers are only able to choose between conventional, organic or GM food if an identity preservation system allows for a proper labeling.

To protect such a freedom of choice, it must be prevented that the different product lines mix with each other. This could happen during cultivation by cross-pollination, through wind or bees, during harvest by contaminated equipment and during processing or transportation by (un)intentional mixture.

There is no uniform definition of "coexistence" and "identity preservation." Consequently, the meaning of those terms varies significantly (Doshi and Lee, 2008, p. 305). Here, they are understood as concepts that build on each other but at the same time are distinct in nature.

The term "coexistence" is used hereinafter only for measures applied in the period from sowing to harvest and intended to ensure the coexistence of different plant organisms. Coexistence measures are, for instance, isolation distances or buffer zones between different crops, a required approval from neighboring farmers if minimum isolation distance is not respected, information duties (registration of areas in database, prior information to authorities, or neighbors), staggered sowing (different plant cycles and rotation intervals of sexually compatible GM and non-GM crops) and the cleaning/separation of equipment or obligatory insurances (Beckmann et al., 2014, p. 376; Lee, 2014, p. 244; Schenkelaars and Wesseler, 2016, p. 6–8).

An identity preservation system, as understood here, ensures that the segregation established by coexistence measures is maintained after the harvest until the product reaches the end-consumer. This is achieved, *inter alia*, with the help of an end-to-end paper trail, segregated production facilities, separate storage and testing procedures (Smyth et al., 2004, p. 140; Kumar and Sopory, 2008, p. 306; Wiseman, 2009, p. 257).

However, it should be borne in mind that coexistence and identity preservation measures can cause a de facto non-existence of genetically modified crops (Sato, 2015, p. 17). On the one hand, this is due to the fact that buffer zones cannot be maintained (Lee, 2014, p. 244) or the liability risk is too high. On the other hand, coexistence and identity preservation requirements increase the production cost (Falck-Zepeda, 2006, p. 1204; Gabriel and Menrad, 2015, p. 482, 484; Schenkelaars and Wesseler, 2016, p. 9). This might lead to a situation where the additional revenue from growing GMOs does not outweigh the extra cost due to coexistence measures (Venus et al., 2017, p. 421).

Another stumbling block for an identity preservation system with regard to GEOs is the fact that it is not possible to distinguish products derived from SDN-1/2 genome editing from naturally occurring mutations.

Here a distinction must be made between the terms "detection," "identification," and "traceability." "Detection" refers only to the possibility to proof a certain genetic alteration. "Identification" means in this context that the origin of the detected genetic alteration can be verified (e.g., naturally or by means of a certain gene modifying technique). "Traceability," on the other hand, stands for the capability to track GM-products at every stage of the supply chain by means of documentation and segregation (Ribarits et al., 2014, p. 185–86).

Keeping this in mind, the genetic alteration as such is detectable. However, at the moment it is not always possible to determine if that alteration occurred naturally or by means of genome editing. A detection of the origin of the genetic modification fails with respect to SDN-1, SDN-2 and certain forms of application of SDN-3 (Ribarits et al., 2014, p. 185–86; Eriksson, 2015, p. 35).

A monitoring of compliance and inspections would therefore be ineffective to some extent, if the competent authority has to prove the actual origin of the genetic alteration. However, this problem does not occur if the producer bears the burden of proof or a prima facie evidence is allowed, since it is implausible that a certain small, site-specific genetic alteration happened on a large scale naturally.

The coherence and consistency of such measures should receive special scrutiny with regard to GEOs as well. It could turn out to be difficult to argue why there should be measures in place to protect organic and conventional crops from GEOs if at the same time no measures are deemed necessary to protect organic farming from the non-organic methods of their conventional neighbors (e.g., a sprayed conventional crop protection agent also reaches the neighboring organic farmland). Concerning those GEOs that are indistinguishable from their conventional counterparts (SDN-1/2), a reasoning in favor of coexistence measures seems therefore to be difficult to uphold in a logically consistent manner.

Labeling

For consumers to have an actual choice between conventional, organic and GM food these products must be labeled. A prerequisite for labeling is the establishment of an identity preservation system as aforementioned.

However, the labeling of food containing material from GEOs faces several different obstacles. First of all, an end product which contains material created by means of SDN-1/2 is not physically different from products produced from a plant with the (theoretically possible) same genetic alteration but bred using conventional methods. A GEO label would therefore only inform about the manufacturing process, but not about the physical characteristics of the product. This makes the conformity of such a provision with WTO law at least questionable (WTO Panel Report, 1991, Para. 5.15; van den Bossche and Zdouc, 2017, p. 388–89).

It should also be borne in mind that labeling requirements cause additional costs (Kaye-Blake et al., 2004, p. 73; Federici, 2010, p. 556) and have a two-fold detrimental effect: On the one hand, they increase the selling price and thus reduce competitiveness. On the other hand, a labeling requirement for GEOs would imply that there is a well-founded reason to inform the consumer of that particular ingredient and might therefore act as a deterrent to the consumer in the same way as a warning notice would do.

Keeping in mind these adverse economic effects and the indistinguishability from conventionally breed plants, a mandatory labeling of GEOs might not be able to withstand a consistency or proportionality test.

Precautionary Principle

The precautionary principle as set out in Principle 15 of the Rio Declaration requires "[w]here there are threats of serious or irreversible damage, lack of full scientific certainty shall not be used as a reason for postponing cost-effective measures to prevent environmental degradation." Even though the legal status of the precautionary principle as customary international law is still unsettled (Fitzmaurice, 2009, p. 4–6; Beyerlin and Marauhn, 2011, p. 284), it has been widely accepted (*Treaty on the Functioning of the European Union (TFEU)*, Art. 191 (2); Freestone, 1991, p. 36).

For the precautionary principle to be applicable there must (1) take place a scientific risk assessment (2) that identifies a potential but uncertain risk (3) whose realization would cause serious or irreversible damage (Andorno, 2004, p. 17–18).

The applicability of the precautionary principle to genome edited plants created by means of SDN-1/2 seems at least questionable. Since these plants are indistinguishable from natural ones, there can be no risk that goes beyond the natural "risk" of evolution. However, the precautionary principle is neither suitable nor meant to tame risks posed by nature.

With regard to the use of SDN-3, a case-by-case determination of the existence and gravity of a potential but uncertain risk should take place, since not every kind of application poses the same risk. Particular caution should be exercised to ensure that a mere hypothetical or perceived risk is not treated as a real but uncertain risk. With other words, the precautionary principle is suitable for the governance of known unknowns but not of hypothetical unknown unknowns.

However, "[t]he precautionary approach should not only consider possible risks, but also possible benefits and possible harms of a range of alternative options and their effect over people" (Rodriguez, 2017, p. 4). Therefore, the precautionary principle requires taking into account possible harms resulting from the non-use of genome editing as well. If those harms of non-use outweigh the risk of use to a certain extent, the actual use could be the "cost-effective measures to prevent environmental degradation." Consequently, the precautionary principle could—under certain circumstances—also be used to justify the need to actually use the genome editing technique.

Opt-out

A viable option to mitigate such normative criteria that oppose an adoption of GEOs is to allow only certain types of usage and to opt-out of others.

This could mean, for example, that the import and sale of GEOs would still be allowed, but cultivation would be banned. Instead of a complete ban, a regional or geographically limited prohibition of cultivation is feasible as well, especially in federal states. In this way, areas that are ecologically particularly sensitive or where a negative public attitude toward genetically modified plants prevails could be exempted. This approach might appeal to a legislator in whose constituency the fear of release into the environment is particularly prevalent, widespread and pronounced.

If the opposition against GEOs is mainly based on the unwillingness to consume food that is derived from GEOs, it could be considered to prohibit the use of GEOs in food products but to allow the marketing of GEO feed instead.

If the public aversion to GEOs is caused by a perceived unnaturalness of genome editing, the legislator could restrict the use of SDN-3 while allowing SDN-1 and SDN-2.

If such restrictions are—as indicated here—not based on scientific grounds but rather on public opinion, political opportunism, or the pressure of interest groups, it might be difficult for advocates of genome editing to accept such constraints. However, it would be too short-sighted, to consider opt-out measures *a priori* as detrimental for the adoption of the genome editing technique. By partially giving in to the demands to regulate GEOs restrictively, the pressure and the mobilization potential to restrain the use of genome editing beyond that is reduced. This form of regulatory tradeoff can make the limited use of the genome editing technique possible in an otherwise rather unfavorable political or social environment. Therefore, a partial opt-out of certain types of application can actually be in the interest of GEO advocates as well.

Proportionality Test

The proportionality principle is enshrined in a wide variety of legal systems worldwide (Sweet and Mathews, 2008, p. 74–75, 112–60; Klatt and Meister, 2012, p. 1–3). It can therefore be assumed that a regulatory measure with regard to GEOs must at the same time comply with the principle of proportionality.

"The principle of proportionality requires that there be a reasonable relationship between a particular objective to be achieved and the means used to achieve that objective" (Clayton and Tomlinson, 2009, p. 323).

It is usually understood as consisting of four distinct parts (Rivers, 2006, p. 181; Craig and de Búrca, 2015, p. 551): (1) a legitimate objective must exist for the measure (legitimacy), (2) the measure must be suitable to achieve that objective

(suitability), (3) the measure must not be more restrictive than necessary (necessity), and (4) the measure must not be excessive in relation to the objective pursued considering competing interests (balancing).

A measure's legitimacy is assumed if its purpose is lawful. Therefore, the pursuit of any of the normative criteria analyzed above should in general constitute a legitimate objective.

The suitability of the individual measure requires closer scrutiny. Even though a regulator is granted a certain margin of appreciation, the assumption of a measure's suitability must be based on factual grounds in order to prevent arbitrariness (Harbo, 2015, p. 72; Henckels, 2015, p. 53–54). Any measure addressing a non-existing risk is therefore *a priori* unsuitable. With regard to uncertain risks, a risk assessment can provide a factual basis for an envisaged measure. In case of a mere hypothetical risk, the permissibility depends on the scope of discretion that a legislator is granted by the applicable legal system.

The necessity test will most likely require a precise differentiation between SDN-1,-2, and−3, since it seems rather unlikely that it is necessary for a measure to encompass all the different genome editing methods in the same manner.

The last step of the proportionality test (balancing) is a suitable instrument to perform the weighing of category 2 normative criteria or to ensure the mutual effectiveness of conflicting category 3 criteria (cf. **Figure 1**).

Consequently, the proportionality doctrine serves the purpose to reconcile different normative criteria. As such, it is predestined to support the legislator when it comes to find a balance between the different interests existing with regard to the regulation of GEOs.

Clear-Cut Regulatory Concepts: Ban or Non-regulation

In contrast to the methods mentioned above, which are based on balancing and reconciliation, clear-cut and one-sided approaches can also be considered regarding the regulation of GEOs. Such an approach could take shape in the form of a ban or even a non-regulation of GEOs.

A regulator might come to the conclusion that one or several normative criteria of absolute validity, which are not in conflict with opposing criteria of the same category (cf. **Figure 1**), make a complete ban of GEOs necessary.

This might be the case in societies where the slightest risk to the ecosystem weighs so heavily that a ban is perceived as the only regulatory option.

The opposite is also conceivable, namely that a regulation of GEOs is not deemed necessary or even that an unregulated status of GEOs is explicitly desired.

This scenario is feasible if possible adverse effects of GEOs do not pass the threshold for absolute validity or if the adoption of GEOs is backed by a normative criterion of absolute validity (cf. **Figure 1**). This might be the case in countries where GEOs are perceived as imperative solution to battle under- or malnutrition of the population.

However, both of these extreme scenarios are rather unlikely to be implemented in any jurisdiction. For a completely unregulated status of GEOs the issue of genetically modified organisms is by far too controversial. Against a complete ban speaks the fact that it seems difficult to put forward objective reasons to outlaw all forms of genome editing when keeping in mind the indistinguishability of SDN-1 and SDN-2 modifications from naturally occurring alterations.

CONCLUSION

The analysis of normative criteria has shown that a regulatory framework for genome edited plants and products derived from them is influenced by a versatile accumulation of different interests.

Since those interests differ from country to country depending on the respective political, economic, and social circumstances, the respective legislator has the task of finding a suitable balance between these normative criteria. Although the interests are partly at odds with each other, regulatory tools are in place to reconcile most of them.

As a result, the individual regulatory outcome might be as manifold as the interests at hand, but should be within the restraint of international law and basic legal principles.

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REVIEW
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The Idea of Precaution: Ethical Requirements for the Regulation of New Biotechnologies in the Environmental Field

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The rapid emergence of new biotechnologies for selectively altering genetic material—so-called genome editing—has sparked public controversy about how their development and application in the environmental fields are to be regulated. Since the use of these new technologies harbors not only considerable potential but also risks of serious damage whose occurrence is uncertain due to their application in complex environmental systems, many national and international legal authorities are currently adhering to policies of precaution. According to critics, however, precautionary measures and the legal principle of precaution on which they are based are unduly restrictive in the case of the new biotechnologies, hindering advancements in both research and various fields of application. At the same time, legal notions of precaution are highly ambiguous within and across different national and international formulations, thereby further complicating the controversy about their implications. This paper goes beyond the concept of precaution as found in environmental law by examining the *ethical* significance and the *ethical* justification of precautionary measures in the environmental field. In particular, it clarifies the criterion of potential damage, disambiguates different types of epistemic bases in precaution decisions, and considers the relevance and implications of different ethical risk theories as to their response to epistemic uncertainty and vagueness. The two main conclusions are that, first, irrespective of the ethical risk theory embraced, there is an ethical obligation to take precautionary measures whenever serious damage is possible and the probability of damage occurring epistemically uncertain or vague. Regarding the risk assessment, it is argued that the burden of proof lies not with those who fear the occurrence of serious environmental damage. Rather, it is up to those whose actions give rise to such fears to demonstrate that serious damage is extremely improbable or scientifically absurd. Second, the moral responsibility to determine precaution situations and to specify appropriate precautionary measures is attributed not only to state authorities but also to industrial players as well as research communities. Based on these two conclusions, recommendations are given as to how the precautionary principle should be incorporated in political and legal decision-making.

Keywords: precaution, ethics, new technologies, biotechnology, regulation, risk evaluation, precautionary measures

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INTRODUCTION

The rapid development of new techniques which allow us to selectively alter genetic material, and are thus termed *genome editing*[1], has sparked public discussion about how such biotechnologies are to be regulated. On the one hand, the new biotechnologies appear to harbor considerable potential for research and for many areas of application. In the mosquito that spreads malaria, for example, it is now feasible to produce so-called gene drives[2] which could be deployed to diminish disease carrier populations (cf. for example Hammond et al., 2016). On the other hand, due to their application in complex environmental systems in which the occurrence of serious damage is typically uncertain, many national and international legal authorities are currently adhering to policies of precaution. In Switzerland, for example, authorities currently assume that all so-called new procedures are genetic engineering procedures, and therefore fall under previously established genetic engineering regulations that require relatively strict authorization procedures. According to critics, however, precautionary measures and the legal principle of precaution on which they are based are unduly restrictive because the intended alterations to the genome are either no longer detectable in the product or may well be the result of natural mutations.

Legal notions of precaution are highly ambiguous within and across different national and international formulations, thereby further complicating the controversy about their implications. This paper goes beyond the concept of precaution as found in environmental law by examining the *ethical* significance and the *ethical* justification of precautionary measures in the environmental field[3]. It shows how precaution is a (morally) significant action-guiding principle in the regulation of new biotechnologies, and describes the broader conditions and (moral) responsibilities across a wide range of actors for precautionary measures to have their desired effect. In doing so, the scope of the considerations and arguments presented here is limited in at least two respects. First, the main aim of this paper is to show how the idea of precaution bears ethical relevance in the regulation of new environmental (bio-) technologies, thereby foregoing any attempt to offer a full (philosophical) defense of the principle. Note that in bioethical debates in particular, ideas about the (moral) value of precaution are only beginning to be developed (cf. for this assessment of the debate Munthe, 2015). The paper contributes to the debate within environmental politics and, hence, is intended primarily for an interdisciplinary, policy-oriented audience. Second, since the ethical analysis of the idea of precaution focuses on the context of environmental (bio-) technology and decision-making, it is up to further discussion whether its conclusions apply also to other areas in which reference to precaution are increasingly made, such as in medical health care or climate policy.

As a starting point of the ethical analysis, this paper will draw on both the concept of precaution as it is originally found in environmental law as well as on the everyday understanding of precaution and precautionary measures (section Precaution as a Concept in Environmental Law and the Term "precaution" in Specialist and General Parlance). Since, however, neither environmental law nor everyday language provide an answer to the question of how a precautionary approach in the environmental field can be *ethically justified,* the paper will look more closely at whether, and to what extent, legal and day-to-day criteria for introducing precautionary measures are also relevant from an ethical point of view. In particular, it clarifies the criterion of potential damage, disambiguates different types of epistemic bases in precautionary decision-making, and considers the relevance and implications of different ethical theories of risk as to their response to epistemic uncertainty and vagueness (section The Ethical Idea of Precaution). The two main conclusions are that, first, irrespective of the ethical theory of risk embraced, there is an ethical obligation to take precautionary measures if serious damage is possible, and if the probability of damage occurring is epistemically uncertain or vague. Regarding the risk assessment, it is argued that the burden of proof lies not with those who fear the occurrence of serious environmental damage. Rather, it is up to those whose actions give rise to such fears to demonstrate that serious damage is extremely improbable or scientifically absurd. Second, the moral responsibility to determine situations of precaution and to specify appropriate precautionary measures is attributed not only to state authorities but also to industrial players as well as research communities (section Precautionary Obligations). Based on these two conclusions, recommendations are given as to how the precautionary principle should be incorporated in political and legal decision-making (section Recommendations).

PRECAUTION AS A CONCEPT IN ENVIRONMENTAL LAW AND THE TERM "PRECAUTION" IN SPECIALIST AND GENERAL PARLANCE

Precaution as a Concept in Environmental Law

The classic legal model to protect the public from damage comes from hazard prevention. Toward the end of the twentieth century, the conviction became established in environmental policy that in certain situations it is not enough to react only when a threat is imminent or when a threat of damage is certain. Protective measures should also be taken—as a precautionary

[1]The so-called CRISPR/Cas systems are among the genome editing methods currently under discussion. They allow the targeted modification, insertion or removal of individual DNA building blocks. The method was scientifically documented for the first time in 2012 and can be applied to almost all organisms.

[2]In organisms with sexual reproduction, a gene drive is the (naturally occurring or engineered) mechanism by which particular genes or suites of genes and the corresponding phenotypes are propagated throughout a population with a chance greated than (the normal, i.e., Mendelian) 50%.

[3]This paper is based on a report of the Swiss Federal Ethics Committee on Non-Human Biotechnology (ECNH). The report was published in May 2018 (http://www.ekah.admin.ch/en/ecnh-opinions-and-reports/ecnh-reports/). Its current members, elected by the Federal Council for a four-year legislature, are Markus Arnold, Monika Betzler, Christine Clavien, Eva Gelinsky, Greta Guarda, Gérald Hess, Tosso Leeb, Matthias Mahlmann, Jean-Marc Neuhaus, Klaus Peter Rippe, Otto Schäfer, and Markus Wild. The authors would like to thank Andreas Bachmann for his critical inputs throughout the process of writing as well as Nina Scherrer for her support concerning literature research.

measure—even if it is not yet known whether and with what probability such damage will occur. This idea of precaution was increasingly included in the discussion on environmental law and has subsequently become firmly established in various legal documents at national and international level.

An important milestone in the establishment of the principle of precaution in law at international level was the 1992 Declaration of the United Nations Conference on Environment and Development of Rio de Janeiro (Rio Declaration)[4]. Principle 15 formulates the idea of precaution: "In order to protect the environment, the precautionary approach shall be widely applied by States according to their capabilities. Where there are threats of serious or irreversible damage, lack of full scientific certainty shall not be used as a reason for postponing cost-effective measures to prevent environmental degradation." The European Commission addressed the concept of precaution in its Communication in the year 2000[5]. In the meantime, it has become an established regulatory principle of environmental legislation. Precaution is applied when preliminary risk assessment indicates that there are reasonable grounds for concern that something has a potentially dangerous impact on the environment, human, animal or plant health, even when scientific evidence is insufficient, inconclusive or uncertain[6]. Swiss environmental legislation also addresses the issue of precaution. The Federal Constitution requires that damage or nuisance be avoided[7]. The Environmental Protection Act[8] and the Gene Technology Act[9] state that such damaging and disturbing impacts are to be limited at an early stage.

These documents differ in the way in which they formulate the concept of precaution. Whereas the European Commission talks of the *precautionary principle* in its communication, the Rio Declaration uses the term *precautionary approach* in the English version, *Vorsorgegrundsatz (engl. precautionary policy/principle*) in German, and *mesure de précaution (engl. precautionary measure)* in French. The Swiss formulations talk of avoiding damage and nuisance to the environment. The Environmental Protection Act and the Gene Technology Act state that such impact is to be limited at an *early* stage.

It is conceivable that different things are intended with these different formulations, and that the idea of precaution may not involve one principle or approach, but a whole array of different principles or approaches (cf. Hartzell-Nichols, 2013). Alternatively, it may be that the idea of precaution is formulated differently in varying contexts, but that the same set of legal instruments is ultimately established. In any case, it can be said that all formulations have a common core (cf. Gardiner, 2006). Precautions should be taken to avoid damage when two criteria are met: (1) it is feared that damage (of a certain extent) may occur and (2) knowledge about the probability of such damage is restricted. According to the Rio Declaration, the possible damage must be serious or irreversible and the restricted knowledge must constitute scientific uncertainty. In the European Commission's communication, the severity of the damage is not qualified, and a preliminary scientific risk assessment must give cause for concern.

The formulations in Swiss law differ from the internationally established understanding of precaution in a variety of ways. They state that not only harmful effects but also nuisances must be prevented, and the criterion of restricted knowledge is not explicitly mentioned. Furthermore, there is no mention of scientific uncertainty or of preliminary scientific risk assessment[10]. It may be said that the idea of precaution, as it has been discussed since the Rio Declaration in 1992, only finds expression in Swiss environmental law in individual pieces of legislation such as the Gene Technology Act.

This paper aims to respond to the core requirement of all these formulations, namely the need to react to the fear of potential harmful effects, and to the question of how such a requirement and the resulting obligations can be ethically justified.

Precaution and Prevention

In German, the terms *Vorsorge* (precaution) and *Prävention* (prevention) are widely used synonymously, both in technical jargon and in everyday language. German-language legal texts sometimes use the term *Prävention* in the context of precaution. In French and Italian, the two terms are also generally used synonymously in everyday usage. On the other hand, specialist literature in these two languages distinguishes clearly between *précaution*/*precauzione* and *prévention/prevenzione*: if the probability of occurrence of damage is known, the term used is *prévention*/*prevenzione*; if, however, the probability of damage occurring is uncertain, the term *précaution/precauzione* is employed[11]. As this paper examines the question of how uncertainty is to be addressed, it also looks at the ongoing discussion in French and Italian specialist language of *précaution/precauzione,* respectively.

[4]http://www.un.org/documents/ga/conf151/aconf15126-1annex1.htm

[5]http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:52000DC0001&from=EN

[6]Directive 2001/18/EC of the European Parliament and of the Council of 12 March 2001 on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC (https://eur-lex.europa.eu/eli/dir/2001/18/oj); Cartagena Protocol on Biosafety (https://bch.cbd.int/protocol/text/)

[7]Swiss Federal Constitution, Article 74 (https://www.admin.ch/opc/en/classified-compilation/19995395/index.html)

[8]Swiss Federal Act on the Protection of the Environment, Articles 1 and 11 (https://www.admin.ch/opc/en/classified-compilation/19830267/index.html)

[9]Swiss Federal Act on Non-Human Gene Technology, Article 2 (https://www.admin.ch/opc/en/classified-compilation/19996136/index.html)

[10]The criterion of restricted knowledge could perhaps be construed from the formulation that measures shall be taken *early*. This would have to mean that action should be taken not at the time when imminent danger is to be averted, but earlier, when there is no certainty as to the probability of the damage or nuisance occurring.

[11]Only specialist language, in other words, aims at capturing the shift from a (preventive) approach in which "the decision-maker intervenes provided that the threats to the environment are tangible" to a (precautionary) approach under which "authorities are prepared to tackle risks for which there is no definitive proof that there is a link of causation between the suspected activity and the harm or whether the suspected damage will materialize" (de Sadeleer, 2010).

The Broad Understanding of Precaution in Everyday Language and the Narrow Understanding of the Precautionary Requirement in Environmental Law

In contrast to the (international) concept of environmental law, in our day-to-day lives we not only invoke precautionary measures when there is a threat of serious, major or irreversible damage. Rather, we typically consider precautions and corresponding measures even in response to minor harmful scenarios: for example, if unsettled weather is forecast and—as a precautionary measure—we take along a raincoat. Moreover, according to this general colloquial understanding, we also speak of precaution when a situation that is to be assessed negatively might occur not only possibly, but with a very high probability, or even with a probability bordering on certainty. In everyday language, in other words, we use the term "precaution" for situations in which one could (also) speak of prevention. Saving for an old-age pension provides an example of this: even if a person does not know with certainty whether they will reach retirement age, it is rational to take precautionary measures for the loss of income associated with retirement. Or if a single parent knows there is a probability bordering on certainty that they will soon die, and if they can prevent or alleviate some of the negative consequences for the family members left behind after their death, they have a moral duty to take appropriate precautions. Similarly, if a person must assume with near certainty that their behavior will result in others becoming infected with a dangerous disease, she is obliged to take (preventive) measures.

This broad and general understanding of precaution thus means preparing based on one's own or another's assessment of the risk to avoid or alleviate harmful effects that could occur as a result of subjective or objective assumptions of probability. Precautionary measures are decided on this basis. Leaving aside the question of moral duty toward oneself, precaution can also be generally understood as an *ethical duty* either to protect others from harm or to avoid risks of harm that we inflict upon others.

However, even with this general understanding of precaution, it may well be that it also incorporates the idea that possible harmful effects must be of a certain quality in order to justify an obligation to take precautionary measures. On the other hand, according to this broad understanding, there is no precautionary situation and therefore no obligation to take precautionary measures if there is no indication that harmful effects may ensue. This does not mean that no harmful effects can occur; only that it is at present unknown that something is unknown. Moreover, one is not required to be aware of not knowing. This means that even in the everyday understanding of precaution, no one has a moral duty to take precautionary measures against previously unobserved harmful effects or harmful effects that have not yet been observed or deemed possible.

In environmental law, the understanding of precaution is somewhat narrower. Here, the demand for precaution arises in the face of the fact that the scope of our knowledge is restricted. Either the understanding of precaution in environmental law thus refers to a special case of the everyday concept of precaution, or it designates an ethical principle that is distinct from the broad and general understanding of precaution described above.

A look at both environmental law and everyday language serves as a first approach to the possible meaning(s) of the precautionary idea and provides indications as to which situations can call for precautionary measures. However, neither environmental law nor everyday language can provide an answer to the questions of how a precautionary obligation can be *ethically justified*, who bears an obligation, and what this obligation consists in. Thus in the following, we will examine whether and to what extent the criteria for introducing precautionary measures found in the law are also relevant from an ethical point of view, and whether there may be grounds for further obligations beyond these legal criteria. This analysis takes the criteria in environmental law as a starting point, but then continues separately from the legal considerations. A link to the law is re-established after the conclusion of the ethical analysis, in order to reflect these considerations in existing law and to clarify possible need for action.

THE ETHICAL IDEA OF PRECAUTION

The Criterion of Potential Damage

The core idea of precaution is that certain harmful effects should not occur and that one should take measures to prevent or limit them whenever possible. In formulations in internationally relevant environmental legal texts, the duty to take precautionary measures does not relate to all harmful effects, but only to those of a certain quality. According to the Rio Declaration, the duty to protect in the sense of precaution only extends to potentially serious or irreversible damage to human health and the environment. The communication of the European Commission accords this particular quality to damage to the environment and human, animal and plant health if it exceeds a certain level. This damage can be understood to constitute the impairment of legally defined objects of protection or protection goals. Besides damage to health and the environment, there may be other (also serious) effects of an economic nature. However, under international environmental law there seems to be no precautionary obligation to protect against such effects.

For an ethical examination of the idea of precaution, the criterion of potential damage raises two main questions. On the one hand, it may be asked how an obligation to precaution, which relates to damage that is not certain but possible to occur (in the sense that there are plausible grounds to fear its occurrence) can be justified. On the other hand, we must establish what justifies the restriction of these obligations to a particular type or quality of possible damage. In order to answer these questions, we must first look more closely at what constitutes damage.

What Constitutes Damage and Who or What Can Suffer Damage?

A plausible definition of damage is a change that must be judged to be negative. It is irrelevant who causes the damage. The damage is the same whether humans cause it or it is the result of natural forces.

Damage is morally relevant when it affects entities that themselves have moral value. Who or what these entities are depends on the position held in (environmental) ethics. Here, we restrict ourselves to a selection of four options that are most frequently referred to:

- Anthropocentric positions place humans at the center in the sense that only humans count morally for their own sake. Only humans, therefore, can suffer damage for their own sake. According to this position, serious damage to animals, plants or the environment is morally relevant only insofar as it affects humans and, correspondingly, these entities are merely of instrumental or relational value to them.
- Pathocentric positions place a living being's sentience and ability to feel pain as the main criterion to determine whether it can suffer damage. A living being can suffer damage provided it has some form of inner experience or if it can experience something as good or bad.
- Biocentric positions consider all living beings to have moral value for their own sake. For these positions, sentience is not a prerequisite for a being's inherent value. There are two main biocentric approaches. According to one approach, living beings have inherent value and can therefore be damaged, because being alive has value for its own sake. According to the second approach, living beings can be damaged because as bearers of a good life they have a good of their own. This second approach assumes that living beings have, so to speak, an inscribed aim specific to their species.
- Ecocentric positions focus not just on living beings but the whole of nature as a comprehensive, complex interaction between entities. If we interpret this position holistically, collective entities such as ecosystems, biotopes, species and populations, nature, the earth or even the whole universe have inherent value. For advocates of an individualistic reading of this position, all individual beings that are part of nature count morally for their own sake, both living beings and non-living beings such as lakes, mountains or landscapes. All of these collective or individual entities can be harmed.

Depending on the position held in environmental ethics, different entities will be among those beings that can be harmed for their own sake. This, however, does not yet tell us how much the damage caused to such an entity counts. There are essentially two answers to this question. The egalitarian position assumes that equal damage caused to any entity that can be harmed must be assessed equally and unequal damage differently. According to a hierarchical position, all entities that can be harmed should be considered. However, as not all entities have equal value, the damage caused to (hierarchically) different entities counts differently. Either the nature of the species counts, so that interests, such as those of humans, are weighted more highly than equal interests of other entities. Or, the complexity of characteristics counts, and the more similar the characteristics to those of humans in terms of their complexity, the higher the harmful effects are weighted[12].

The Ethical Significance of Qualifying Damage in the Context of Precaution

In contrast to the broad everyday understanding of precaution, according to which precautionary measures should be taken against even the slightest of harmful effects, in a narrower understanding of the concept, as it is formulated in environmental law, the quality of the damage plays an important role[13].

One reason for restricting precautionary obligation in environmental law to a particular type of damage may lie in the fact that the State is under an obligation to intervene in basic rights, in particular rights of freedom. Any intervention in basic rights requires justification. Another or additional reason could be that at international level only a qualified type of damage could be agreed on for political reasons.

For the purposes of this discussion, irrespective of any possible politically motivated reason for limiting the concept of precaution to certain types of damage, we will look at the normative question (which is also relevant for a legal justification) as to how far such a limitation can be ethically justified. Two main positions can be distinguished regarding the normative meaning of damage. The first position assumes that certain types of damage cannot be compared with others; the second assumes that all types of damage can and may be compared:

1. The first position assumes that certain types of damage represent a negative outcome of a type that cannot be compared and therefore not be weighted against other negative outcomes. These types of damage thus form their own normative category. If it is conceivable that damage of this type could occur in a certain situation, there is either a duty to refrain from action or a requirement to act (e.g., to generate knowledge as a prerequisite for a risk assessment). Damage of this kind must always be avoided. Even if the

[12]Cf. ECNH, Dignity of Living Beings with regard to Plants. Moral consideration of plants for their own sake, 2008, p. 19, and ECNH, Ethical Treatment of Fish, 2014, p. 21 f, including criticism of the different positions

[13]In terms of precaution, the Rio Declaration talks not only of possible *serious*, but of *irreversible* damage. Any change is, essentially, irreversible. If, for example, a forest is destroyed, it is not possible to restore it to exactly the same form, even if reforestation takes place over a long period. The living creatures that formed part of the forest cannot be brought back. The forest is a new forest with new living creatures. In an ecological context, however, the concept of irreversibility is not usually understood in this way. A forest that can be restored, or a particular moth which disappears but of which examples of the same species become re-established, are not considered to have been lost irreversibly. According to this understanding of irreversibility, the damage can be compensated. The term is used to qualify a particular type of damage: damage that has long-term effects and affects things that are considered particularly important and valuable to the human community (possibly also to later generations) and its environment. Understood thus, irreversibility serves as an indicator when assessing how serious any damage caused may be, but not as an independent criterion for precautionary measures.

probability of damage occurring is extremely slight, it is the extent of the potential damage that is of relevance. For if risk is a function of damage and probability of occurrence, and if the negative outcome is astronomically severe damage, then even the smallest probability of occurrence would result in an immeasurably great and therefore impermissible risk. The key question to be asked in this position is: what constitutes incomparably severe damage?

There are two variations of this first position. According to the *first variation*, the *physical* destruction of the whole of humanity would constitute incomparably severe damage, whereas according to the *second variation*, it is the *cultural* destruction of humanity, which meets the criterion of incomparably severe damage. Even if, following a catastrophic nuclear event, a large number of people could continue to live biologically, but not in a way that constitutes the cultural nature of humans, then according to the second variation, this would constitute incomparably severe damage and hence an evil that must be prevented at all costs. It is inadmissible to weigh up such damage against other interests.

Advocates of both variations of this first position agree with the second position set out below that a weighing of interests is admissible with regard to all other interests.

2. According to the second position, no damage can be of a quality that does not allow comparison with other types of damage. If different instances of damage can only be distinguished by their extent, it can still be assumed that only once the damage reaches a certain extent is it necessary to act (which may also mean refraining from doing anything). This would then give us a conception of a threshold. Only when the possible damage reaches a certain level does precaution come into play in situations where knowledge is limited, and the obligation arises to take measures to prevent damage of this magnitude. If the possible damage does not reach this threshold, precautionary measures are not required, even in situations of scientific uncertainty. The key question to be asked in this position is: when is this threshold reached?

   A *variant* of this second position also includes small-scale possible damage in the consideration of precaution. According to this position, requiring precautionary measures may also be justified with regard to such types of damage, even if the probability of their occurrence is uncertain or vague. This at least, provided the costs of the measures taken are reasonable.

   A further *variant* of this second position does not require precautionary measures to be taken if the possible benefits of an action are scientifically and plausibly weighted higher than any potential severe damage.

The Epistemic Bases of Precautionary Decisions

A precautionary situation is one in which damage could occur but in which there is only limited knowledge about the probability of this possible damage occurring. The ethical idea of precaution, according to the thesis to be examined, justifies an obligation to take measures to prevent possible damage or to limit it to an extent not exceeding a permissible degree. This obligation exists even if no more is (yet) known about the probability of occurrence other than that it is greater than zero. Precautionary situations can therefore be seen as a particular type of risk situation. Decisions about precautionary situations are thus a type of risk decision.

Firstly, a distinction must be made between four types of epistemic basis on which risk decisions are made.

- It is known that damage will occur with 100% or 0% **certainty**: the damage is either sure to occur or sure not to occur. No statement of probability need be made.
- The damage scenario and its probability of occurrence are fully determinable. There is a situation of **complete or certain knowledge of the risk**. We know the statistical probability with which damage will occur. The risk is therefore calculable. In French and Italian specialist literature on the subject, this type of epistemic basis would be the object of *prevention*, not of precaution[14].
- The damage scenarios are known. The bases on which their probability of occurring can be calculated are, however, imprecise to varying degrees. The probability of occurrence cannot therefore be calculated quantitatively but can only be estimated in qualitative terms. There is a situation of **incomplete or uncertain knowledge of the risk**. An example of this might be the exact prediction of avalanches: We know what the damage scenario is, but despite the various calculation models available, can only make a qualitative assessment of the probability of an avalanche occurring—as "high" or "low."
- There are scientifically plausible indications for possible damage. Unlike type 3, however, it is not possible to estimate the probability of their occurrence. This epistemic situation is referred to below as **vagueness**. An example of such an epistemic situation of vagueness is the risk posed by a nuclear final storage facility. Owing to the time dimension, our geological and biological knowledge and experience are insufficient for us to make even a qualitative estimate of the probability of damage occurring.

To be distinguished from the four epistemic bases are situations of **ignorance**[15]. In such situations we do not know that we do not know. We have neither an idea of the damage potential nor do we have any (scientifically plausible) indications that give rise to fears. Therefore, there is no vagueness, but rather ignorance. A reaction is therefore impossible and there can thus be no obligation to take precautions. As soon as we have some form of hunch or fear, we are in a situation of uncertainty, no longer in a situation of ignorance.

It is important to bear in mind that uncertainty or vagueness refers only to the probability of occurrence, not to the damage scenarios. The damage is always known or at least there must be scientifically plausible indications of the damage scenarios. If the damage is not known or if there are no such indications, a situation of ignorance exists. Even complex situations do not

[14] See also section Precaution and Prevention.

[15] Others deny the relevance of the distinction between uncertainty and risk by arguing that, practically, uncertainty is a case of risk (cf. Roser, 2017).

mean that the damage scenarios are uncertain or doubtful, but rather that assessing their probability of occurrence becomes correspondingly more complex and therefore more difficult.

By the same token, epistemic uncertainty is to be distinguished from psychological uncertainty. If, based on a subjective assessment, someone fears that damage may occur and therefore feels insecure, this does not necessarily mean that there is epistemic uncertainty. There may be sufficient risk data to calculate the risk. Despite the psychological uncertainty, there would then be no epistemic uncertainty, but rather sufficient knowledge of the risk.

In practice, assigning a concrete decision situation to one of the theoretical types of epistemic basis regularly gives rise to controversy. Thus, it is debatable when a certainty of 100% or 0% can be assumed outside of controllable contexts, such as those that can be generated in a laboratory. When technologies are applied in the environment, there will always be a degree of uncertainty or vagueness. In the context of environmental risks, in particular, some people point to the complexity of the system and argue that such risk assessments are not only currently impossible, but that they are not feasible in principle. Others, on the other hand, assume that, even in complex systems, for certain types of events sufficient data may be available to determine the probability of occurrence or at least to provide a rough qualitative estimate. According to this position, even in the case of complex systems one should not therefore generally assume that a risk assessment is impossible.

These assignment issues and their role in precautionary decisions are discussed in section How Can an Ethical Decision be Made When Expert Opinions Differ? For the time being, it suffices to note that the precautionary idea relates to the epistemic bases of uncertainty and vagueness. Accordingly, measures are to be taken under the heading of precaution, although it is (still) uncertain or vague whether the feared damage will occur.

How Do Ethics Theories Respond to the Epistemic Situation of Uncertainty?

What should be done when there is epistemic uncertainty and vagueness with regard to ethically relevant damage in the context of precaution? The answer to this question depends on the ethical theory of risk embraced.

Even if there are many competing ethical theories of risk, they can be assigned to only a limited number of types. Here we will focus on those two theory types which, according to a widely shared view, play the most dominant role in normative ethics, in general, as well as in (applied) attempts of answering the question of how to deal with precautionary situations: the consequentialist theories (the most well-known of which is the utilitarian theory) and the deontological theories[16]. These two theory types can be linked to all the environmental ethics positions mentioned in section What Constitutes Damage and Who or What Can Suffer Damage?

Deontological Ethics Theories

Common to all variants of deontological ethics theories is the notion that an action is morally right if it corresponds to the obligations that we have toward morally relevant entities. According to deontological ethics theories, entities are morally relevant because they have inherent value, i.e., value in themselves, regardless of their use or significance for others. Depending on the position taken, different entities have such inherent value: only humans or only living beings with certain characteristics, or all living beings or all collective entities. The obligations always exist toward the morally relevant individual entity with inherent value.

If there is a possibility that such an entity could suffer damage in an ethically relevant way, this would trigger a precautionary obligation. A precautionary obligation toward this entity does not rule out the possibility that measures must also be taken to protect other protection objectives, which do not have an inherent value. For example, if a precautionary obligation only exists toward people, this does not mean that no measures are to be taken to protect animals or environmental goods. The reason for these measures, however, lies not in the obligation toward these other beings or goods, but in the precautionary obligation toward the person for whom these beings or goods are of instrumental value.

Advocates of absolute deontological theories are obligated to refrain entirely, i.e., under all circumstances, from deeds that (could) damage entities with inherent value. Such absolute forms of deontological theory do not allow for any weighing up, even when there is a conflict of obligations. As inherent value cannot be weighted, making it impossible to calculate which obligation is of greater importance, in such cases advocates of deontological theories find themselves facing a dilemma. One variant of this approach excludes the weighing up of certain qualified goods only, such as human dignity, whereas for all other goods, a *prima facie* approach applies as described below.

Advocates of prima facie approaches of deontological risk theories permit a threshold value for damage, if it does not violate morally justified claims. They justify this by saying that an obligation to act always implies that it can also be fulfilled. An instruction that cannot be fulfilled is not plausible. If all action that could damage morally relevant entities were prohibited, life would not be possible, because with every action there is a probability that an entity with inherent value will be damaged. According to these prima facie approaches, exposing these entities to risks is reasonable if these risks are below the threshold value. If, on the other hand, they lie above the threshold value, measures should be taken to reduce the risks to below this value. If this is not possible, the action must be refrained from completely or at least until the risks can be reduced to below the threshold value. A special case of this variant of a threshold position assumes that, even below the threshold value, there is still an obligation to take further measures, insofar as they are proportionate.

[16]We note that, besides consequentialist and deontological approaches, virtue ethical accounts—which focus not so much on consequences nor on obligations but emphasize the virtues or (moral) character of the (moral) agent—are often considered a genuine, i.e., irreducible, third alternative. Here, however, as we focus on the most basic, underlying logic of competing ethical theories (of risk), we assume that virtue ethical aspects can ultimately be assigned to either consequentialist or deontological types of considerations.

In deontological risk theories, opportunities (i.e., more or less probable benefits) associated with an act may not be weighed against the associated risks[17].

If complete risk knowledge is available, that is to say, it is known with which probability an entity with inherent value will be damaged by a certain action, advocates of deontological risk theories always decide according to the obligations that they have toward this entity. If the risk of being damaged is reasonable for the entity, the action is permissible. If the risk lies above the threshold value and is therefore unreasonable, the action must be refrained from.

If the risk knowledge is incomplete, the reasonableness and thus the admissibility of a risk cannot be determined. It is not known whether a certain action (or the application of a technology as a whole) exceeds the permitted threshold value. In such a situation, deontological approaches will require more data and information on the probability of damage occurring to morally relevant entities. The same is true to an even greater extent for situations in which there are only scientifically based theses that make serious damage appear plausible. In these cases, too, an obligation to carry out research may stem from this theory.

It should be borne in mind that risks must also be taken in order to obtain further risk information. These risks must also be reasonable. It follows from this that, according to deontological theories, this additional information can only be obtained gradually. This is the only way to obtain this information without exceeding the permitted risk threshold[18].

Consequentialist Ethics Theories

There are also many types and variations of consequentialist ethics theories. The most well-known and politically influential is the utilitarian. It is therefore the focus of the following considerations. What all variants of this theoretical family have in common is that an action is assessed *solely* based on its consequences. For example, according to the act utilitarian theory, each action must maximize the expected net utility.

Because only the consequences of an action count, this precludes entities having inherent value in the deontological sense[19]. A change which is judged negative for a morally relevant entity according to deontological theory does not necessarily represent morally relevant damage according to utilitarian theory. Rather, it may be necessary to bring about such a change if it increases the net utility for all morally relevant entities. According to utilitarian theory, there would be morally relevant damage if an act did not increase this net utility.

If there is complete knowledge about opportunities and risks, these can be weighed up against each other and the best possible outcome for all ethically relevant entities can be calculated.

If the risk knowledge, i.e., the knowledge of opportunities and risks, is incomplete, further information is required according to consequentialist theories just as it is in the case of deontological theories, until it is possible to calculate the consequences (i.e., the net utility, according to the utilitarian theory). This is all the more the case when there are situations of vagueness in which there are only (scientifically founded) indications that serious damage may result.

In order to calculate the risk, information about both opportunities and risks for entities with moral value is required. New data is continuously considered in this calculation. Obtaining information also has its price[20]. In situations in which the opportunities are fully known, it may be that the price for additional risk information becomes so high that the calculation requires one to act without further risk information. However, a step-by-step approach must also be taken according to the logic of the consequentialist theories presented here. According to utilitarian theory, a step is taken when the calculation of the available information suggests that the net utility will be greater than if this step is not taken. As long as the data necessary for a calculation is unavailable, and the estimated cost of acquiring the data is not higher than the estimated opportunities, then there is a need for research.

How Can an Ethical Decision Be Made When Expert Opinions Differ?

How do the different ethics theories react to a situation of disagreement or indecision about risk knowledge? If there is knowledge about possible damage, but the data on the probability of its occurrence is interpreted differently in expert circles[21], advocates of both deontological and consequential risk theories will ask about the plausibility of the deviating interpretations. If the degree of plausibility of different interpretations varies, the more plausible expert opinion must be considered.

The degree of plausibility depends on the data available, the state of the art or the care taken in applying scientific methodology. Plausibility is decided based on the criteria for scientific excellence recognized by the scientific community: theory or hypothesis must, among other things, explain a particular phenomenon and be testable, meet coherence requirements and satisfy the principle of organized skepticism (e.g., undergo a peer review). A scientific hypothesis is thus

[17] There is disagreement among advocates of deontological ethics over whether opportunities that enable the fulfillment of positive obligations should be taken into account.

[18] It remains to be seen how these threshold values are to be set and how one knows when enough information is available in order to establish when the risk is no longer reasonable.

[19] For advocates of a utilitarian theory, the individual being or individual entity never has value for its own sake.

[20] See: Christian Munthe, Precaution and Ethics. Handling risks, uncertainties and knowledge gaps in the regulation of new biotechnologies, Report commissioned by the ECNH, published as Volume 12 of the ECNH publication series "Contributions to Ethics and Biotechnology", 2017.

[21] There are many reasons why scientific results are interpreted in a variety of ways. Scientific disagreement often results from ambiguous and inaccurately positive results of research. There is a further problem with interpreting data when studies do not meet statistical relevance requirements. This makes it even more important to create transparency about the basic assumptions on which scientific interpretations are based.

considered plausible if there is much to be said for its correctness. This is, so to speak, the threshold that separates plausibility from non-plausibility.

It is the task of the scientific community to assess scientific plausibility. In order to fulfill this task according to scientific criteria, the institutions need access to the information that led to the formulation of the scientific theses. The data must be presented in a comprehensible manner, including data that does *not* support the scientific thesis. Furthermore, the scientific institutions must be independent to ensure that plausibility is assessed impartially and according to scientific criteria.

What should be done when disagreement or indecision still exists within the scientific community and the question of plausibility cannot be decided in a scientific manner? If there are two or more competing positions that all meet the plausibility criteria and have large groups of advocates in the scientific community, it is usually also accepted within the community that there is a state of disagreement. From an ethical standpoint, therefore, research is required. More information is required to find out which of the interpretations is more plausible.

If, on the other hand, a large majority of the scientific community considers the data situation to be clear, the role of a deviating minority opinion must be examined nevertheless. Must the majority opinion be followed or is there a situation of scientific uncertainty? First of all, it should be noted that neither the fact that a scientific position is held by a majority nor by a minority is a criterion for its correctness. Even when everyone agrees, this does not mean the position is true *for this reason*. Conversely, the plausibility of a position cannot be determined independently of the sciences. If this were possible, it would be possible to make an objective and unequivocal decision on which theories are plausible based on criteria independent of science. It is conceivable that there are several plausible theses concerning the same facts or phenomena. Theoretically, it should be possible to use plausibility criteria to decide which of the gradually differing plausible positions is the most plausible. In practice, however, the scientific community is generally unable to judge so easily either the question of plausibility or the question of the degree of plausibility.

Nevertheless, in such undecided and indecisive situations, decisions have to be made. For this reason, it is imperative that decision-makers, such as public authorities, check whether the criteria for scientific research have been adhered to, and to what extent competing positions are plausible, in order to be able to understand the assessments of the scientific institutions and classify them appropriately. They therefore also require access to the necessary information in a comprehensible form, including diverging data that does not support the scientific theses. These authorities should therefore also have employees with this kind of scientific training. It is not their responsibility to carry out a plausibility assessment themselves, but they must be able to critically understand those made by the scientific community. It should be noted that these employees act as representatives of the political decision-making authorities and thus play a role different to that of the academic institutions.

Different Theories, Converging Practices

There are different approaches to justifying the concept of precaution depending on the ethical theory of risk. Nevertheless, if there are indicators of a precautionary situation, and if the criteria that trigger measures are met, advocates of both deontological and consequentialist theories largely agree over the implications of the precautionary measures and the form that they should take. They agree on this in spite of all their theoretical differences, including the relevance they assign to the consequences which new technologies may have. According to both risk theories, there is an obligation to act in a precautionary manner. Both demand an obligation to obtain comprehensive information in order to reduce uncertainties with the aim of enabling suitable risk assessment.

PRECAUTIONARY OBLIGATIONS

Precautionary situations differ from other risk situations in that, firstly, *serious damage* is possible and secondly, the *probability of occurrence* is *epistemologically uncertain*. If both these criteria are met, there is an ethical obligation to take precautionary measures. Precautionary measures can and must be taken, therefore, if the existence of the two criteria is established. There are two conceivable options:

- According to the *first option*, those who fear that serious damage may occur must show that their fear lies within a plausible range.
- According to the *second option*, the burden of truth is reversed. It is not up to those who fear the occurrence of serious damage to demonstrate plausible grounds for this fear. Rather, it is the responsibility of those whose actions give rise to the fear of serious damage occurring to demonstrate plausibly why such damage is extremely improbable or scientifically absurd.

If there are plausible indications of serious damage, the reversal of the burden of truth is justified. Furthermore, in precautionary situations, i.e., in situations in which it is feared that possibly serious damage may occur, the obligation to ensure that precautionary measures are taken lies primarily with the state authorities responsible for safeguarding the protection objectives in question.

The issue of how to apply new (bio) technologies in the environment and identify the role of precaution in this context is more than a purely legal or scientific one. Owing to the far-reaching consequences of these technologies, such as the (unintended) rise of new and unknown animal and human diseases or the reduction of biodiversity in the attempt to combat malaria using CRISPR/Cas-based gene drives, not only are the state authorities called upon, but the answers must be negotiated by society in the political process. The decision whether to use genome editing to fight malaria in endemic areas, by way of example, neither belongs solely to science nor to legal authorities, but also requires engagement of the local communities who are particularly prone to foreign economic interests[22]. While

[22]cf. Patrão Neves and Druml (2017) a report on the UNESCO Chair of Bioethics' meeting at the Medical University of Vienna in September 2016, which gathered

the state is solely responsible for the political decision-making processes[23], this is not inconsistent with the fact that the public authorities rely on the involvement of others[24] in order to fulfill their responsibilities.

Various instruments of precaution are conceivable considering both the political decision-making processes and actual proposals for regulations. No attempt is made here to provide a definitive list of these instruments.

Taking precautionary measures in favor of protection objectives or ensuring that an ethically unjustifiable occurrence of damage is (highly) unlikely often involves prohibiting or refraining from an activity or a certain application, and thus raises the question of if, and to what extent, precaution can be ethically reconciled with basic and more specific freedom rights. Arguably, the answer to this question depends on a more in-depth account of the moral value of precaution, of freedom, and of their relation, which goes beyond the scope of this paper. However, restricting freedom rights in some way may be justified if the measures taken are proportionate with regard to the protection of freedom rights. If, for example, plausible fears exist, but owing to a lack of knowledge or unanimity about the knowledge available it is still unclear whether these fears will continue to be justified in the future, the appropriate measure is not a general prohibition, but a temporary one (moratorium). Furthermore, rather than general prohibition, spatial or application-specific prohibition should be considered.

However, there is a need to counter the frequently expressed reservation that precautionary measures necessarily only involve proscription. Precautionary measures can also exist as *orders* to act. The obligation to proceed step by step, for example, means that missing knowledge can be acquired and potential serious damage restricted at an early stage. When the first astronauts landed on the Moon, it was feared that they might bring back microbes, which could lead to catastrophic effects on earth. This fear, which was plausible relative to the state of knowledge at that time, did not mean that the moon landing was prohibited. Instead, the astronauts had to spend 3 months in quarantine upon their return, a precautionary measure that effectively assuaged the fears.

Besides the state agencies responsible for determining precautionary situations and for the binding definition of measures, other players also have a moral duty. These might be companies and manufacturers that produce potentially harmful substances or that introduce them into the environment as well as agricultural holdings. Businesses and manufacturers have the duty to work with such substances in accordance with the regulations and rules of good professional practice. The idea of precaution also obliges them to report any unexpected adverse effects noticed, so that appropriate precautions can be taken. As a result, the state also has a duty to create agencies to which such observations can be reported, and to react in good time.

Research scientists and research institutions also have a responsibility, as they are often the first or the only ones able to recognize the damage potential of their research activities. They have a duty to work in compliance with the rules set within their scientific field, and to take precautionary measures to avoid serious damage in the context of their research activities. This may mean that precautionary measures are already called for when research projects are appraised or funded, if scientifically plausible damage scenarios are apparent. For example, state research funding may not be one-sided and a range of research prospects and research paradigms should be considered. Furthermore, researchers and research institutions are required to draw the attention of the authorities and the public at an early stage to developments which may have precautionary relevance. Here also, it is the state's duty to receive such information and to react expeditiously.

If all the players involved are to be able to observe their precautionary obligations, the responsible actors in the education system are also called upon. Pupils and students should be made aware of the issues in a way appropriate to their level of competence, and taught how to deal with knowledge, uncertainty and risk situations. This should happen above all at tertiary level, i.e., in universities, and in vocational training for occupations, which are confronted with such precautionary situations. In the context of biotechnology, this includes agricultural colleges.

Dealing with new technologies in the environment does not only affect those in the research field or those who apply these technologies in their work. Because of their potential impact, how to deal with new technologies in the environment and the extent to which it is permissible to expose third parties to risks are issues of importance to the whole of society. In Switzerland, therefore, they are regularly the subject of political popular votes.

RECOMMENDATIONS

1. **Consistent strengthening and application of the idea of precaution.** With regard to new biotechnologies, the applicability of the legal concept of precaution is frequently questioned. However, the idea of precaution can also be legitimized ethically, irrespective of the underlying ethical risk theories. This leads to the first recommendation, namely to adhere to the concept of precaution in the regulation of new biotechnologies,[25] to establish it firmly in the further development of environmental law and to support its application at international level.

infectious disease experts with a focus on malaria, entomologists and ethicists to discuss the advantages and disadvantages of genome editing applied to mosquitoes to fight malaria.

[23]A further option is theoretically conceivable, namely that a precautionary situation can always be assumed, i.e. that it is always clear that the criteria are met. Such a position, which means a general reversal of the burden of proof for all actions, would however encroach on freedom rights to a disproportionate degree and cannot therefore be ethically justified.

[24]Wareham and Nardini (2015), for example, propose a method of public deliberation to discriminate negligible from non-negligible risks with respect to the application of synthetic biotechnology.

[25]cf. the current legal discussion in Switzerland, in which adherence to the precautionary principle is recommended (Errass, 2018).

The question of how to deal with epistemic uncertainties and thus with precautionary situations is closely related to the question of how we generate knowledge. It also affects the political culture in which we make decisions involving technologies and uncertainty. The following recommendations therefore relate to the conditions under which knowledge is acquired and political decisions are made.

2. **Improving the reliability of risk assessments.** The data on which a risk analysis is based must satisfy scientific criteria. It is the responsibility of the scientific institutions to comply with these criteria, and they have their own mechanisms for doing so. The framework conditions of the scientific institutions should be strengthened in such a way that they are able to meet the criteria in a scientifically independent manner and can consistently demand that all actors comply with the scientific standards and justification requirements. Scientific data and assessments must also be verifiable and comprehensible in order to meet internal scientific controls, and thus satisfy scientific criteria. This involves granting access to all information necessary for scientific evaluation, including to divergent data that does *not* support a scientific thesis[26]. Furthermore, attention must be paid to promoting and cultivating diversity of perspectives and cross-sectional competences.

   Access to data and transparency of scientific assessments are also essential for decision-making authorities; they must be able to understand the plausibility of scientific data and how they have been assessed, in order to be able to make reasoned decisions. Moreover, they must be able to present the risk-related decisions that affect the public in a transparent and understandable manner.

   This is the only way to ensure that voters can form free and informed opinions, and thus that risk decisions in the political process can be reliable.

3. **Respecting the different roles of expert committees, on the one hand, and of decision-making authorities and the courts, on the other.** Decisions about dealing with new (bio)technologies in the environment have far-reaching consequences, which are of relevance to the whole of society. The decisions may therefore not be left to individuals, nor may the democratically legitimate authorities in charge of making these decisions delegate them to others.

   This also means that decision-making within specialized bodies advising the competent authorities must be subject to democratic control. This requires their decision-making process to be transparent and comprehensible, and majority opinions and minority positions must be presented openly and comprehensibly with justifications. Furthermore, given both the plurality of scientific opinions and the fact that the state may not delegate decisions in such matters, it follows that neither the decision-making authorities nor jurisdiction automatically accept the expert opinions of specialized advisory bodies. The decision-making authorities must therefore also have appropriately trained staff capable of critically following the plausibility checks and assessments made by the scientific institutions.

4. **Strengthening political awareness in dealing with technologies and uncertainties.** Decisions on how to deal with technologies involve uncertainties and possibly have far-reaching consequences. The decisions are based on risk assessments that involve making decisions about values. In democratic societies, the responsibility for these value decisions lies with the citizens, not with scientists. Awareness of this fact must also be raised among the employees of authorities who implement such value decisions when assessing individual cases. If they are involved in this decision-making process as specialists, they do so on behalf of the political authority. Their role as scientists in this context is thus different from that of their colleagues in scientific institutions.

CONCLUSION

The rapid development of new biotechnologies such as CRISPR-Cas systems and other genome editing processes opens up new opportunities and promises a wide range of applications, although it is yet to be seen whether all this potential can be realized. At the same time, the new technologies and their application potential confront us with considerable uncertainties. On the one hand, we do not know everything about how the new technologies function or about their impact to organisms on which they are applied. If the technologies and organisms, which have been altered by the processes, come into contact with the environment, this not only increases the complexity of possible interactions, but also our uncertainties.

Environmental law responds to this epistemic situation of uncertainty with the legal concept of the precautionary principle or precautionary approach. If serious damage is not merely conceivable, but there is also a scientifically plausible foundation for the fear that such damage could occur, then a precautionary obligation exists. It is concluded that the concept of precaution in environmental law and the precautionary measures to which it gives rise can also be justified ethically, irrespectively of the underlying ethical theory of risk.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

[26]In view of recent developments in science and education policy, care must be taken to ensure that conflicts of interest do not restrict impartial research at universities. Such restrictions not only compromise the independence of scientists but also alter the self-conception of scientific institutions. They may affect the quality of scientific data, influence the choice of research approaches and, at worst, lead to interest-based solutions and results. In all cases, such restrictions undermine confidence in the independence of science and the scientific quality of data and data assessment.

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Revisiting Risk Governance of GM Plants: The Need to Consider New and Emerging Gene-Editing Techniques

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New and emerging gene-editing techniques make it possible to target specific genes in species with greater speed and specificity than previously possible. Of major relevance for plant breeding, regulators and scientists are discussing how to regulate products developed using these gene-editing techniques. Such discussions include whether to adopt or adapt the current framework for GMO risk governance in evaluating the impacts of gene-edited plants, and derived products, on the environment, human and animal health and society. Product classification or definition is one of several aspects of the current framework being criticized. Further, knowledge gaps related to risk assessments of gene-edited organisms—for example of target and off-target effects of intervention in plant genomes—are also of concern. Resolving these and related aspects of the current framework will involve addressing many subjective, value-laden positions, for example how to specify protection goals through ecosystem service approaches. A process informed by responsible research and innovation practices, involving a broader community of people, organizations, experts, and interest groups, could help scientists, regulators, and other stakeholders address these complex, value-laden concerns related to gene-editing of plants with and for society.

Keywords: genetically modified plants, crop breeding, risk assessment, CRISPR (clustered regularly interspaced short palindromic repeats)/Cas9, transgenic plants

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INTRODUCTION

New and emerging gene-editing techniques being developed include clustered regularly interspaced short palindromic repeats (CRISPR), oligonucleotide directed mutagenesis (ODMs), meganucleases (EMNs), zinc finger nucleases (ZFNs), and transcription activator-like effector nucleases (TALENs). These new techniques open the possibility for editing genetic information and modulating gene expression in organisms in faster and more targeted ways. Gene-editing techniques raise the possibility of targeting, *in vivo*, a specific gene or sequence in the genome of virtually any species. Targeted gene modification can be the deletion, insertion or alteration of nucleotides in an existing molecule of DNA or RNA, as well as insertions or deletions of large sequences in specific target locations.

Regulators and scientists discuss whether gene-edited organisms should be subjected to the same risk assessment and management requirements as genetically modified organisms (GMOs). In general terms, GMOs require regulatory approval before environmental release and use in food and feed. Regulatory approval is informed by an assessment of risks to human health and the environment. An open question is thus whether and how the EU current framework applying to GMOs needs to be applied, adapted, and updated for new and emerging gene-editing techniques.

In this paper, we discuss the potential challenges new and emerging gene-editing techniques pose to established risk governance strategies. We focus on regulatory requirements for assessing health and environmental risks as established under EU Directives, and elaborate how biosafety research can strengthen risk assessment (RA) and management. At present, national frameworks in the EU Member States are transposing the EU-level framework laid out by the respective EU Directives and thus are harmonized with the general community framework. There are challenges with traceability and monitoring of products developed using new and emerging gene-editing techniques. In addition, risk assessment and management of genetically modified (GM) plants is constrained by limitations in transparency regarding public disclosure related to product development. We propose that the framework of responsible research and innovation (RRI) offers a useful way to improve GM risk governance research and practice for biosafety of crop development with new and emerging gene-editing techniques.

OVERVIEW OF THE REGULATORY LANDSCAPES FOR GMOs

The Scope of Current GMO Regulation

In considering challenges with risk governance of new and emerging gene-editing techniques, it is instructive to start with current regulations related to GMOs. European regulatory requirements that address environmental release of GMOs and of GM foods and feeds are established in EU Directive 2001/18/EC (originally 90/220/EC), in regulation (EC) No. 1829/2003 and its sister regulations (**Figure 1**), as well as in various national frameworks. Central to any regulatory requirement is an element of assessing risks to human, animal, and environmental health. At the pan-European-level, such risk assessments are based on a case-by-case approach and a stepwise procedure. The European Food Safety Authority (EFSA) provides scientific review and assessment of safety and environmental impact of GMOs, while the European Commission is responsible for risk management decisions.

Other countries, for example the United States, have not developed a new regulatory process for GMOs or gene-edited organisms. In the United States, depending on the genetic modification and the host organism, one or several United States federal agencies would be involved in GMO regulation, for example the Food and Drug Administration (FDA), the Environmental Protection Agency (EPA) and the Animal and Plant Health Inspection Service (APHIS) (Schuttelaar and Partners, 2015; Ishii and Araki, 2017). In the United States, gene-edited plants are not subject to specific regulatory requirements unless they have novel traits expressing, for example, herbicide tolerance or antibiotic resistance. Thus, for the United States framework not all novel traits trigger regulatory oversight, but only a defined range of traits of specific concern, e.g., compositional differences that are not GRAS, pesticidal properties or traits and genetic elements derived from organisms which are plant pathogens or that may induce plant pathogenicity.

Similarly, Canada has proceeded without developing new, GMO-specific regulatory requirements and uses already adopted regulatory frameworks. In Canada, GMOs fall under consideration of "plant with novel trait" a category which includes not only GMOs, but also plants with induced mutations, natural mutations, and exotic germplasm not previously grown in Canada (Smyth, 2017). The United States and Canadian regulatory frameworks focus primarily on human safety and environmental risk, the efficacy of the novel trait, and the intended use of the product. By contrast, other countries, for example, Norway, consider non-safety-related aspects of GMOs such as socio-economic considerations, ethical issues, and potential contribution to sustainable development (see the Norwegian Gene Technology ACT, 1993)[1].

Where Canada has adopted a product-based regulatory system and the United States has a hybrid system, Argentina and Europe have a process-based system. By adopting a process-based system, GMOs are regulated differently than other products (e.g., organisms and plants developed by other methods than GM technology) and according to a specific regulatory framework: this is the case in Europe. Those who argue against novel regulation to new and emerging gene-editing techniques object on the grounds of a product-based system of regulation. A main argument of this group is the final product—gene-edited organisms— contain comparable types of genetic changes (or mutations) to organisms originating from established methods of genetic modification, such as random mutagenesis techniques (e.g., irradiation).

Reviewing the regulatory landscape for GMOs reveals how fundamentally different approaches to regulation may continue, independent of the regulatory system the country has adopted, to divide national responses to risk governance of new and emerging gene-editing techniques. Based on this observation, Ishii and Araki (2017) argue for international efforts of regulatory harmonization, for example by the Cartagena Protocol on Biosafety (CP). At the international level, the Convention on Biological Diversity (CBD) has served as the umbrella treaty for the CP since 2000 (entry into force in 2003). The CP agreement aims to ensure the safe handling, transport, and use of living modified organisms (LMOs) resulting from modern biotechnology, taking into account possible adverse effects on biological diversity as well as risks to human health. The CP treaty offers a benchmark and guide for many developing countries exploring adoption of GMO regulatory frameworks. Further, the

[1]Norwegian Gene Technology Act is available in English at: https://www.regjeringen.no/en/dokumenter/gene-technology-act/id173031/

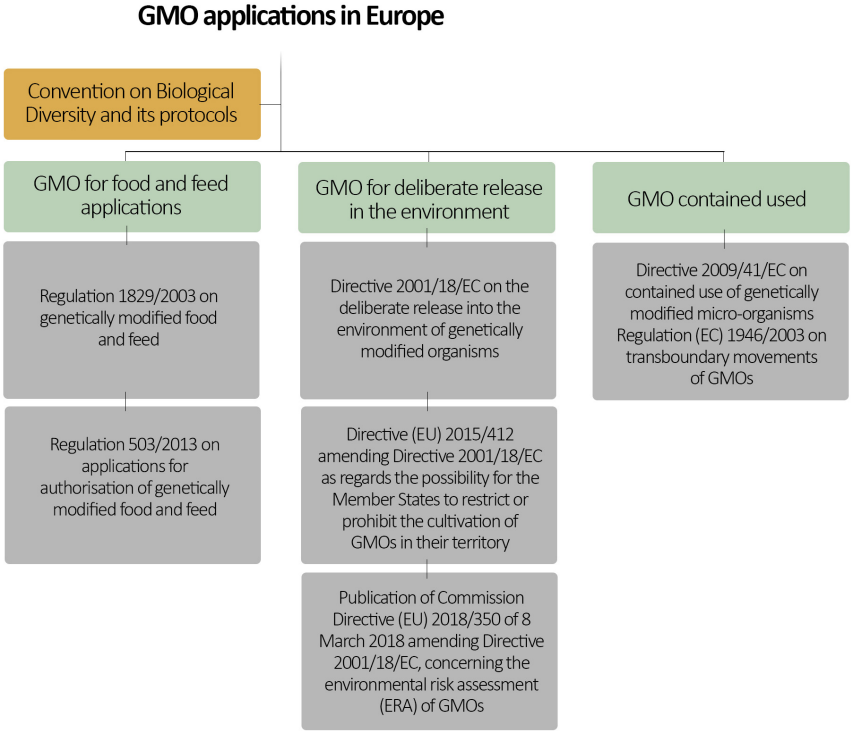

FIGURE 1 | European regulatory framework for GMOs. Note that European Union is also a signatory to the United Nations Convention on Biological Diversity and its related protocols. Therefore, it can be also considered as part of the European GMO regulatory framework.

CBD and CP have established interactive platforms for sharing information and knowledge about international biosafety issues, including unintentional transboundary movement of LMOs and emergency measures for unauthorized GMO escape.[2] Under the CP treaty, organisms altered with new and emerging gene-editing techniques would seem to fall under the agreement for safe handling, transport and use of LMOs—indeed, the LMO definition was left intentionally open to remain relevant for precisely such future developments (Mackenzie et al., 2003). The treaty has been signed and ratified by some 170 countries, including the EU and Norway. Several countries, however, including Russia, United States, Canada, and Argentina are not parties to the CP, which may hamper any international effort of regulatory harmonization.

The first country to adopt regulation specifically for new and emerging gene-editing techniques was Argentina (Resolution No.173/2015). Argentina has issued a Resolution which specifies criteria to assess whether certain products are covered by the definitions included in their biosafety law. An important criterion is whether a product contains a "novel combination of genetic material" (Whelan and Lema, 2015). Brazil issued a similar resolution earlier this year (Resolution No. 16/2018), which includes a criterion to determine the regulatory status of new and emerging gene-editing techniques, for example if products using these techniques will be considered a GMO as per Brazilian Biosafety Law. Despite these early actions, most countries in Europe and elsewhere, and at international levels (e.g., European Union, OECD, CBD, the CP) are still discussing whether and how to adapt GMO risk governance frameworks to account for new and emerging gene-editing techniques.

Regulatory Challenges for New and Emerging Gene-Editing Techniques

National responses to the growing use of new and emerging gene-editing techniques in plants raise questions of whether such developments (a) might be exempt from current GMO regulations, and/or (b) if existing regulations require revision and adaptation to appropriately manage new techniques and resulting products (Wolt et al., 2016). As noted above, the main argument for exemption from current GMO regulation is the similarity of organisms altered with new and emerging gene-editing techniques to organisms originating from random mutagenesis (e.g., irradiation). The argument of exemption based on similarity posits that gene-edited organisms are indistinguishable from products created by already exempted processes (Jones, 2015b; Davison and Ammann, 2017). A central assumption of this argument is that any risks associated with new and emerging techniques for gene-editing will also be similar and equal to, or less significant than risks associated with exempted techniques or products (Hartung and Schiemann, 2014; Sprink et al., 2016; Globus and Qimron, 2018).

[2]The Convention on Biological Diversity Clearing House Mechanism platform at https://www.cbd.int/chm/ and the Biosafety Clearing House platform set up by the Cartagena Protocol on Biosafety at https://bch.cbd.int/.

BOX 1 | European authorities' definition and categorization of gene-editing techniques.

Site-Directed Nucleases-1 (SDN-1) generates site-specific random mutations (changes of single base pairs, short deletions and insertions) by non-homologous end-joining. During SDN-1, no repair template is provided to the cells together with the SDN. Therefore, in the case of insertions, the inserted material is derived from the organism's own genome, i.e., it is not exogenous.

Site-Directed Nuclease-2 (SDN-2) generates site-specific desired point mutation by DNA repair processes through homologous recombination (specific nucleotide substitutions of a single or a few nucleotides or small insertions or deletions). During SDN-2, an exogenous DNA template is delivered to the cells simultaneously with the SDN for achieving desired nucleotide change via homology dependent repair.

Site-Directed Nuclease-3 (SDN-3) targets delivery of transgenes (insertions) by homologous recombination. Exogenous DNA fragments or gene cassettes up to several kilo base pairs (kbp) in length can be inserted to a desired site in the genome or a gene.

The EFSA GMO Panel opinion addressing the safety assessment of plants developed using Zinc Finger Nuclease and other Site-Directed Nucleases with similar function (EFSA Panel on Genetically modified organisms (GMO), 2012) and the Institute for Prospective Technological Studies and Institute for Health and Consumer Protection (both from the Joint Research Centre at the European Commission) (Lusser et al., 2011) have set forth three major categories of new and emerging gene-editing techniques (**Box 1**).

European Food Safety Authority holds that products developed using SDN-3 techniques would be categorized as GMOs and regulated under EU Directive. There has been a disagreement as to whether products arising from use of SDN-1 or SDN-2 might be exempt (Sprink et al., 2016). For example, while waiting for a decision from the European Court of Justice (EJC), Sweden decided that gene-editing products with no recombinant DNA insertions may (e.g., SDN-1), on a case-by-case basis, be exempted from GMO regulation (Nature Editorial, 2017). The recent EJC ruling,[3] however, now clarifies that all SDN techniques fall under the EU Directive.

The scope of the EU legislation and Article 2(2) of the Release Directive (Directive 2015/412 amending Directive 2001/18/EC) provide the definition of a GMO. These laws define a GMO as, "An organism, with the exception of human beings, in which the genetic material has been altered in a way that does not occur naturally by mating and/or natural recombination." In Annex IA, part 1 scopes techniques of genetic modification, stating:

> *"Techniques of genetic modification referred to in Article 2(2)(a) are inter alia [not an exhaustive list]: recombinant nuclei acid techniques involving the formation of new combinations of genetic material by the insertion of nucleic acid molecules produced by whatever means outside an organism, into any viruses, bacterial plasmid or other vector system and their incorporation into a host organism in which they do not naturally occur but in which they are capable of continued propagation; techniques involving the direct introduction into an organism of heritable material prepared outside the organism including micro-injection, macro-injection and micro-encapsulation; cell fusion (including protoplast fusion or hybridization techniques where live cells with new combinations of heritable genetic material are formed through the fusion of two or more cells by means of methods that did not occur naturally."*

Whereas Article 3 and Annex IB specifies exemptions to the Directive (Zetterberg and Björnberg, 2017). Excluded techniques include mutagenesis and cell fusion, including protoplast fusion, of plant cells of organisms which can exchange genetic material through traditional breeding. Annex IB lists techniques that do produce a GMO under the Directive but are exempt on the condition that they do not involve the use of recombinant nucleic acid molecules or GM organisms other than those produced by one or more of the techniques/methods listed in Annex IB.

In summary, the EU Directive provides grounds for the argument for exemption of gene-edited plants due to the potential similarity between gene-edited plants and those originating from mutagenesis techniques. However, argument for exemption may be limited because (a) the Directive does not define mutagenesis (Eriksson et al., 2018), and (b) the argument solely lies on the technique used (i.e., mutagenesis). Ambiguity arises because there are a variety of mutagenesis techniques that can be applied (e.g., irradiation, CRISPR, ODM, etc.) and thus, the Directive does not acknowledge whether the process of gene-editing by each of these techniques leads to the formation of an organisms covered by the GMO definition. The use of the term 'mutagenesis' may therefore lead to the false impression that there is only one mutagenic technique in place.

Heinemann (2015) argues that the reasoning based upon distinguishability of products and not genetic engineering techniques is not relevant to the Cartagena Protocol or the EU Directive because technique is neither relevant to the definition of a GMO nor to the description of a process by which a GMO is made. Moreover, distinguishability is a function of existing technology. As technologies change, so might the ability to distinguish products from each other. We acknowledge that not all products of new and emerging gene-editing techniques are indistinguishable. For example, in certain cases of multiplexed editing, where edited genes are located in multiple chromosomal sites, or other products where characterization and traceability is possible (e.g., large deletions with SDN-1 techniques) (c.f., Duensing et al., 2018). In the context of debates about regulation of new and emerging gene-editing techniques, however, it seems problematic to be at once a new technique and a technique associated with a long history of safe use. This issue is a core focus of the recent ECJ ruling on the interpretation and validity of Articles 2 and 3 of, and Annexes IA and IB to, Directive 2001/18/EC on the deliberate release into the environment of GMOs.

[3]Provisional text of the ECJ ruling is available in English at: http://curia.europa.eu/juris/document/document.jsf?text=&docid=204387&pageIndex=0&doclang=EN&mode=req&dir=&occ=first&part=1&cid=709582#Footnote*

According to the provisional text of the ECJ ruling, organisms and products of new and emerging gene-editing techniques will fall under GMO Directive. The court is clear that these new techniques, "Alter genetic material of an organism in a way that does not occur naturally" (paragraph 28, page 8 of the ECJ provisional text in English). Moreover, the ECJ opinion draws on the fact that these new techniques are not like "those which have conventionally been used in a number of applications and have a long safety record" (paragraph 26, page 8 of the ECJ provisional text in English). Instead of focusing on how mutagenesis techniques might produce "undistinguishable" products, the ECJ viewpoint is that it is impossible to determine with certainty the existence and extent of risks presented by new directed mutagenesis techniques without a premarket RA. The ruling further states, "For the purpose of interpreting a provision of EU law, it is necessary to consider not only its wording but also the context in which it occurs and the objectives pursued by the rules of which it is part" (paragraph 42, page 9 of the ECJ provisional text in English). The ECJ further reiterated the precautionary principle which was taken into account in the drafting of the directive and so also must be taken into account in implementation.

SUITABILITY OF CURRENT RISK GOVERNANCE OF GMO PLANTS

Current Guidance on Risk Assessments of GMOs Under European Regulation

Guidance for evaluating the impact of genetically modified (GM) plants and plant-derived products in the EU is provided by two documents based on Directive 2001/18/EC and Regulation (EC) No. 1829/2003: guidance on Environmental Risk Assessment (ERA) of GM plants (EFSA Panel on Genetically modified organisms (GMO), 2010) and guidance for risk assessment of food and feed (RAFF) derived from GM plants (EFSA Panel on Genetically modified organisms (GMO), 2011; **Figure 2**) (**Box 2**). The ERA focuses mainly on the impact of GMOs on the environment, including humans and animals as components of the environment. By contrast, RAFF focuses only on the health of humans and animals upon consumption of GM foods and feeds.

Comparative safety assessment, as a general principle of risk assessment, is applied in both guidance documents. In risk assessment, hazards are defined as characteristics of the GM plants (or food and feed) which may cause adverse effects. Comparison is made to understand potentially harmful differences between a genetically modified plant (or food and feed) and the unmodified parent (or appropriate comparator).

In ERA and RAFF, risk assessment seeks to identify and characterize intended and unintended effects of genetic modification with respect to potential impact on environmental, human, and animal health. Data that can reveal these effects are derived from molecular characterization; compositional analysis; studies of interactions between genetically modified-plant and the environment as well as agronomic and phenotypic characterization.

For ERA, the process of correctly identifying potential hazards begins with systematic description of the case under assessment. Three components are considered, namely (i) the plant; (ii) the new trait and its intended effects as well as the phenotypic characteristics of the GM plant; and (iii) the receiving environment (**Box 2**, Step 1), which is when the scope of an ERA is defined. Scientific data to identify potential hazards, which are generated by practical testing of the GM plant, as well as the extent to which the receiving environment could be exposed to any identified hazard is estimated (**Box 2**, Steps 2 and 3) within the scope defined in Step 1. Resulting data are fed into subsequent steps to inform the overall outcome of ERA.

As stated in **Box 2**, EFSA has identified specific risk areas for which hazard characterization of a GM plant must be conducted, guided by specific protection goals (e.g., biodiversity conservation and ecological functions) formulated in Step 1, **Box 2**. Specific risk areas include persistence and invasiveness of the GMO, plant-to-plant gene flow, plant to microorganism gene transfer, interaction of the GMO with target organisms, interaction of the GMO with non-target organisms, impact of the specific cultivation, management and harvesting techniques, and effects on human and animal health (EFSA Panel on Genetically modified organisms (GMO), 2010). For example, hazard characterization in the risk area of "persistence and invasiveness" would require species-specific background knowledge of reproductive biology, weediness, invasive and persistence characteristics, hybridization and introgression potential with any compatible relatives. For viable propagating GM plants, i.e., GM plants that can germinate and thrive in the receiving environment, additional information according to a tiered 3-stage approach is required under current EU regulations.

The purpose of the information in stage 1 is to deduce whether the GM plant and its progeny can grow, reproduce and hybridize under the climatic and growth conditions of the specific receiving environment in the EU, and how the phenotypic characteristics (in particular growth and reproduction) compare to conventional counterparts. Answers to these questions are provided by collating information on seed germination characteristics, phenotype under agronomic conditions, reproductive biology and seed persistence (EFSA Panel on Genetically modified organisms (GMO), 2010). Information is further required in stage 2 for plants that can grow overwinter and/or can transmit genes to compatible relatives. The most direct way to answer this question is to conduct experiments in representative sites over a 2-year minimum period in the proposed receiving environment, as relative fitness is a function of environmental context (Birch et al., 2007). For GM plants with existing relatives or able to form feral population in the receiving environment, additional information is required in stage 3 to determine whether the GM trait confers fitness advantages to the GM plants, and whether the GM traits is capable of altering the fitness of compatible relatives or feral population in the new environment (EFSA Panel on Genetically modified organisms (GMO), 2010).

For RAFF from GM plants, hazard identification and characterization begin with molecular characterization of the GM plant. Molecular characterization is followed by comparative

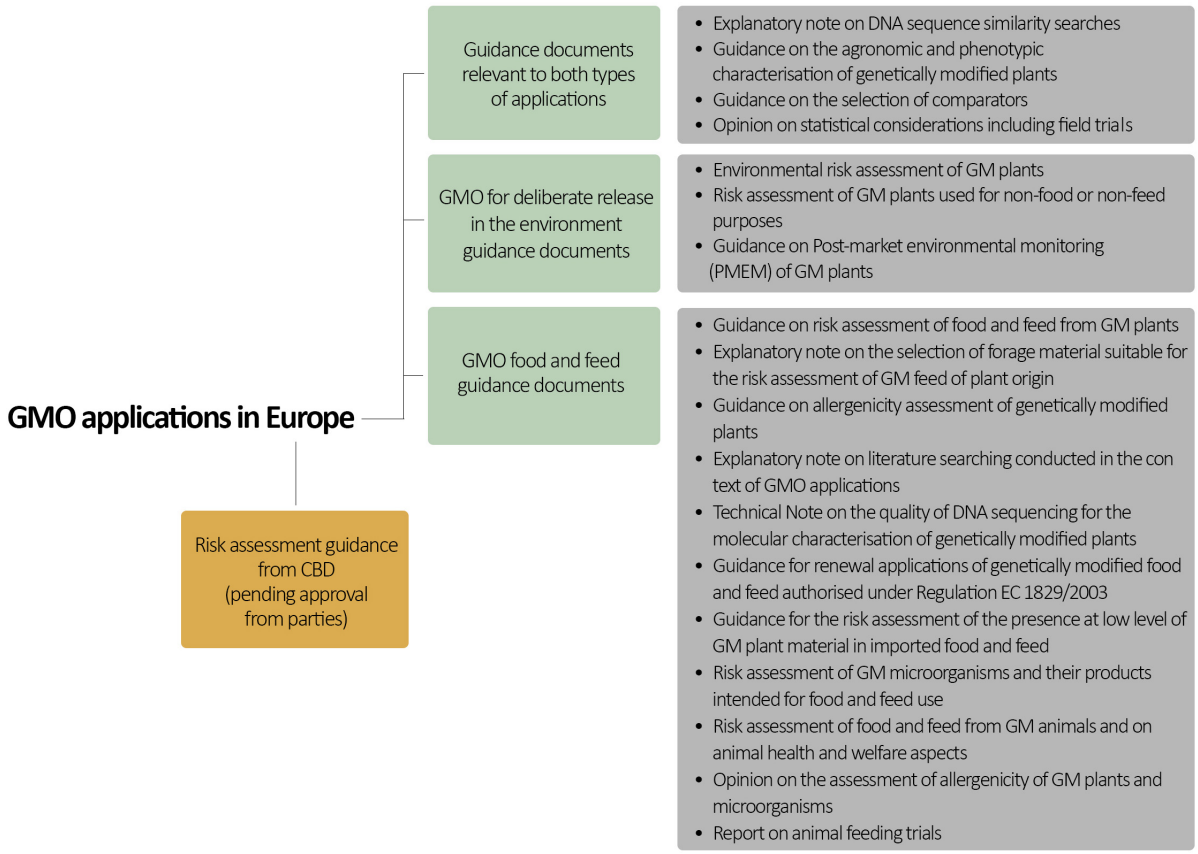

FIGURE 2 | European scientific guidelines for GMO risk assessment. Note that this is not an exhaustive list of all relevant guidance documents.

analysis of relevant characteristics of the GM plant and its comparator(s). The aim of these activities is to identify and characterize both intended and unintended effects on human and animal health (excluding other components of the ecosystem). The unintended effects may be due to genetic rearrangement or metabolite changes due to genetic modifications and can be detected by analysing the flanking regions of the inserts and by proteomic and/or metabolomic analyses of the end-product. Inserts are likely to affect known or predicted functions of endogenous genes. The EFSA guidance document requires in-depth information describing the identity of the nucleic acid intended for transformation, vector sequences potentially delivered to the GM plant, and characteristic of the DNA insert (EFSA Panel on Genetically modified organisms (GMO), 2011). In general, molecular characterization seeks to provide information on whether genetic modifications raise health concerns with regard to the interruption of endogenous genes, leading for example, to production of toxins, allergens, and/or anti-nutrients.

Challenges for Risk Assessment of New and Emerging Gene-Editing Techniques

The present debate on how new and emerging gene-editing techniques will be regulated lacks a fundamental discussion on whether current risk assessment methodologies are adequate to analyze organisms arising from these techniques. A consequence of the recent ECJ ruling, which considers products of new and emerging gene-editing techniques as GMOs, is the question of adoption or adaptation of the current GMO risk assessment and risk management procedures for products arising from the new techniques. In this section, we look first at the potential challenges of adopting the current EU GMO framework for ERA of plants arising from the new directed mutagenesis techniques, and subsequently highlight the challenges of using the current guidelines for food and feed products from new techniques. We close with challenges to risk assessment in general, in particular with traceability and detection.

Environmental Risk Assessment

The current EU ERA framework was designed for GMOs produced via classical techniques of genetic modification (e.g., biolistic particle delivery or agro-bacterium mediated methods). Products of new and emerging techniques, according to the ECJ ruling, are all classified as GMOs, thus, raising a question of how the framework will be implemented. In particularly, an open question remains how to adapt guidance to support assessment of products arising from new and emerging gene-editing techniques (Lusser et al., 2011; EFSA Panel on Genetically

BOX 2 | Steps in ERA of GM plants.

Problem formulation and hazard identification

In this first step, the assumptions underlying the ERA are explicitly formulated in the form of a problem statement, involving identification of the potentially hazardous characteristics of the GM plant, the nature of the hazards and exposure paths of the environment to harm associated with the hazards. By comparing the GM plant to its non-modified parent (or other appropriate comparators), differences in the GM plants that may constitute harm and their potential environmental consequences can be identified. Quantifiable assessment endpoints and testable hypotheses that will guide data generation and assessment are also defined.

Hazard characterization

During hazard characterization, the environmental harm potentially associated with each identified hazard is evaluated according to the set out hypotheses, and expressed quantitatively and/or qualitatively. In qualitative expression, the categorical terms "high," "moderate," "low," or "negligible" are employed to express the scale of severity of identified hazards.

Exposure characterization

In this step, the likelihood of the adverse effect occurring is estimated. Similar to hazard characterization, "likelihood" is denoted using ordered categorical descriptions of "high," "moderate," "low" or "negligible." Quantitative expression of 0 to 1 can also be used to express likelihood where 0 represents impossibility and 1 represents certainty.

Risk characterization

An estimate of the risk of adverse effect is made for each identified hazard at this stage. This is achieved by combining the magnitude of the consequences of the hazard and the likelihood that the consequences related to the hazard will occur, and expressed quantitatively or semi-quantitatively.

Risk management strategies

The risk management strategies aim to reduce the identified risks to a level of no concern, and considers defined areas of uncertainty. The risk management is described in terms of hazard and/or exposure reduction, and the consequent reduction in risk quantified when possible. Additionally, the reliability and efficacy of the measures used to mitigate the risks are assessed at this stage.

Overall risk evaluation

This is the overall risk evaluation of the GM plant taking into consideration the estimated risk, levels of uncertainty, knowledge gaps, assumptions made in arriving at the risk level, and the proposed risk management strategies. The overall risk evaluation results in informed (in qualitative or quantitative terms) guidance to risk managers. Justifications for why certain risks are acceptable are also provided at this stage, and may give rise to certain specific activities such as post market environmental monitoring.

In addition to the above six steps, the EFSA identified seven cross-cutting consideration and specific areas of risks to be addressed during ERA of GM plants (EFSA Panel on Genetically modified organisms (GMO), 2010).

Note: Steps in RA of food and feed from GM plants are described in EFSA Scientific Committee (2011).

modified organisms (GMO), 2012; Jones, 2015a; The Norwegian Biotechnology Advisory Board, 2018).

Given that a framework is only as good as its weakest elements, one strategy to determine the suitability of the current EU ERA framework for new directed mutagenesis techniques is to focus in particular on elements persistently critiqued by the scientific community (EuroActive, 2008; Hilbeck et al., 2011). Based on contemporary scientific critiques, the following elements of ERA of new directed mutagenesis techniques might be adopted or adapted: the focus of risk assessment; test-organisms; effect testing; post-release monitoring; and risk management.

The focus

Environmental Risk Assessment of new directed mutagenesis techniques may necessitate change in focus to include the entire crop plant, given that products of new and emerging gene-editing techniques may differ in complexity from conventional GM plants. This difference will depend on the extent of alterations engineered into a product using new techniques. At present and based on the concept of substantial equivalence, only change in trait or the newly expressed protein is emphasized in the implementation of the framework (Eu-Directive, 2001; European Commission, 2002). In addition, expansion of the scope of test compounds to include toxins and antitoxins may be necessary. Related, a lack of clear guidelines on cut-off, i.e., limit of concern, for substantial equivalence between GM- and non-GM plants is another element of test focus receiving critique (Millstone et al., 1999).

Test organisms

Choice of test organisms for evaluating target and non-target effects of products of new and emerging gene-editing techniques may necessitate a case-by-case selection of suitable testing species. Suitable testing species need to be representative of relevant ecological functions of the receiving environment, different from the current standard set of universal testing species that are representative of trophic levels of a generic ecosystem (OECD, 1981). This position has also been proposed as a remedy to the deficit inherent in the use of the current framework for ERA of GM plants (Hilbeck et al., 2011).

Effect-testing

In the current framework, where substantial equivalence is established, the stressor for which chronic effect, indirect effect and interaction effect testing is conducted is the new trait (e.g., an expressed protein or toxin, and not the whole plant) (Romeis et al., 2006, 2007). For products of new and emerging gene-editing techniques with a targeted knockout mutation, with no *a priori* known altered primary compound, no stressor may be identified, therefore no effect test can be deemed relevant. In this specific type of example, a focus on the entire GM plant also becomes necessary for robust effect-testing in ERA (Romeis et al., 2006, 2007).

Post-release monitoring

With new and emerging gene-editing techniques, it may be difficult to carry out post-release monitoring if similar mutations can also be found in conventional, not genetically modified varieties. This is a challenge unique to new and emerging gene-editing techniques, for example in the case of CRISPR/Cas9 where mutations involving a few nucleotide base-pairs which can also be achieved by conventional breeding techniques, or can occur naturally, is engineered into the target. For such products (especially SDN-1 category), it will be impossible to identify and associate the engineered modifications with a specific technique without prior knowledge of the type of modifications (or the techniques used to achieve the modifications). Thus, certain products of the new techniques of site directed mutagenesis cannot be detected, traced or monitored based on the requirements of the current framework, which needs the presence of marker sequences to identify a modified organism.

Many crops are changed using gene-editing techniques to delete various parts of target genes for either knocking out or change the gene functions. These crops are sometimes referred to as transgene-free crops, because even though the genetic composition is changed, no transgene DNA is integrated in the genome of these plants (Ricroch et al., 2017). The aim of deletion is most often elimination or changing the gene expression implicated with virus infections or other plant pests, rendering the crop more resistant to the particular infectious agent (Ricroch et al., 2017).

While advantageous for cultivated crops, such genetic changes may infer a huge selective advantage and thus create a high positive selection if pollen from cultivated fields are spread to wild relatives. Such gene flow is a major concern for GM crops and may be a realistic outcome of cultivation of disease resistant gene edited crops, unless co-existence measures are enforced (growing distance to wild population etc.). Since many genes have several functions, it is possible that knocking out or changing a specific gene function, may in addition to the intended effect, also alter unintended pathways. Assessing unexpected, unintended changes requires untargeted whole-genome profiling, post-release monitoring and general surveillance.

Risk management

In ERA, decisions of the risk managers are guided by the outcome of the scientific risk assessment (**Box 2**), which has risk management strategies as a part of the framework (Step 5, **Box 2**), where the Applicant outlines measures (including the reliability of the proposed measures) to reduce any identified risks. Therefore, if risk assessors lack experience evaluating the potential risks of new and emerging gene-editing techniques, this will reasonably impact the information provided to risk managers.

Risk Assessment of Food and Feed

Beyond the challenges with ERA of new and emerging gene-editing techniques listed above, current regulatory requirements are based on risk assessment developed and available when regulatory discussions on GMOs were just starting in the 1990s. It is therefore also necessary to discuss how to revise and adapt existing methods to better cover such challenges at the frontiers of biotechnology. Investigating the suitability of new methods implies assessing whether new tools, such as bioinformatics, and next generation sequencing or other -omics techniques, can complement or replace and thus contribute constructively to comparative assessment—or even to the assessment of whole gene-edited organisms when appropriate comparators are unavailable.

When it comes to new methods for RAFF, molecular characterization of a gene-edited organism may therefore need to take into consideration two main aspects of the genetic modification. One aspect is related to the spectrum of changes at the intended site (i.e., the nucleotide changes at target sequence). The second aspect refers to the spectrum of sites that have been changed. Both considerations are necessary because confining the change to the intended template only is not yet possible. Unintended effects might arise from both target site and off-target sites. Thus, after the procedure, intended products must be separated from unintended products (**Figure 3**).

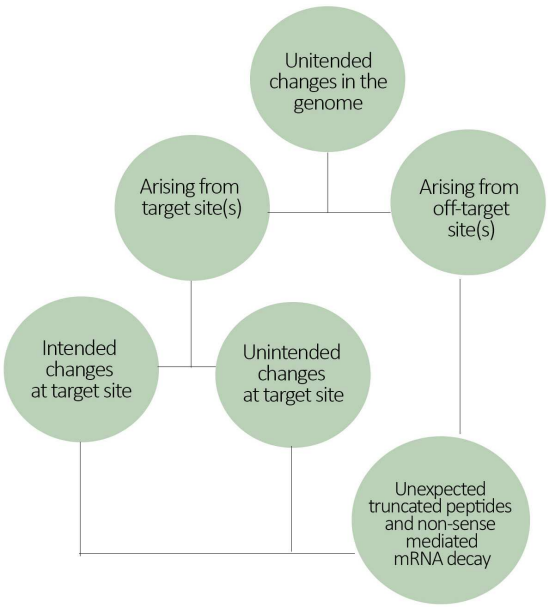

FIGURE 3 | Flowchart illustrating the potential source of gene-editing mediated genome changes. Genome changes can vary in location (target and/or off-target sites), in quantity (how many sites were changed) and also in quality (deletion, insertion, substitution of nucleotides in a sequence). Therefore, genome changes at target site can have both intended and unintended effects depending on the quality/type of the change.

Risk Assessment: Detecting Unintended Changes From New and Emerging Gene-Editing Techniques

Current EFSA guidelines for environmental risk assessment and risk assessment for GM foods and feeds start from identifying potential hazards associated with intended and unintended molecular changes. Potential hazards are assessed based on molecular description, comparative data with a non-GM counterpart followed by toxicological, allergenicity and

nutritional assessments (EFSA Panel on Genetically modified organisms (GMO), 2010, 2011), as well as routine PCR and sequencing protocols and standard protein quantification protocols such as Western blots, ELISA testing or other spectrophotometry methods for assessing expression of newly introduced proteins (e.g., EFSA Panel on Genetically modified organisms (GMO), 2011; AHTEG, 2016). The idea behind hazard characterization and identification is to provide sufficient information on the description of the techniques used for the genetic modification, the source and characterization of nucleic acids used for transformation, nature and source of vector(s) used including nucleotide sequences intended for insertion, information on the sequences actually inserted/deleted or altered and the expression of the sequences as well as genetic stability of the inserted/modified sequence and phenotypic stability of the GM plant.

New and emerging gene-editing techniques might generate truncated polypeptides and/or non-sense-mediated mRNA decay either intentionally or unintentionally as part of a knockout process. Whereas such products are considered an unintended effect in transgene-based GMOs (Rosati et al., 2008), the desired phenotype in this case (i.e., resistance to a pest or an herbicide) is obtained by the nucleotide change in the gene of interest that generates the production of the non-functional gene products (Hussain et al., 2018).

In silico analysis can help identify mRNA variants and putative peptides derived from truncated DNA sequences or from potential read-through events, which should be then followed by *in vivo* RNA sequencing analysis. Characterizing peptide or protein variants is technically challenging because it relies on prior knowledge about binding sites to isolate the protein from an extract. If the binding site is lost or altered due to the genetic transformation, it means that this peptide variant will not be picked up for further analysis. If detected, it may not be fully distinguished from wild-type peptide variants that are also present in the sample. A recent review of detection methods for on-target changes generated by CRISPR and other sequence-specific nucleases is provided in Zischewski et al. (2017). As a specific example, MON810 and RR Soybean transgene cassettes have been found to produce read-through products which were further processed, resulting in four different RNA variants from which the transcribed region of the nopaline synthase terminator (tNOS) was completely deleted in soybean (Windels et al., 2001). In the case of MON810, RT-PCR performed in the 3′ end region of the transgene cassette produced cDNA variants of different length. An *in silico* translation of these transcripts identified 2 and 18 putative additional amino acids in different variants, all derived from the adjacent host genomic sequences, added to the truncated CRY1A protein with no homology with any known protein (Rosati et al., 2008).

Detecting unintended off-target changes can be more challenging than detecting changes at target sites because the number and position of nucleotide changes are unknown. There are also no data or guidance documents on test-methodologies to addresses unintended effects occurring due to off-target activity. If off-target effects occur within a gene, loss of gene function (truncation or gene deletion) or alteration of protein affinity or function (amino acid substitution) could be a possible outcome. Outside of protein coding genes, unintended alterations in promoters, introns or terminators could significantly alter gene expression. Plant allergens are also a major concern (Hoffmann-Sommergruber, 2000) and alterations of such allergens may constitute a health risk for human or animal consumption of plant foods. Screening for off-target sites at a genome-wide scale may be daunting, but in light of new directed mutagenesis techniques may be a necessary task for assessing the safety of commercialized products. A few approaches have been developed to investigate off-target activity of CRISPR modifications. These have been categorized into four major approaches: (i) *in silico* prediction, (ii) *in vitro* genome-wide assays, (iii) cell-based assays and (iv) *in vivo* screening.

In silico tools basically include all available software which have their own computational algorithms that identify likely off-target sites based on the sequence of the guide RNA. Pre-selected sites can be checked using the same methods described for target site detection and identification. Addgene's team has created an online spread-sheet-based tool that compares these softwares and provides scores to each of their features so that a user can choose according to her or his needs. As a result, the tool generates a ranking of most suitable software (Addgene, 2017).

Many of the CRISPR/Cas9 design tools include information about potential off-target sites in the genome of interest, but it is important to keep in mind that not every algorithm searches for every kind of off-target effect (e.g., DNA or RNA bulges). It has also been observed that analyses from *in silico* predictions are not always correct and their results don't always align because the CRISPR/Cas9 system is not completely understood (Zischewski et al., 2017).

In vitro and cell-based assays are mainly developed to search for CRISPR/Cas9 DSBs fingerprints. Digested genome sequencing, or Digenome-seq, is an *in vitro* assay that has become increasingly popular since its introduction in 2015 (Kim et al., 2015). Two newer methods are now also available, CIRCLE-Seq and SITE-Seq (Cameron et al., 2017; Tsai et al., 2017). Yet, these methods collectively remove genomic structural context. On the other hand, cell-based assays use different techniques to identify double-stranded breaks in genomic DNA within the cell environment. There are currently three approaches: BLESS (breaks labeling *in situ* and sequencing), GUIDE-Seq (genome-wide unbiased identification of DSBs enabled by sequencing), LAM-HTGTS (linear amplification-mediated high-throughput genome-wide translocation sequencing) (Crosetto et al., 2013; Tsai et al., 2015; Hu et al., 2016). However, Cas9 pharmacokinetic profile of the delivered components across cell and tissue types, especially the form factor of the gene editing components (DNA, RNA, or protein) and the delivery vehicle (viral or non-viral) is still a critical and underexplored determinant of Cas9 specificity (Tycko et al., 2016). Every time a different *in vitro* or cell-based assay is performed, a different off-target outcome might thus be expected. This potential variability makes it difficult to integrate across observations in a systematic, data-driven way. Consequently, these parameters are not taken into account by the majority of available off-target prediction tools.

Recently, the successful use of CRISPR in human cells has been connected to a selection process in CRISPR treated cells and shows that there may be other unique risk related factors to gene-editing, which are not discovered by searching for off-target DNA changes. Two papers showing that human polypotent stem cells that are treated with CRISPR may acquire mutations in P53 (Ihry et al., 2018) and immortalized human retinal pigment epithelial cells successfully treated with CRISPR may be exposed to a selection process against functional p53 (Haapaniemi et al., 2018). Even though these are experiments in human cells, the potential relevance for other species, including crops, should not be overlooked. The results may indicate that the successful integration of CRISPR edits could be impacted by genes connected to cell cycle arrest and DNA repair. If that is the case, the CRISPR induced selection of mutant cells may also occur in other species. A number of studies claim high precision and low to no off-target activity of CRISPR/Cas9 (e.g., Feng et al., 2018; Wei et al., 2018); however, whole genome sequencing (WGS) has recently documented off-target activity does in fact occurs in animals (Anderson et al., 2018) and plants (Braatz et al., 2017). When it comes to reducing off-target activity, gRNA design including RNA to DNA nucleotide replacements (Yin et al., 2018), length and composition of gRNA binding domain (Cho et al., 2014) as well as mismatched between gRNA and target DNA (Fu et al., 2014) seem to play a role. However, off-target activity has not been investigated to the extent of understanding thoroughly what governs changes outside the intended loci in the genome.

According to current understanding, the PAM (protospacer adjacent motif) sequence and its immediate upstream and downstream nucleotides, GC content of the gRNA, and epigenetics and chromatin structure of the target, each also have potential roles in off-target activity (reviewed in Jamal et al., 2016). Recently it has become evident that not only at which nucleotide CAS9 cuts, but also the sequence composition at the target, determines the outcome of the plant repair process (Vu et al., 2017). This indicates that not only gRNA binding but also the targeted sequence composition, will dictate factors such as the size of deletions and incorporation of mosaicism at the cut site.

DISCUSSION: THE NEED FOR NEW MOLECULAR CHARACTERIZATION AND TRACEABILITY METHODS AND A RESPONSIBLE RESEARCH AND INNOVATION APPROACH TO RISK GOVERNANCE OF NEW AND EMERGING GENE-EDITING TECHNIQUES

Molecular Characterization and Traceability of New and Emerging Gene-Edited Plants

Despite rapid progress of Cas9 specificity with marked improvements in guide RNA selection, protein and guide engineering, novel enzymes, and off-target detection methods; Cas9 protein still has been shown to bind and cleave DNA at off-target sites. To address the present limitations associated with *in silico* predictions as well as *in vitro* and cell-based testing of potential off-target sites, the ultimate unbiased method for measuring Cas9 off-target activity across the genome is WGS on the actual gene-editing organism (**Figure 4**). WGS provides a high-resolution, base-by-base view of the entire genome and is able to capture large and small variants that might otherwise be missed (e.g., if other targeted approaches were used). Consequently, WGS helps to identify potential unintended variants for examination in follow-on studies of gene expression and phenotypic analysis (Wang et al., 2018).

Whole genome sequencing strategies are based on high-throughput sequencing technologies such as Illumina dye sequencing, pyrosequencing, and SMRT sequencing. All of these technologies employ a basic shotgun strategy, namely, parallelization and template generation via genome fragmentation. More recently, nanopore sequencing has emerged as a new technique that performs "strand sequencing" in which intact DNA polymers through a protein nanopore, sequencing in real time as the DNA translocates the pore (Ambardar et al., 2016).

There are only a few studies that have applied WGS to investigate off-target activity of CRISPR *in vivo* systems. WGS has been applied for detecting off-target mutations by Cas9 in Arabidopsis (Feng et al., 2014), rice (Zhang et al., 2014), and tomato (Nekrasov et al., 2017). Unfortunately, these studies either looked only at potential off-target sites predicted by computer programs (bias analysis) or fell short of full analysis of all the

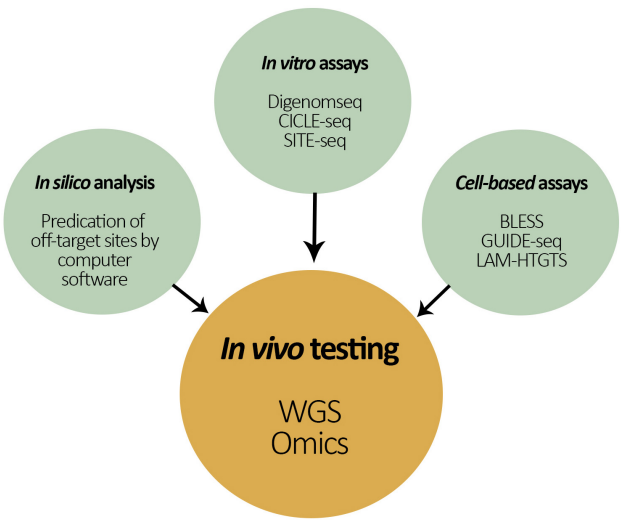

FIGURE 4 | Schematic representation of the different approaches to test for unintended changes in the genome of a gene-editing organism. In the center is the proposed '*in vivo* testing approach' by both whole genome sequencing (WGS) and 'omics' techniques for the assessment of off-target effects in the recipient's genome.

mutations identified by WGS in edited plants (Tang et al., 2018). A recent paper investigated the degree to which GUIDE-Seq analysis predicted off-target changes by sequencing the whole genome of gene-editing mice embryos (Anderson et al., 2018). The results showed that 30 out of 43 off-target sites were predicted using a somewhat adapted version of GUIDE-Seq, meaning that remaining 13 off-target changes were not predicted and thus only detected due to the unbiased WGS approach performed.

Proper consideration of Cas9 genomic specificity for risk assessment should include not only the aggregate number of potential off-target sites for a given guide RNA, but also the physiological impact of individual off-target events (both detected or not) (Tycko et al., 2016). In particular, when it comes to hazard identification, characterizing the scope of off-target changes might not be enough to assess the potential adverse effects of gene-edited organisms. An evolving view of the use of omics techniques in addressing the biological relevance of molecular data is growing among risk assessors and regulators (Heinemann et al., 2011). 'Omics' techniques—for example proteomics, transcriptomics, metabolomics, etc.—are molecular profiling techniques used to screen for a certain type of molecule in a given sample and, thus, allow the simultaneous measurement and comparison of thousands of plant components without prior knowledge of their identity. There are different types of approaches to omics techniques, ranging from untargeted approaches (e.g., profiling all proteins present in a protein extract) to targeted approaches in which a specific feature in a class of molecules is targeted (e.g., screening and quantification of already known proteins) (Heinemann et al., 2011).

A combination of targeted and untargeted methods could allow a more comprehensive approach, and thus provide additional opportunities to identify unintended effects of new and emerging gene-editing techniques applied to plants. Different kinds of questions can be answered using profiling, as it can be used to identify and then characterize new molecules in a GMO (e.g., RNA, protein, metabolite) or molecules at very different concentrations (e.g., anti-nutrients). Profiling can also be used to detect pathological or other responses in a test organism that indicate an unintended change in the GMO and may also be useful for forming hypotheses to determine if the unintended changes were the cause of the adverse effects (Mesnage et al., 2016).

A recent initiative organized by EFSA in April was particularly interested in advancing ways of implementing omics techniques to current EFSA risk assessment guidelines.[4] The outcome of this event, which drew on some 150 experts in the field, was supportive of the idea of adopting omics approaches toward risk assessment guidelines. In fact, EFSA started mapping the use of omics tools in the risk assessment related to food and feed safety back in 2014 (European Food Safety Authority [EFSA], 2014; EFSA Panel on Plant Protection Products and their Residues, 2017) but only recently started to build further toward a concrete path of implementation through guidance.

The regulation (EC) No 1830/2003 provides a framework for the traceability of GMOs and its products with the objectives of facilitating accurate labeling, monitoring the effects on the environment and the implementation of the appropriate risk management measures. This and the aforementioned regulations (**Figure 1**) established that GMO detection and identification methods have to be in place to allow GMOs to be traced and labeled. The method, which must be validated and published by the European Community reference laboratory established under Regulation (EC) No 1829/2003, is based on the detection of unique DNA sequences in the GMO. In other words, it must ensure the identification of the GM event and its reliable quantification. The framework and guidelines have been adequate to the GMOs being approved this far because they all contain the insertion of a foreign DNA sequence in a random genomic region. The variety of endogenous neighboring genomic sequences and the new transgenic DNA provided unique sequences that could differentiate each of the GMOs in the market to date.

However, as gene technology are developed it can be expected that a gene-edited organism containing one or a few nucleotide deletions or insertions at a specific genomic region might not be distinguished, at least using current methods, from a related variety or wild relative. This is because current GMO detection methods focus on a single DNA amplification sequence for its identification, i.e., the inserted and surrounding sequences. While specific methodologies to overcome this challenge will no doubt evolve since the decision of the ECJ, there are already developed plant variety and cultivar identification systems that can be adapted to gene-editing detection. The International Union for the Protection of New Varieties of Plants (UPOV) have refined biochemical and molecular techniques, as well as statistical tests and software for DNA-profiling which could serve as a basis for the gene-editing identification for both GMO traceability and labeling as well as for GMO patent rights (Korir et al., 2011). The main adaptation to this strategy from the previous GMOs methods is the need to perform more than a single DNA amplification test and the probability test to be conducted regarding the level of certainty of identification of a particular product. The presence of off-target DNA changes can also serve as a basis for the development of DNA amplification tests. In addition, plant variety and cultivar identification methods target the recognition of a single plant variety/cultivar while a gene-editing organism might be crossed to an infinitum of commercial crop varieties worldwide, which might then compromise referable results for gene-editing labeling.

Different strategies for DNA identification analysis, identity testing, profiling, and fingerprinting might have to be developed depending on the discrimination power that will be required by each gene-edited organism. Organisms with nucleotide insertions might have new and unique sequences that can be differentiated from any other species genotype using one or a few DNA amplification tests. While

[4]EFSA Colloquium on omics techniques for GMO risk assessment. Available at: https://www.efsa.europa.eu/en/events/event/180424-0.

others, might require further sequencing tests of several DNA fragments.

A Role for Responsible Research and Innovation Approaches to Formal Risk Governance Mechanisms

We have presented and reviewed a number of challenges related to risk governance of new and emerging gene-editing techniques. In addition to arguments for and against different types of regulatory frameworks we have, in light of the recent ECJ ruling, focused on limitations within current risk assessment approaches (ERA and RAFF alike). Beyond technological advances related to WGS and omics approaches for hazard and effect detection and monitoring, we have identified serval gaps in the knowledge base with regards to application of new and emerging gene-editing techniques to plants. In particular, we discussed knowledge gaps on the appropriate focus, selection of test organisms, and use of comparators when it comes to risk assessments of GMOs.

There are a diverse set of opinions on how knowledge gaps should be resolved in the application of new and emerging gene-editing techniques to plants in society. Risk analysis is value-based and "subjective," meaning there is no absolute way for the process to move from scientific information to decision, despite more robust technical inputs such as from WGS or omics approaches. This issue relates to a classic example of an ill-structured "messy" challenge in science and technology policy (Metlay and Sarewitz, 2012), or a "post-normal science" issue (Funtowicz and Ravetz, 1993). The ways in which governments, industries, research institutes, and others decide to address thorny issues and knowledge gaps going forward is vital: what is chosen for knowing means also choosing what may remain unknown, and such intentional or accidental social production of ignorance will affect societal ability to assess, manage, and respond to social and environmental hazards (see for example Kleinman and Suryanarayanan, 2013).

Stepping back and presenting risk governance challenges in this way opens a larger conversation on what it means to responsibly research and innovate around agricultural biotechnologies (c.f., Hartley et al., 2016). Scholars and philosophers of scientific knowledge production have for decades been investigating such questions (c.f., Sismondo, 2008 for a review, and Kuhn, 1962; Winner, 1986; Laird, 1993 as specific examples). Broadly speaking, these communities recognize three overarching challenges related science and technology governance that make resolving the issues above a challenge (see von Schomberg, 2013; and Keeler et al., 2018): (i) why pursue research and innovation (orientation); (ii) who should be involved in research and innovation processes and why (legitimacy); and (iii) how to manage research innovation to achieve a desired outcome (control).

Recently, especially in Europe, the term "responsible research and innovation" (RRI) has come to describe a set of processes and outcomes intended to help resolve these general issues of science and technology governance in, with, and for society (see Stilgoe et al., 2013; von Schomberg, 2013; Foley et al., 2016 for a more detailed discussion on alignment of processes and outcomes for responsibility). Although it can first sound as if talking about responsibility means conversations about blame and accusation for 'irresponsibility,' the core of RRI conversations involve a set of questions directly related to the challenges like those we have presented with regulation and guidance of new and emerging gene-editing techniques: how to govern activities implicating existing, emerging, and new biotechnologies. In this context, a widely respected and accepted definition states that RRI is, "*A transparent, interactive process by which societal actors and innovators become mutually responsive to each other with a view to the (ethical) acceptability, sustainability and societal desirability of the innovation process and its marketable products (in order to allow a proper embedding of scientific and technological advances in our society)*" (von Schomberg, 2013, p. 64).

Responsible research and innovation approaches don't presume to offer singular answers to scientific and societal questions. Instead, RRI encourages new ways of asking questions, exploring potential consequences of choices, and seeking answers when governing research and innovation activities. What is expected of benefits associated with application of new gene-editing techniques? How are intended and unintended effects to be assessed? When do assessed risks and promised benefits mean that a further research and innovation are justified? When not? What specific protection goals must be managed to avoid or mitigate unintended effects? As the Research Council of Norway, which strongly encourages adoption of RRI in its biotechnology funding, suggests: "Looking forward, thinking through, inviting along, and working together" (The Research Council of Norway [RCN], n.d.) can help address questions like the above associated with agricultural biotechnology risk governance.

National and international life sciences communities recognize the need for broader conversations about responsibility as well (c.f., The National Academies of Sciences, Engineering and Medicine [NASEM], 2016; Chneiweiss et al., 2017). Importantly, and as Hartley et al. (2016) have noted, a much broader community of people, organizations, experts, and interest groups will need to be involved in resolving questions like the above when approaching new and emerging gene-editing techniques through RRI. Evaluation on the state of RRI implementation in the Excellent Science Pillar of the EUR 77 billion eighth research and innovation (R&I) program highlights limited progress in adoption of inclusion of varied expertise in research and innovation activities (Bernstein et al., 2018). Indeed, beyond traditional industry and minimal civil society organization stakeholder engagement, engagement with non-traditional expertise in R&I is most commonly referenced as a unidirectional activity—for example, public outreach. In such one-way forms of "engaging" the public there is rarely opportunity for systematic reflection on or learning from diverse groups of people and expertise related, for example, to values associated with risks (Sturgis and Allum, 2004).

As a case in point specifically related to risk governance, we can return here to the challenge of communication between risk assessors and risk managers [a challenge recognized by

EFSA in its 2016 guidance on specific protection goals for environmental risk assessment (EFSA Scientific Committee, 2016)]. EFSA guidance holds only that assessors and managers of risk are appropriate authorities to set specific protection goals (SPGs), identify stressors and hazards, and determine appropriate exposure pathways and adverse effects for risk assessment. On one hand, SPGs are defined as, "Explicit and unambiguous targets for protection extracted from legislation and public policy goals" (EFSA Scientific Committee, 2016, p. 9). On the other hand, the very approach that EFSA guidance states should be used to set these "explicit and unambiguous targets"—ecosystem services valuation—depends on complex, ambiguous, uncertain, and contested methodology (c.f., Millennium Ecosystem Assessment [MEA], 2005; Department for Environment, Food and Rural Affairs [DEFRA], 2007). This is not to say that attempting valuation beyond standard economic analysis is futile. Quite the opposite; but our point is to say that it is the very ambiguity and subjectivity of these environmental risk assessment processes that make an RRI approach so potentially useful (c.f., Funtowicz and Ravetz, 1993). From an RRI perspective, the scientific input into such processes is necessary but not sufficient: more diverse expertise and value-sets are needed to help respond to the ambiguity and contested-ness of risk governance challenges (Wickson and Wynne, 2012).

As Preston and Wickson (2016) have argued, new opportunities associated with contemporary approaches to genetic modification offer a chance to "improve governance through informing, shaping and guiding the actual development of emerging technologies (rather than just their regulation)" (p. 55). This chance is especially relevant with new and emerging gene-editing techniques because they are easy to apply, cheaper to use, and much faster than previous GM plant techniques, in addition to raising issues with potential detection, traceability, and labeling.

As we have noted above, efforts could be directed to improvements in regulatory guidance on ways that biosafety is studied and assessed. Wickson and Wynne (2012) identified needs for greater opportunities to enhance the robustness of independent scientific peer review of risk assessment dossiers; the transparency and openness with associated data; and the "time, resources, materials, and terms of reference" for independent biosafety researchers and advisors, "to perform the type of meaningful 'independent assessments' that such bodies are supposed to perform" (p. 335). These needs are greater today than ever, and directly related to responsible societal responses to addressing knowledge gaps arising from application of new and emerging gene-editing techniques in plants.

Beyond these scientific questions, the regulatory science and broader life science, biotechnology, and other communities associated with GM plant risk governance can look to other societal actors for help. Policy communities have long experience and expertise with developing processes to combine scientific, political, and public inputs into decision-making about science and technologies (c.f., Metlay and Sarewitz, 2012). Industries have vested interests in supporting responsible and inclusive approaches (to demonstrate concern for safety, beyond profit, and retain their permissions from society to operate) and are very adept at adapting their research, production, and wider value-chains to societally determined guidelines [c.f., research on the idea of companies seeking and working to keep a "social license to operate" (Moffat et al., 2016)]. Social and political scientists have a breadth of expertise, theories, and tested methods for engaging heterogenous and diverse groups of people on controversial and important topics, and the role of scientific experts in this process (c.f., Pielke, 2007). Humanists, artists and philosophers have perhaps the deepest traditions of grappling with and constructively raising the types of questions humans unearth at the expanding frontiers of engineering life. We agree with the conclusions of Hartley et al. (2016) that when it comes to enhancing guidance in current GMO regulations, the way forward will require, "commitment to candor, recognition of underlying values and assumptions, involvement of a broad range of knowledge and actors, consideration of a range of alternatives, and preparedness to respond" (p. 2 of 7) to support responsible use of gene-editing in plants with and for society.

CONCLUSION

The current risk assessment framework was developed for products of classical GM techniques. As the 25 July 2018 ECJ decision points out, new and emerging gene-editing techniques lack a long-history of safe-use in any organism. Indeed, the scientific literature reveals the biotechnology community is still focused, at a fundamental level, on improving the efficiency and the applied uses of such techniques. Given this reality, a key question for the field going forward is not whether to regulate for safe use of biotechnologies, but how.

Several aspects of the current framework and its implementation stand to benefit from reconsideration in light of progress in the broader field. Examples of these aspects include: choice of test organisms for identification of target and non-target effects; use of the whole edited plant/derived-product as stressor in effect-testing; and expansion of the repertoire of molecular techniques to include omics in molecular characterization of hazards (Ramon et al., 2014; Casacuberta et al., 2015). In particular, the risk assessment guidance may need to be revised to enhance suitability for evaluating impacts of products by new and emerging gene-editing techniques on environmental, human, and animal health.

The present moment offers an opportunity to advance GM plant risk governance anchored in biosafety research and RRI approaches. This is especially true as the ECJ reminds the field of biosafety approaches structured by the guiding European Union application of the precautionary principle. Considering such approaches points to the need for more, better resourced, transparent, and independent risk assessment of products of gene-editing techniques, intended and unintended effects, as well as target and off-target activity.

Responsible research and innovation, the Commission's approach to applying the precautionary principle in research and innovation funding (Bourguignon, 2015), is well suited to supporting risk governance of the complex, value-laden issues associated with gene-edited plants. Through an RRI

approach—supplemented with technological advances of WGS and omics approaches—could involve a broader community of people, organizations, and interest groups when reflecting on, anticipating, and responding to risk governance challenges (Baltimore et al., 2015). In pursuit of broader societal consensus, the scientific community came together to use its moral authority and remain in control of the pace of research on inheritable changes in human germ lines (c.f., Wade, 2015). The broad plant-biotechnology community could similarly explore more open and coordinated pursuit of societally desirable, ethically acceptable, and sustainable changes to plant life, grounded in principles of biosafety.

AUTHOR CONTRIBUTIONS

AM provided a first draft of this article and wrote the regulatory section. AO wrote the environmental section. SA-T wrote the food and feed and molecular characterization and traceability section. O-GW wrote the post release monitoring section. MB wrote the RRI section and led on English-language revision. All authors contributed to the final draft of this manuscript.

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DNA-Free Genome Editing: Past, Present and Future

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Genome Editing using engineered endonuclease (GEEN) systems rapidly took over the field of plant science and plant breeding. So far, Genome Editing techniques have been applied in more than fifty different plants; including model species like Arabidopsis; main crops like rice, maize or wheat as well as economically less important crops like strawberry, peanut and cucumber. These techniques have been used for basic research as proof-of-concept or to investigate gene functions in most of its applications. However, several market-oriented traits have been addressed including enhanced agronomic characteristics, improved food and feed quality, increased tolerance to abiotic and biotic stress and herbicide tolerance. These technologies are evolving at a tearing pace and especially the field of CRISPR based Genome Editing is advancing incredibly fast. CRISPR-Systems derived from a multitude of bacterial species are being used for targeted Gene Editing and many modifications have already been applied to the existing CRISPR-Systems such as (i) alter their protospacer adjacent motif (ii) increase their specificity (iii) alter their ability to cut DNA and (iv) fuse them with additional proteins. Besides, the classical transformation system using *Agrobacteria tumefaciens* or *Rhizobium rhizogenes*, other transformation technologies have become available and additional methods are on its way to the plant sector. Some of them are utilizing solely proteins or protein-RNA complexes for transformation, making it possible to alter the genome without the use of recombinant DNA. Due to this, it is impossible that foreign DNA is being incorporated into the host genome. In this review we will present the recent developments and techniques in the field of DNA-free Genome Editing, its advantages and pitfalls and give a perspective on technologies which might be available in the future for targeted Genome Editing in plants. Furthermore, we will discuss these techniques in the light of existing– and potential future regulations.

Keywords: DNA-free, Genome Editing, RGEN, CRISPR/Cas, CRISPR/Cpf, plant, TALEN, RNPs

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INTRODUCTION

Genome Editing for targeted gene improvement is widely used in the field of plant science for basic research as well as for specific improvement of desired traits in commercial crops. Mainly five tools have been used for targeted Genome Editing so far. Besides Oligonucleotide Directed Mutagenesis (ODM), which had its origin in the early 80s of the last century and found its way in plant science ∼15 years ago, mainly engineered nuclease (ENs) are used (Wallace et al., 1981). There are four kinds of

engineered nucleases (i) Zinc-Finger Nucleases, (ii) Meganucleases (iii) Transcription Activator Like Effector Nucleases (TALENs) and (iv) Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-Systems. The latter is more a collection of different closely related techniques which all have been adapted for the use in Genome Editing (Puchta, 2017). Nowadays, most applications in plants (and in animals) are done by using either TALENs or CRISPR-Systems. In the majority of cases plants are stable transformed to introduce the Genome Editing tools into the plant genome (**Figure 1A**). Subsequently the plants are self-pollinated or crossed to get rid of the incorporated DNA, only the intended mutation remains. In some cases, transient expression of the tools e.g., via plasmids, initiate these mutations but all of these techniques make use of recombinant DNA at least in an intermediate step. Lately tools have been developed using solely RNA, preassembled Cas9 protein-gRNA ribonucleoproteins (RNPs) or TALEN-proteins for mutation induction (**Figure 1B**). All of these are completely free of DNA so the risk of DNA integration into the genome can be excluded. Due to this we will focus on these in the following article.

TARGETED NUCLEASES

Bacteria have been altering genomes since ages by using e.g., TALEs or CRISPR in combination with CRISPR associated (Cas) nucleases or other techniques such as classical restriction enzymes or Meganucleases (Roberts and Murray, 1976; Jacquier and Dujon, 1985; Stoddard, 2005; Römer et al., 2007). The aims of the bacteria using site-directed nucleases (SDNs) as tools are as diverse as ours, by using altered versions of these natural occurring mechanisms. TALEs e.g., have their origin in *Xanthomonas spec*. which manipulate cellular processes of the host by introducing TALE-proteins into plant cells via a type III secretion system (Göhre and Robatzek, 2008). Once recognized their target, TALEs alter gene expression e.g., for sugar transporters to supply the bacteria with enough resources to grow which is triggering an infection in the host such as bacterial blight (Lahaye and Bonas, 2001). Scientist revealed the hidden code of these natural occurring tracing devices and fused them with a nuclease (FokI) creating TALE-Nucleases (TALENs) (Boch et al., 2009; Cermak et al., 2011). By using them as pairs a precise induction of a DNA double strand break is possible in many organisms (Sprink et al., 2015).

CRISPR-Systems have a different origin and are ubiquitously present. Around 40% of bacteria *spec*. and 90% of archaea *spec*. sequenced so far possess one or more CRISPR-Systems (Marraffini and Sontheimer, 2010; Shmakov et al., 2017). They have been described first by Ishino et al., 1987 in the model organism *Escherichia coli* but it took additional 30 years until their function as a kind of adaptive immune system of bacteria against invading nucleic acids such as plasmids or phages have been revealed in bacteria for yogurt production (Ishino et al., 1987; Barrangou et al., 2007). Today CRISPR is still used in dairy industry to prevent phage infection in starter cultures (Grens, 2015). Additional applications have been derived from this mechanism, Jinek et al. (2012) described the ability of this technology for precise RNA guided genome modification and started the CRISPR-era (Jinek et al., 2012). Their ideas have been adopted by many scientists working in various fields and led to a new age of Genome Editing. Till now hundreds of genomes have been edited in all kinds of kingdoms and clades ranging from small viruses to trees such as Poplar (Fan et al., 2015; Yuan et al., 2015).

Besides the classical Cas9-System from *Streptococcus pyogenes* several Cas-variants from different species like *S.aureus*, *S.thermophilus* and others have been used for Genome Editing in plants (Steinert et al., 2015; Endo et al., 2016). The classical Cas9 System consists of a dual RNA-complex, CRISPR (cr) RNA and *trans* activating CRISPR (tracr) RNA. Jinek et al. (2012)., fused these two RNAs for easier cloning and handling, creating the single guide RNA (sgRNA), for which multiple vector systems are currently available.

Other systems like the CRISPR/Cpf1 (Clustered Regularly Interspaced Short Palindromic Repeats from *Prevotella and Francisella*), recently named Cas12a, differ in several aspects from the classical CRISPR/Cas-Systems as (i) the nucleases are smaller (135 vs. 158 kDa) (ii) the systems possess a natural occurring single guide RNA (iii) cutting of Cas12a results in staggered cuts Cas9 cutting in blunt (iv) the Protospacer adjacent motifs has to be rich in thymine for Cas12a and rich in guanin for Cas9 and (v) the DNA is cut distal from the recognition site by Cas12a and proximal by Cas9.

Cas13 a CRISPR-variant which is able to recognize and cut specific RNA instead of DNA has recently been exploited for RNA editing and tracking in bacteria, mammals and also plants (review Ali et al., 2018). But additional studies have to be performed to test this system for commercial applications. It offers great potential for medicine as well as for agriculture. An initial study in bacterial cells showed non-target, collateral RNA degradation, but these effects have not been reported for recent studies performed in plants and mammals (Cox et al., 2017; Aman et al., 2018).

CURRENT APPLICATIONS

Currently in several publications' authors promote their work as transgene-free but by taking a closer look at these publications reveal that the status of transgene-freeness focuses only on the end product. In many cases the mutation has been initiated by transient expression of plasmid based CRISPR-DNA or stable integration with subsequent backcrossing. For both techniques, integration of DNA into the host genome is still possible as plasmids are degraded in the cells and could integrate into cut sites (Woo et al., 2015). In this paper we focus on work which has been performed completely without the use of DNA for mutation initiation, meaning either RNA, RGEN RNPs or TALEN- proteins have been used for mutation induction. All of these techniques have been used successfully in plants. DNA-free editing has its origin in editing of animal cell lines or embryos where it is frequently used and is being adapted for more and more species (Hur et al., 2016; Park et al., 2017). DNA-free

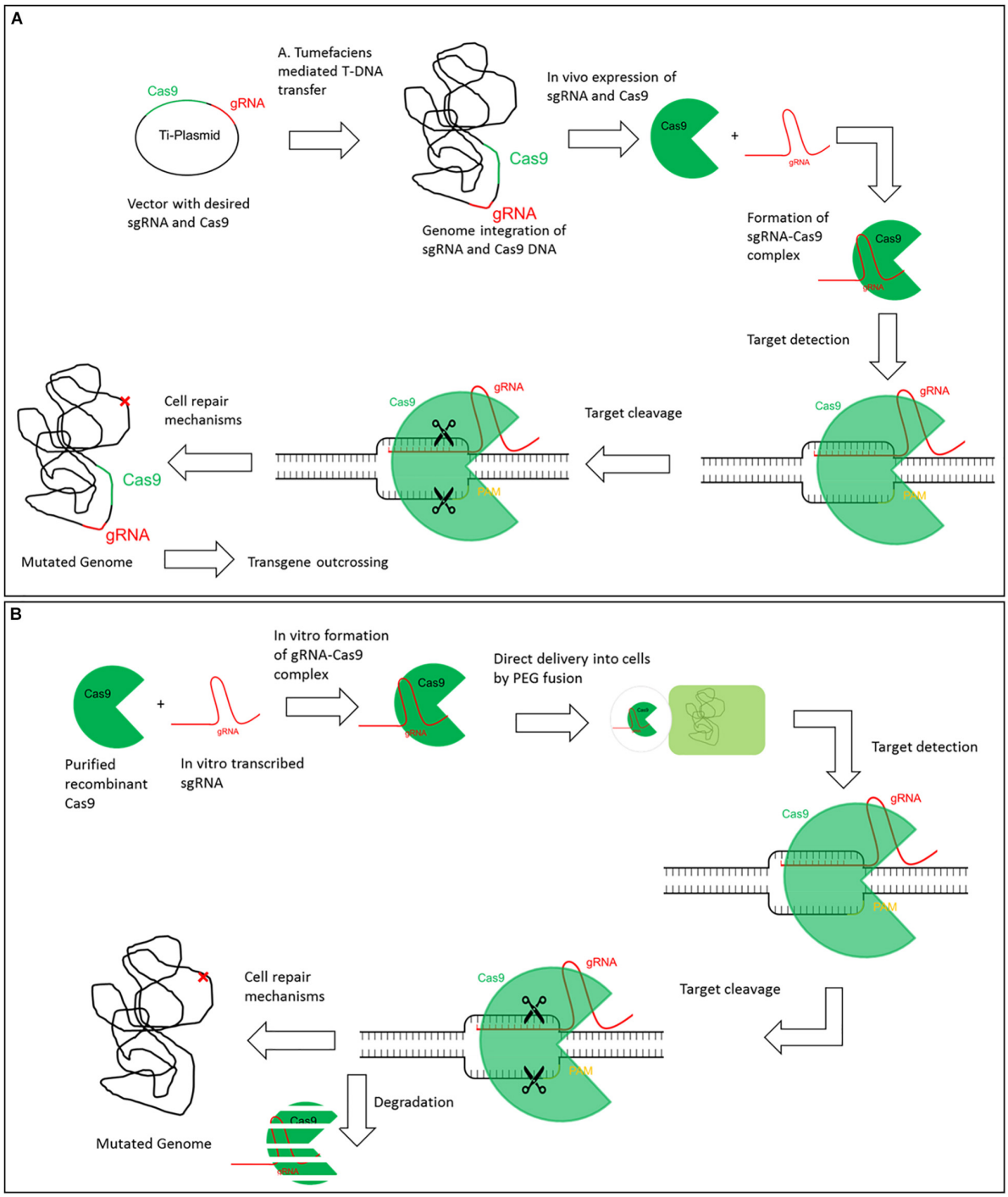

FIGURE 1 | Exemplary comparison of classic CRISPR/Cas9 and DNA-free CRISPR/Cas9. Comparison of classic CRISPR/Cas9 through the example of **(A)**. tumefaciens transformation and DNA-free CRISPR/Cas9 exemplified by PEG mediated protoplast fusion. **(A)** In the classic CRISPR/Cas9 technique a T-Plasmid is designed that includes the desired gRNA and Cas9 coding sequences. Via Agrobacterium tumefaciens mediated transfer both gRNA and Cas9 sequences can be integrated in the host genome. *In vivo* gRNA and Cas9 are translated and the gRNA-Cas9 RNP complex is formed. Upon target detection, a double strand break is induced and mutations can arise by internal cell repair mechanisms. The CRISPR/Cas9 complex is constantly expressed and active in the cell. Finally, the genome can contain both the desired mutation and sequences for gRNA and Cas9. The transgene can be outcrossed but this is less practical or even impossible in vegetative propagated crops. **(B)** For DNA-free CRISPR/Cas9 recombinant Cas9 and *in vitro* translated gRNA are required. The RNP complex is formed *in vitro* and is directly delivered to protoplasts by e.g., PEG fusion. The complex is already active and can directly detect its target to induce double strand breaks. Cell repair mechanisms can lead to a mutated genome at the desired target without addition of any foreign DNA. The CRISPR/Cas9 complex is degraded within the cell and no longer available.

TABLE 1 | Recent publications using DNA-free Genome Editing approaches.

Reference	Plant species	Trait	GE-technique	Tissue	Delivery	Editing efficiency	Off-targets	Method
Woo et al., 2015	*Arabidopsis thaliana*, *Lactuca sativa*, *Nicotiana attenuata, Oryza sativa*	POC	CRISPR/Cas9 RNPs	Protoplasts	PEG-fusion	Calli: Monoallelic mutations: 5.7%; Biallelic mutations: 40%	0/104 (2–5 MM)	Targeted deep seq.
Baek et al., 2016	*Chlamydomonas reinhardtii*	Yield, abiotic stress	CRISPR/Cas9 RNPs	Single cells	Electroporation	Cells: Up to 0.56%	0/17 (2–4 MM)	Targeted deep seq.
Malnoy et al., 2016	*Malus domestica*, *Vitis vinifera*	Biotic stress	CRISPR/Cas9 RNPs	Protoplasts	PEG-fusion	Protoplasts: 0.1–6.9%	n.d.	n.d.
Stoddard et al., 2016	*Nicotiana benthamiana*	POC, herbicide resistance	TALEN mRNA	Protoplasts	PEG-fusion	Protoplasts: No-UTR DNA control: 70.5%, No-UTR mRNA control: 5.8%	n.d.	n.d.
Shin et al., 2016	*C. reinhardtii*	POC	CRISPR/Cas9 RNPs	Single cells	Electroporation	Up to 3×10^{-5}	0/333 (1–3 MM)	WGS
Svitashev et al., 2016	Zea mays	Male sterility, herbicide tolerance	CRISPR/Cas9 RNPs	Immature embryos	Biolistics	Embryo: Cas9 only: 0.002–0.02 DNA delivery: 0.18–0.56 RNP delivery: 0.01–0.69	n.d.	n.d.
Subburaj et al., 2016	Petunia hybrid	Herbicide resistance	CRISPR/Cas9 RNPs	Protoplasts	PEG-fusion;	Protoplasts: Cas9 protein: 0.03–27.13, NR-RGEN: 5.3–34.69	n.d.	n.d.
Zhang et al., 2016	*Triticum aestivum*	Yield	CRISPR/Cas9 mRNA	immature embryos	Biolistics	No. of transgen-free frequency: 0–100% No. of homozygous transgen-free frequency: 0–36.8%	0/8 (2–4 MM)	PCR- RE
Ferenczi et al., 2017	*C. reinhardtii*	POC, Gene replacement	CRISPR/Cpf1 RNPs	Single cells	Electroporation	Colonies: 0.12–16%	n.d.	n.d.
Grahl et al., 2017	Non-albicans candida	POC	CRISPR/Cas9 RNPs	Single cells	Electroporation	n.d.	n.d.	n.d.
Kim et al., 2017	Glycine max, *N. attenuata*	Fat-synthesis; POC	CRISPR/Cpf1 RNPs	Protoplasts	PEG-fusion	Soybean: Protoplasts: LbCpf1: 0–11.7% AsCpf1: 0–1.6% Tobacco: LbCpf1 and AsCpf1: $< 0.1\%$ LbCpf1 + crRNA and AsCpf1 + crRNA: $< 1\%$	0/23 (4–6 MM)	Targeted deep seq.
Liang et al., 2017	*T. aestivum*	Yield	CRISPR/Cas9 RNPs	Protoplasts; immature embryos	PEG fusion, Biolistics	Protoplasts: 33.4 and 21.8% Embryo cells: gw2-RNPs: 0.18–0.21% pGE-TaGW2: 0.99–1% gasr7-RNP: 45.3%	0/20 (2–5 MM)	Sanger seq.
Andersson et al., 2018	*Solanum tuberosum*	Starch syntheses	CRISPR/Cas9 RNPs	Protoplasts	PEG-fusion	Protoplasts: 1–25%	n.d.	n.d.
Liang et al., 2018	*T. aestivum*, *O. sativa*	Yield/ POC	CRISPR/Cas9/ Cpf1 RNPs, TALEN-Proteins	Protoplasts; immature embryos	PEG fusion, Biolistics	Protoplasts: 10.9–33.6%	n.d.	n.d.

GE-Technique, Genome editing technique; RNPs, Ribonucleoproteins; WGS, Whole Genome sequencing; POC, Proof of concept; n.d., not determined.

editing of plants is a new but emerging field which arose 2015, as concerns raised that plants transformed using DNA might be covered by gene technology laws. To date, DNA-free editing is used in at least 14 plant species, to test the ability in proof of concept studies or for improvement of yield or tolerance against biotic and abiotic stress (**Table 1**). The system is especially useful for species which propagate vegetative or have a long generation cycle as backcrossing is time consuming or impossible such as for potato, grapevine and apple (**Table 1**; Malnoy et al., 2016; Andersson et al., 2018).

Besides the elimination of DNA integration which circumvents the need for backcrossing and screening of the progeny, the DNA-free systems offer some additional advantages compared to the DNA-based systems as till now no off-target effects (non-target cutting) have been observed neither using targeted nor untargeted approaches for identification (Baek et al., 2016; Shin et al., 2016; Kim et al., 2017). Further advantages are that (i) it can be used without further adaption in a majority of species (even those without established genomic alteration systems) as no coding sequence or promotor have to be adapted (Grahl et al., 2017) (ii) the amount of editors can be controlled in a better way as promotor efficiency is avoided, (iii) the editors are ready to introduce mutations directly after transfection (no lagging phase). Most of these effects seems to be a result of the defined relatively short (∼48 h) persistence of the tools in the targeted organism.

But the systems also have to deal with some drawbacks as to date it is not possible to use it in all species, mainly due to undeveloped or unsuited *in vitro* techniques. Furthermore, the efficiency is lower compared to classical methods and a selection of positively edited plants is only possible by genomic selection such as sequencing. These points result in higher costs for the technique, but further optimization will result in better *in vitro* protocols and dropping costs.

TRANSFORMATION METHODS

DNA-free Genome Editing is currently performed using CRISPR/Cas9 and TALENs and reagents are introduced by either transient expression of mRNAs encoding for TALENs or Cas nuclease and guide RNA or by direct delivery of isolated RNPs. When using RNPs the complex is already preassembled and active upon delivery, when using RNA, the editors have to be transcribed and the complex has to assemble which result in a short delay in activity. DNA-free transformation challenges two major problems: (i) Delivery through the plant cell wall and (ii) regeneration of plants from tissue or cell-wall free cells. To avoid the plant cell wall barrier most edits, use isolated protoplasts, single plant cells which cell wall has been enzymatic digested. Protoplasts were the first tissue which has been used for DNA-free Genome Editing as they can be targeted easily by polyethylene glycol (PEG) mediated fusion. Therefore, the RNP complex or mRNA is enclosed in PEG vesicles and fused with protoplasts. This system enables an average editing rate of around 10% which is lower compared to DNA-based systems (Svitashev et al., 2016; Andersson et al., 2018). In potato the system is efficient from the transfection to regenerated plants, it was possible to alter all four copies of a single gene in 2–3% of the regenerated shoots (Andersson et al., 2018). In other crops such as apple or grapevine the transformation system is working but regeneration protocols for edited lines are still not available as protoplast regeneration and identification of successfully modified lines is tricky and differ even between cultivars of the same species (Malnoy et al., 2016). The single-cell alga *Chlamydomonas reinhardtii* was successfully transformed with RNPs by electroporation. Although, functional protocols are available for potatoes, lettuce, tobacco, soy and petunia regeneration rate is often low (Woo et al., 2015; Subburaj et al., 2016; Kim et al., 2017). Lately also immature embryos are being used for DNA-free transformation systems. Immature embryos can be target by biolistic delivery of both RNPs and mRNA (Svitashev et al., 2016; Zhang et al., 2016; Liang et al., 2017).

More methods are available to transfer naked DNA to plants but need to be adapted to transform DNA-free Genome Editing tools. Protoplast microinjection is described since 1983 (Griesbach, 1983) and has recently been relighted for DNA delivery in oil palm (Masani et al., 2014) but have not been tested for RNP delivery so far. An optimization of biolistic delivery to plant cells where proteins are loaded into the pores of gold activated mesoporous silica nanoparticles has been described (Martin-Ortigosa and Wang, 2014) but not published for Genome Editing yet. To overcome regeneration of immature cells *in planta* particle bombardement (iPB) that targets mature plant tissue was introduced in wheat (Hamada et al., 2017). A new method for the transformation of mature plant tissue is infiltration with cell penetrating peptides (CPPs). CPPs are a class of short, positively charged peptides that can translocate across cellular membranes. Recently they have been shown to be capable of binding site-specific nucleases (Rádis-Baptista et al., 2017). Still their potential for DNA-free Genome Editing in plants needs to be exploited.

Additional methods have also been tested to porate single cells and deliver macromolecules to the cell, such as microfluidic cell deformation or sonication and furthermore such as intensive light beams are being discussed but haven't been tested for plants so far (Han et al., 2015; Schlicher et al., 2006).

The field of DNA-free Genome Editing is still evolving and besides new delivery methods for the reagents, proteins coupled to engineered nucleases are being developed. These approaches have been tested and used in stable transformation systems but seem to be also suitable for a DNA-free approach. Besides additional Cas-systems such as Cas12a from different organism also Cas13a could be adapted for a transient RNA-editing in an DNA-free approach, leading to a transient change in expression. This is comparable to the coupling of TALEs and other activators or repressors to Cas9 to alter the expression of genes for a defined time. Furthermore, nickases are frequently used to introduce single stranded DNA breaks in plants, to enhance specificity of Cas-systems (Fauser et al., 2014). Due to the already high specificity of the DNA-free systems,

nickases are not expected to be used in DNA-free approaches. Likely, other systems will be used in the near future e.g., base editors such as cytidine or adenosine deaminases, which have been used in plants already and offer great potential to be adopted for DNA-free approaches (Gaudelli et al., 2017; Zong et al., 2017). A new and highly discussed approach is to alter methylation or acetylation for Epigenome Editing, these approaches could also be used in DNA-free approaches (Maeder et al., 2013). The newest development in the field is the guidance of integrases by Cas9, to achieve a targeted recombination. This approach is still depended on integrase sites and has been tested only in yeast and mammalian cells but an intensive search for altered integrase sites is ongoing, so that in the future targeted recombination might be possible even in plants (Chaikind et al., 2016; Merrick et al., 2018).

REGULATORY CONCEPTS AND CONCERNS

Although several European authorities proposed ways, how to handle and interpret new plant breeding technologies in the current or an updated European legal framework (e.g., Advisory Committee on Releases to the Environment [ACRE], 2013; Swedish Board of Agriculture [SBA], 2015; Commissie Genetische Modificatie [COGEM], 2017; Federal office for consumer protection and food safety [BVL] , 2017) the European court of justice (ECJ) decided fairly unscientifically on July 25th this year, that plant products derived from Genome Editing processes (other than modified by chemical or physical mutagenesis) fall under the strict regulatory framework applied for GMOs (ECLI:EU:C:2018:583)[1]. The judgment triggered strong displeasure in the European scientific community, who forecasts noticeable economic disadvantages for European plant- and seed industry (European Plant Science Organization [EPSO], 2018; European Seed association [ESA], 2018; Vlaamsche Institute Biologie [VIB], 2018). Additionally, also the Scientific advise mechanism of the European commission published a statement on the ruling in which they recall the product-based aspects of the European gene technology law and recommend "*revising the existing GMO Directive to reflect current knowledge and scientific evidence, in particular on gene editing and established techniques of genetic modification*" (The Scientific Advice Mechanism [SAM], 2018). Due to the ruling European plant breeders need to undergo expensive and time-consuming approval procedures before their products improved by GEENs can be placed on the market. In particular, for DNA-free Genome Editing approaches, this regulation is intangible due to the lacking difference to a conventionally bred plant, as no DNA from non-related crops or organisms is introduced into the plant genome and detectable, neither in the final plant product, nor in its progenies. At no time-point during generation process, the plant genome encounters foreign, recombinant DNA, which usually triggers current European GMO-regulations. The genome edits are usually indistinguishable from natural mutations (Cao et al., 2011). In addition, off-targets play a minor role in DNA-free approaches: compared with stable and transient expression, GEENs are degraded within hours and thus the GEEN's mode of action is only present in the original cells (protoplasts) of the edited plant (Liang et al., 2015). However, as long Europe sets its focus on the generation process during approval of new plant products, also DNA-free genome edited plants will fall within the same scrutiny as the few legal GMO-plants grown in Europe. This could lead to potential trade issues and impede innovation as stated by members of the WTO lately (World trade organization [WTO], 2018).

Notwithstanding, several countries started to update their legal interpretation of GEEN. Among them are the South-American ABC: Argentina, Brazil, Chile - the United States, Canada and Israel while in Japan and Australia new regulations and a possible exemption of Genome Editing approaches from strict rulings adopted for conventional genetically modified plants are still under discussion. Giving rise to a worldwide regulatory patchwork for genome-edited plants with a diverse set of interpretations and definitions for genome-edited plants resulting in reservations between international trade partners and trade restraints between economic regions. International harmonization of regulations and definitions thus is essential to close the risk-benefit gap between precaution and innovation potential of genome edited plants (Duensing et al., 2018). Argentina pioneered with a straightforward regulation for the new Genome Editing technologies already in 2015, 2 years after the first application of CRISPR in plants. The Resolución 173/2015[2] defines a case-by-case dependent approach, in which applicants can request the responsive authority CONABIA already during product development to determine if their products will fall under GMO regulation. Following the Cartagena protocol definitions for living modified organisms; this is only the case when the new plant product contains a **-novel-** combination of genetic material – similar to conventional transgenic approaches when a transgene is permanently detectable in the final plant product. In case of SDN-1 (NHEJ based deletion/change of a few, often less than 20 nucleotides (Lusser et al., 2011) DNA-free Genome Editing approaches act without introducing foreign DNA that would be detectable in the final plant product. SDN-2 approaches (HDR based replacement of usually less than 20 nucleotides) using a short repair DNA sequence as template, are accordingly not completely DNA-free, although in the final plant product the template is not traceable anymore. In Argentina, plant products derived from GEENs thus will become less strictly regulated than classical GMOs.

[1]Ruling in the Case C-528/16: http://curia.europa.eu/juris/document/document_print.jsf?docid=204387&text=&dir=&doclang=EN&part=1&occ=first&mode=lst&pageIndex=0&cid=5226816

[2]Resolución 173/2015: http://www.fao.org/faolex/results/details/en/c/LEX-FAOC144508

Likewise, similar guidelines are expectable in Brazil and Chile, which subsequently introduced similar case-by-case, mainly product-focused regulations. Brazil for example interprets GEENs explicitly as SDN as one of several "new precision breeding innovation technologies," which may create a product not considered a GMO in the annex I of the normative resolution no. 16/2018[3]. Recently, together with the former mentioned ABC, also Paraguay and Uruguay declared their intention to harmonize their Genome Editing-friendly regulations and to establish genome-edited plants analogous to conventionally bred plants[4]. This initiative will transform South America into a hot spot for further Genome Editing innovations. Plant products derived by GEENs still lack on the market in these countries, but it is commendable that more and more countries worldwide clarify their legal status to pave the way for the next green revolution.

[3] Resolution no. 16/2018: https://agrobiobrasil.org.br/wp-content/uploads/2018/05/Normative-Resolution-16-of-January-15-2018.pdf

[4] XXXVI RO CAS Declaración II: http://consejocas.org/wp-content/uploads/2018/09/XXXVI-RO-CAS-Declaraci%C3%B3n-II.-T%C3%A9cnicas-de-Edici%C3%B3n-G%C3%A9nica.pdf

AUTHOR CONTRIBUTIONS

TS, JM-S, and JM wrote the manuscript. DM provided and conducted the data search. JM-S provided and constructed the figure. All authors read and approved the final manuscript.

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Genetic Alterations That Do or Do Not Occur Naturally; Consequences for Genome Edited Organisms in the Context of Regulatory Oversight

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The ability to successfully exploit genome edited organisms for the benefit of food security and the environment will essentially be determined by the extent to which these organisms fall under specific regulatory provisions. In many jurisdictions the answer to this question is considered to depend on the genetic characteristics of the edited organism, and whether the changes introduced in its genome do (or do not) occur naturally. We provide here a number of key considerations to assist with this evaluation as well as a guide of concrete examples of genetic alterations with an assessment of their natural occurrence. These examples support the conclusion that for many of the common types of alterations introduced by means of genome editing, the resulting organisms would not be subject to specific biosafety regulatory provisions whenever novelty of the genetic combination is a crucial determinant.

Keywords: genome editing, regulatory oversight, natural genetic alterations, GMO, classification, future policy

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INTRODUCTION

The advances presented by genome editing including oligonucleotide-directed mutagenesis (ODM) and site-directed nuclease (SDN) technology have been widely recognized as a true revolution in our abilities to alter and improve genomes. One of the fields where these techniques are predicted to have a significant impact is plant breeding. Humans have selected genotypes more adapted to their needs and have improved agricultural practices ever since the early Neolithic period. The use of SDNs, and in particular CRISPR/Cas technology, will allow the introduction of additional genomic alterations efficiently and with an unprecedented level of precision. This technological innovation also presents regulatory challenges and leads to a number of questions. First, are such genome edited organisms subject to specific regulatory provisions related to biosafety? In many jurisdictions around the world, there is still legal uncertainty about this. And second, if the answer to the first question is no, should genome edited organisms nevertheless be subject to regulatory oversight that is stricter in any aspect than those which apply to conventionally bred organisms? The impact these techniques will have on plant breeding will greatly depend on how we answer these questions. To unlock the promise and potential of genome editing and to take responsibility for its development to benefit society there is an urgent need for a legal clarification based on correct scientific understanding. To support the decision-making process we aim to describe what types of genetic alterations do and do not occur

naturally and estimate to what extent various alterations along a range from small to large genetic changes may occur in nature. Genome editing also has a lot of potential for the introduction of epigenetic changes. The scope of this paper, however, is limited to alterations in the primary sequence of the genetic material.

GENOMES IN EVOLUTION

Genomes have evolved over millions of years starting from the first development of living organisms, leading to the evolution of a wide variety of species. Indeed, naturally occurring mutations together with natural selection are the key factors driving evolution. Many organisms have had the capacity to adjust to specific environmental conditions and the plasticity of genomes has been one of the factors contributing to the ability to survive changing conditions. Over the years our understanding of the mechanisms underlying this evolution has grown significantly. On top of that, modern genome sequencing technology has revealed to us what type of alterations have occurred during evolution, domestication and breeding. If we want to determine what types of combinations of genetic material should be considered novel and are beyond what can "occur naturally by mating and/or natural recombination"—the phrase used in the EU GMO definition—it is important to have a closer look at these alterations.

Genetic information has to be faithfully transmitted during each cell division to allow the correct development and functioning of each organism. It also needs to be faithfully transmitted to the offspring in order to maintain species boundaries and biodiversity. However, some level of change needs to be generated to endow genetic information with the plasticity required for organisms and species adaptation to a changing environment. For this reason, most organisms have evolved mechanisms to ensure a high but imperfect fidelity in DNA replication, causing spontaneous mutations at a low rate, and equally efficient and imperfect mechanisms for repairing the DNA when damaged by endogenous or environmental mutagenic agents such as UV or radiation (Kunkel and Erie, 2015). This leads to a certain amount of natural mutations continuously being introduced into the genomes of all organisms. In the annual model plant *Arabidopsis thaliana*, with a genome size that is about 24 times smaller than the human genome, this mutation rate has been estimated to be 7×10^{-9} base substitutions per site per generation (Ossowski et al., 2010), which is approximately one substitution per genome per generation. In addition to these continuously arising mutations, genomes are equipped with repetitive and mobile genetic elements that promote additional mutations and genome rearrangements, which are much more discontinuous during evolution (Lisch, 2013). Finally, genomes are not completely isolated within the species boundaries and different species can exchange genetic information in nature through horizontal transfer, as it has been shown for rice to millet (Diao et al., 2006) or for the *Poa* to *Festuca* grass genera (Vallenback et al., 2008), or even between kingdoms such as from *Agrobacterium* to sweet potato (Kyndt et al., 2015). Moreover, the combination of two complete genomes through interspecific crosses, has also frequently occurred during plant genome evolution (Wendel, 2015).

All these fine or crude genetic changes are the raw material on which selection operates allowing species adaptation and evolution, both in the wild and under human direction. A look at the mutations that are at the origin of new characters selected during crop domestication and breeding shows that they cover a wide range of mutation types and mechanisms (Olsen and Wendel, 2013). These include point mutations causing amino acid changes, premature translation terminations and changes in transcript splicing or gene regulation. They also include transposon insertions and large deletions causing changes in coding or regulatory sequences in genes involved in plant and inflorescence architecture, seed shattering and dormancy, grain size and color, among many other characters that have been subject to farmer-driven selection (Olsen and Wendel, 2013). The domestication and genetic improvement of crops to serve human needs has also necessitated the incorporation of new genes from other species or even the combination of two complete genomes from different species. In fact, the domestication of most crops is the result of the combination of many different types of mutagenic events (see for instance Table 1 in Olsen and Wendel, 2013). As an example, bread wheat domestication required many independent mutations, including those at the two genes controlling seed shattering in the wild emmer wheat (Avni et al., 2017), different introgressions from wild related species, a whole genome duplication event in emmer wheat, and an interspecific cross between the domesticated emmer wheat and the wild goatgrass (*Aegilops tauschii*) (Gornicki and Faris, 2014). In general, crop domestication has required a significant number of mutations and genome rearrangements accumulated in genomes, and this has also been true during the whole history of crop breeding.

Whereas, all these individual mutations happened in nature spontaneously, they were artificially selected and combined by humans, who ultimately have made possible the large phenotypic diversity of today's crops and the radical differences crops present when compared to wild plants. One may even argue that the specific combinations of enhanced traits in all our crops are something that never would have occurred nor maintained without human intervention.

Plant breeding has been widely influenced by scientific progress during modern history, which has allowed expanding the range of techniques incorporated and boosted its sophistication. One of the key factors during this process was the increase in genetic variation available for breeding which was achieved both by expanding the gene pool that could be used for breeding and by enlarging the variability within the species by mutagenesis. The expansion of the gene pool was obtained by forcing crosses with increasingly distant species with the help, among others, of *in vitro* culture techniques allowing the rescue of the offspring of otherwise sterile crosses. Embryo rescue plays an important role in plant breeding programmes and is expected to retain or even broaden its significance, since plants obtained by embryo rescue do not have to be considered as genetically modified (Winkelmann et al., 2010).

The increase in variability within species through mutagenesis has been particularly successful and during the past 70 years more than 3,200 new crop varieties have been developed through mutation programs (cf. IAEA/FAO mutant variety database, https://mvd.iaea.org/), predominantly using γ-ray irradiation, but also other physical or chemical mutagens (Jankowicz-Cieslak and Till, 2015).

It is clear that during evolution, domestication and plant breeding a wide variety of genetic alterations have occurred and are still being introduced and further exploited. But not every type of alteration does or is likely to occur naturally. Alterations that cannot occur naturally are considered novel. It is for instance highly unlikely that organisms that are unrelated at any higher taxonomic level exchange large amounts of genetic material, although the examples given above show that horizontal gene transfer (HGT) is known to occur also among sexually incompatible eukaryotic/plant species.

THE NOVELTY CRITERION IN GMO REGULATORY REGIMES

The Cartagena Protocol on Biosafety to the Convention on Biological Diversity serves as a framework promoting international harmonization in the legislation of GMOs (Cartagena Protocol, 2000). This Protocol does not use the term GMO but defines a "living modified organism" (LMO) as "any living organism that possesses a novel combination of genetic material developed through modern biotechnology." In the Cartagena Protocol, the mere use of a technique of modern biotechnology is not enough to trigger regulatory oversight. The resulting organism additionally needs to possess a novel combination of genetic material. The combination of genetic material needs to be beyond what can occur naturally by mating and/or natural recombination. We deliberately use these latter words because also the EU GMO definition uses a similar phrasing. But what does "beyond what can occur naturally by mating and/or natural recombination" actually mean? As a next step we will therefore go over a list of concrete examples of genetic alterations in different species and determine, based on our current understanding of biology, whether these alterations do occur naturally.

ALTERATIONS BEYOND WHAT CAN OCCUR NATURALLY

In **Table 1** we provide 15 concrete examples of genetic alterations. We do not specify by what means these alterations have been introduced, but in line with the Cartagena Protocol definitions they would only result in the formation of a specifically regulated organism, if—besides having resulted in the formation of a genetic combination that does not occur naturally by mating and/or natural recombination—they also have been achieved by a method that does not occur naturally. We propose that the wording "does not occur" should be interpreted such that the alterations are extremely unlikely to occur. Those that are more likely to occur in nature or as a result of human intervention via conventional breeding approaches would not be considered to result in the formation of a specifically regulated organism.

The first two examples in **Table 1** are about point mutations of which we state that the likelihood of occurrence is very high. This can be easily substantiated by performing sequence analyses to estimate the occurrence of single nucleotide polymorphisms (SNPs) in genes in different crops. In most genes one will find dozens if not hundreds of SNPs. The more varieties of a crop are subject to the analysis, the more SNPs are generally found. In a separate appendix to this paper we provide such an analysis for the acetolactate synthase (ALS) gene in Arabidopsis, wheat and rice. This can be seen as an illustrative example for the occurrence of SNPs in any gene in a crop. Similarly, one can perform sequence analyses for determining the natural occurrence of the other types of genetic alterations that we describe in **Table 1**, as well.

EMERGING CONCLUSIONS AND PRINCIPLES

From what we have described above it is apparent that quite a lot of alterations are already occurring naturally. If we then go over the more concrete examples of genetic alterations (**Table 1**), in many occasions we get a rather clear picture. When such alterations are deliberately introduced by means of techniques that do not occur naturally, and the regulatory framework uses novelty of the genetic combination as a criterion, then the resulting organisms either would clearly classify as a specifically regulated organism or would clearly not classify as such. This is for instance the case for single point mutations or for deletions of any size, which in that context would not be considered to result in the formation of a specifically regulated organism, or for the introduction of a transgene, which does result in the formation of such an organism. The deliberate and simultaneous introduction of a very large amount of specific point mutations would under such legal approach also be considered to result in the formation of a specifically regulated organism, even though each individual mutation can occur naturally.

But there are also areas that are less clear. Exactly how many point mutations can be simultaneously introduced before the organism would become a specifically regulated organism? And how many sequential base pairs of exogenous or recombinant DNA, stemming from a non-crossable source, can be introduced before the organism becomes a specifically regulated organism? For sure, the more point mutations and the longer the stretch of exogenous DNA, the less likely it is that this would occur spontaneously in nature. Drawing a line will inevitably be arbitrary, however for regulatory purposes this will be necessary.

Concerning the number of point mutations one could perhaps debate why this is important to discuss in detail. Yet, it might still be relevant to determine how many point mutations are allowed to happen because it will largely determine whether the resulting organism would have to be classified as a specifically regulated organism or not. Are 10 simultaneous point mutations acceptable? For sure the introduction of 40 SNPs is acceptable if they are introduced by means of an

TABLE 1 | Genetic alterations and the proposed classification of organisms in the context of GMO regulatory oversight.

	Organism	Description of the alteration	Does it occur in nature?	Likeliness of occurrence	Alteration beyond what does occur naturally by mating and/or natural recombination*	Motivation
1	Any plant	Single point mutation	YES	Very likely	NO	The mutation rate has been estimated in different plants to be on average 1 mutation per genome per generation, meaning that every single plant will contain, at least, one new mutation. See also the **Appendix** in supplementary material.
2	Any plant	2,000 point mutations	Perhaps only in a nuclear fall-out	Extremely unlikely	YES	This is the example of classical mutagenesis using a radioactive source to induce random mutations. The organisms resulting from this intervention are exempted from the scope of the EU GMO directive on the condition that no recombinant nucleic acid molecules or GMOs have been used.
3	Any plant	Short deletions (a few bp)	YES	Very likely	NO	Such one base pair deletions occur on a regular basis. Mostly after the occurrence of a double-strand break.
4	Any plant	Larger deletions (a few kbp)	YES	It does happen	NO	Larger deletions also do occur, but with a lower frequency. In viruses such deletions occur rather frequently during processes of attenuation.
5	Any plant	Short insertions (a few bp)	YES	Very likely	NO	Small insertions also occur on a regular basis. Similar as with small deletions such insertions may occur, for example after a double-strand break.
6	Maize	50 kb exogenous DNA insertion	YES	Very unlikely	YES	Such size of insertions do occur in very rare occasions, but the larger the insertion the more unlikely they become.
7	Any plant	2 kb exogenous DNA insertion from a non-related source (a transgene)	YES	Very unlikely	YES	Even though there are rare events of horizontal gene transfer, it is very unlikely that such an event occurs. This is also what the legislation truly intended to have covered by the GMO legislation.
8	Any plant	A cisgene flanked by the T-DNA left and right borders	NO	Extremely unlikely	YES?	There are no known examples of such types of alterations occurring in nature. Where T-DNA has been introduced spontaneously, it was with sequences stemming from *A.tumefaciens* in between.
9	Any plant	An allele swap	YES	It does happen	NO	Allele swaps do occur, especially as a result of recombination during meiosis.
10	Any plant	Cisgene addition	YES	It does happen	NO/YES	The difference with an allele swap is that the cisgene may be present anywhere in the genome, not necessarily on its 'natural' location. It is known however that in plants genes on a regular basis get duplicated, resulting in an extra copy of the gene being inserted at another location in the genome, away from its original 'natural' location.

(Continued)

TABLE 1 | Continued

	Organism	Description of the alteration	Does it occur in nature?	Likeliness of occurrence	Alteration beyond what does occur naturally by mating and/or natural recombination*	Motivation
11	Any plant	Intragene addition	?	Extremely unlikely	YES	The rearrangement of regulatory sequences does occur in nature and is actually important in evolutionary terms. But it is extremely unlikely to occur within a few generations in the context of plant breeding.
12	Plants that cannot naturally be infected by *A. tumefaciens*	T-DNA insertion	NO	Extremely unlikely	YES	When a plant cannot naturally be infected by *A. tumefaciens*, then the insertion of T-DNA cannot occur.
13	Plants that can be naturally infected by *A. tumefaciens* such as *Nicotiana* spp., sweet potato and *Linaria* spp	T-DNA insertion	YES	Unlikely, but it has happened	NO	*A. tumefaciens* can naturally infect a wide variety of plants, but this usually is a local infection resulting in the formation of crown galls, without the T-DNA being inserted into the reproductive DNA. However, this has happened during evolution of for example sweet potato.
14	Any plant	Genome duplication	YES	It does happen	NO	Genome duplications do spontaneously happen in nature but have even more been exploited by man to strengthen certain characteristics of crops.
15	Any plant	Transposon insertion	YES	It does happen	NO	Transposons mobile genetic elements that account for an important fraction of all plant genomes. Its movement generates mutations that are at the origin of many traits selected by humans to domesticate wild plants of breed new varieties.

**As proposed in the article organisms should only be considered as GMO subject to specific regulatory oversight when they fulfill two criteria: (1) the alteration must have been achieved by means of a technical intervention that does not occur naturally, and (2) the genetic combination formed must be beyond what does occur naturally by mating and/or natural recombination.*

allele swap, where one existing allele is replaced by another allele originating from the organisms' gene pool. Another question is whether the introduction of different mutations in consecutive rounds of intervention at a certain point would trigger the legislation. In other words: would the simultaneous introduction of 200 point mutations be considered to lead to the formation of a novel combination of genetic material and therefore become a specifically regulated organism, whereas the accumulated introduction of 200 single point mutations would not? These intricate but realistic questions need pragmatic answers.

RECOMMENDATIONS

The scheme and the concrete examples that we have presented will help to clarify the regulatory status of genome edited organisms within different regulatory frameworks. Several competent authorities in the world have recently clarified the scope of their legislation. USDA-APHIS has stated that organisms with single point mutations, deletions of any size, and in which genes from compatible species have been introduced are not a regulated article under its biotechnology regulations. In their view such organisms do not require specific regulatory scrutiny because they could otherwise have been developed through traditional breeding techniques. Conventionally bred varieties are considered by the US regulators to present a risk level one should not be forced to go below. In Argentina and Brazil regulatory procedures have been introduced through which the regulatory bodies can determine on a case-by-case basis whether something would be regulated as a GMO (Whelan and Lema, 2015). They use the novelty criterion from the Cartagena Protocol LMO definition as their guiding principle. The outcomes of these procedures so far show that also they do not regard organisms with point mutations or (small) deletions, or any other that could have occurred through conventional breeding or by natural, spontaneous mutations, as GMOs that require specific scrutiny.

In the EU, the recent ruling of the Court of Justice of the European Union considers genome edited organisms as GMOs that do not fall under the existing exemption for organisms resulting from conventional mutagenesis (CJEU).[1] The motivation of the ruling leaves very little room for a more product-oriented interpretation of the phrase "has been altered in a way that does not occur naturally by mating and or natural recombination" in the EU GMO definition. The Court has not used the novelty criterion, even though it could have. This implies that organisms that have edits that can or do occur naturally, will have to follow the same regulatory procedures as GMOs, including a detailed analysis of possible risks. This does not comply with the principles set out in the Cartagena Protocol on Biosafety and would be disproportionate and scientifically unsound. In the last few months, different proposals to solve this situation have been proposed. First, the existing EU GMO legal framework could be modified following for instance the proposal put forward by the Netherlands (Eriksson et al., 2018). This would allow including genome editing techniques in the list of techniques exempted from the regulation. Another option that has also been proposed would be to revert the trend of the last 15 years limiting the latitude of scientist and risk assessors applying the case-by-case approach to the risk analysis of GMOs as laid down in Directives 90/220/EEC and 2001/18/EC (Casacuberta and Puigdomènech, 2018). Whatever the path followed, the principles developed in this article should help to adjust the legal framework to each use of genome editing techniques and perform a more proportionate risk assessment of genome edited organisms.

It is important to note that being classified or not as an LMO under the Cartagena Protocol, a GMO under EU legislation, a regulated article under the USDA-APHIS plant pest legislative framework, is not *per se* a safety related issue. However, it is true that especially the EU GMO regulatory framework is foremost applied to organisms that contain novel genetic combinations beyond what does occur naturally by mating and/or natural recombination, and subject these to pre-market risk assessment under the pretext that these organisms may carry with them a risk due to the genetic novelty *per se*. Mutations, whether naturally occurring or man-made, such as limited nucleotide changes could also result in phenotypic changes presenting a hazard and the legislator has not seen a need to place them under additional scrutiny. This is because the regulator has considered the products of traditional breeding techniques including mutagenesis to present a risk level that is acceptable.

Breeders are not required by law to perform a pre-market risk assessment and get a market authorization for new varieties based on the use of conventional breeding techniques including conventional mutagenesis. But their products nevertheless need to be safe for human consumption and the environment. If they are not, other food and environmental legislation such as the US legislation on food safety, the EU general food law and the EU environmental liability legislation enters into force to correct them and, if necessary, hold the developer accountable (De Jong et al., 2018).

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[1] http://curia.europa.eu/juris/document/document.jsf?text=&docid=207002&pageIndex=0&doclang=EN&mode=req&dir=&occ=first&part=1&cid=6947981

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frontiers in Plant Science

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Plant Genome Engineering for Targeted Improvement of Crop Traits

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To improve food security, plant biology research aims to improve crop yield and tolerance to biotic and abiotic stress, as well as increasing the nutrient contents of food. Conventional breeding systems have allowed breeders to produce improved varieties of many crops; for example, hybrid grain crops show dramatic improvements in yield. However, many challenges remain and emerging technologies have the potential to address many of these challenges. For example, site-specific nucleases such as TALENs and CRISPR/Cas systems, which enable high-efficiency genome engineering across eukaryotic species, have revolutionized biological research and its applications in crop plants. These nucleases have been used in diverse plant species to generate a wide variety of site-specific genome modifications through strategies that include targeted mutagenesis and editing for various agricultural biotechnology applications. Moreover, CRISPR/Cas genome-wide screens make it possible to discover novel traits, expand the range of traits, and accelerate trait development in target crops that are key for food security. Here, we discuss the development and use of various site-specific nuclease systems for different plant genome-engineering applications. We highlight the existing opportunities to harness these technologies for targeted improvement of traits to enhance crop productivity and resilience to climate change. These cutting-edge genome-editing technologies are thus poised to reshape the future of agriculture and food security.

Keywords: CRISPR/Cas systems, genome editing, genome engineering, crop improvement, climate change, food security, synthetic biology

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FOOD SECURITY: ADDRESSING OLD CHALLENGES AND EMERGING THREATS

To sustain life, food must provide an adequate supply of calories and nutrients. Food insecurity, the lack of access to an adequate food supply, threatens millions of people worldwide with malnutrition. Moreover, the problem is getting worse; the global population is growing rapidly and is expected to reach 8.3 billion by 2030 (United Nations, Department of Economic and Social Affairs, Population Division, 2017). As a result, the demand for food, animal feed, and fuel will increase (Sundström et al., 2014). Challenges to food security, such as increasing population, have been joined by new threats such as increases in abiotic stresses due to climate change, decreases in arable land due to desertification, salinization, and human use, and emerging diseases. To enhance food security for future generations, the world must double the current crop production rate in spite of the

predicted threats, including climate change (Godfray et al., 2010; Jones et al., 2014). Plant breeders have harnessed natural and artificial mutations, as well as important tactics such as breeding for hybrid vigor, to address food insecurity. However, additional work will be required to meet current and emerging challenges.

To improve crop yield, current approaches aim to increase the amount of food produced per unit of area cultivated, and to prevent crop failures. To increase yield per area in grain crops such as rice, breeders have targeted traits that increase the number of grains produced per plant, the number of plants that can be cultivated per unit area, and the size of each grain. Many of these traits involve manipulation of plant architecture through balancing meristem activity and hormone signaling. To prevent crop failures and thus improve yield stability, breeders have targeted traits that help crops tolerate stresses. For abiotic stress, researchers have targeted tolerance to heat, cold, high light, high salt, heavy metals, and other stresses. For biotic stresses, which have become an increasing problem as globalization and weather accelerate the spread of pathogens, researchers have identified loci conferring resistance to various viral, bacterial, and fungal pathogens, as well as loci affecting interactions with animal and plant pathogens, including nematodes and parasitic plants such as Striga (Butt et al., 2018). The challenge in disease resistance is twofold, identifying the essential loci to introduce, and introducing the key resistance loci into elite varieties in a timely manner. Moreover, balancing the energy requirements for resistance and growth to minimize yield penalties remains difficult.

To increase the nutrition of crops, current approaches aim to provide diverse and balanced diets with adequate levels of vitamins and minerals that enhance human health. Recent developments in crop biotechnology make it possible to manipulate the key enzymes in certain metabolic pathways, thereby enhancing the contents of key nutrients such as vitamins and iron, and reducing the contents of unfavorable compounds such as phytic acids and acrylamide-forming amino acids. Several biofortified crops such as rice, maize, and wheat have been produced to solve the problem of nutrition deficiencies (Ye et al., 2000; Gil-Humanes et al., 2014; Mugode et al., 2014). A well-known example is Golden Rice, which is genetically engineered to produce a significant level of β-carotene to help people at risk of vitamin A deficiency (Ye et al., 2000).

A HISTORICAL PERSPECTIVE ON PLANT GENOME ENGINEERING

Nature has been altering genomes for thousands of years, with natural selection enabling plants with certain genomic variants to survive. Moreover, humans have been using artificial selection to domesticate crops for more than 10,000 years. This process produced modern corn from its wild ancestor teosinte, among many other examples. Indeed, all crops grown today have undergone extensive genetic changes. Genetic changes or variations are key to crop improvement, but our ancestors had to make do with naturally occurring mutations. In the twentieth century, once it was recognized that DNA and genes shape all life, it became clear that altering DNA sequences induces phenotypic variations. Therefore, researchers developed and tested reagents, including radiation and chemical mutagens, to induce DNA mutations and have examined the resulting phenotypic variations (Shu et al., 2012). This mutation breeding concept was established in the 1940s and yielded noteworthy successes, such as the wheat varieties with significantly improved yields that were key to the Green Revolution of the 1970s.

A major advance in genetic modification was made with the discovery that *Agrobacterium tumefaciens* (Agrobacterium), the bacterium that causes crown gall disease, is a natural genetic engineer that introduces a piece of its own DNA into the genome of a plant it infects, potentially carrying along a DNA sequence provided by a researcher (Nester, 2014). This bacterium injects a so-called tumor-inducing (Ti) plasmid into the plant cell, where it integrates into the genome (Yadav et al., 1982). Engineering of Ti-plasmid-derived "binary vectors" that can replicate in *Escherichia coli* as well as in Agrobacterium, and still integrate into plant genomes, provided the basis for plant biotechnology. Using these tools, it is possible to incorporate into a plant genome even genes from distantly related organisms, in a process called transgenesis; if the genes come from related plant species, this process is called cisgenesis (Schubert and Williams, 2006). However, this approach has many drawbacks, including the random nature of the gene insertion, the possibility of disrupting functional genes, public concerns over genetically modified organisms (GMOs), and the failure to make use of the native genetic repertoire of the plant. There was therefore a pressing need for techniques to precisely change DNA sequences at the single-base level. Such technologies for adding, deleting, and editing existing DNA sequences to develop traits of interest are essential to crop bioengineering for various purposes, including improving crop performance to withstand the hotter and drier environments expected to arise under climate change.

In the 1980s, Mario Capecchi first established gene-targeting technology, along with the concept of harnessing double-strand breaks (DSBs) for genome editing (Capecchi, 1980). A later development was the ability to engineer genomes by generating site-specific DSBs (Jasin, 1996). After DSBs are generated, the cell's own repair machinery can be harvested to dictate the genetic outcome through the imprecise repair process of non-homologous end joining (NHEJ) or the precise repair process of homology-directed repair (HDR) (Trevino and Zhang, 2014; Baltes and Voytas, 2015; Bortesi and Fischer, 2015; Schaart et al., 2016) (**Figure 1**). For example, NHEJ can cause insertion or deletion of a few bases and thus create functional knockouts of genes (Gorbunova and Levy, 1997; Charbonnel et al., 2011; Lloyd et al., 2012). By generating more than one DSB, it becomes possible to produce even more types of changes, including chromosomal deletions, gene inversions and, with DSBs on two different chromosomes, chromosomal translocations (Morgan et al., 1998; Ferguson and Alt, 2001). In contrast to NHEJ, HDR produces a precise repair and enables the sequence to be rewritten in a user-defined manner (Puchta et al., 1996; Puchta, 2005) (**Figure 1**). HDR can be used for genome editing and precise modification of the genome with various types of repair templates, ranging from short oligonucleotides to those a few

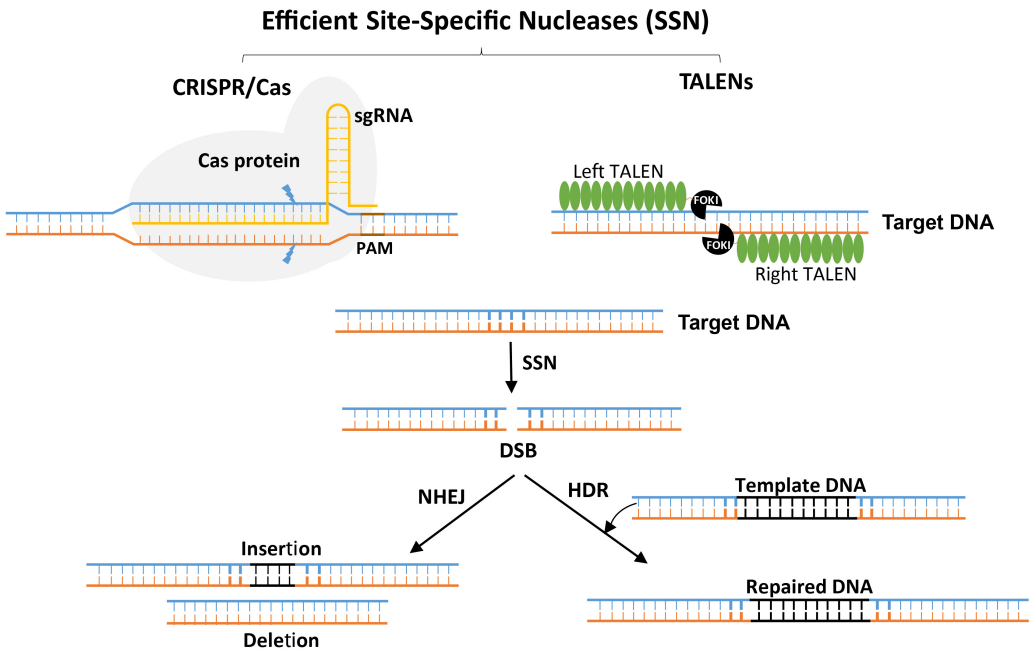

FIGURE 1 | Site-specific nuclease-induced genome editing. The double-stranded breaks (DSBs) introduced by CRISPR/Cas or TALEN complexes stimulates the endogenous DNA repair machineries, non-homologous end joining (NHEJ) or homology-directed repair (HDR). The NHEJ machinery repairs the DNA imperfectly and introduces frameshifts by insertion or deletion leading to loss-of-function mutations. However, the HDR pathway precisely inserts a piece of DNA (from an exogenous template DNA with enough similarity to the DSB flanking sequence) by homologous recombination, which is useful for the introduction of specific point mutations or a new gene sequence.

hundred base pairs in length up to full genes with homologous ends or arms flanking the DSB site (Song and Stieger, 2017; Boel et al., 2018).

Generating DSBs allows many possible mechanisms of genome editing to be accomplished by harnessing the cell's repair machinery. The big question was how to generate a site-specific DSB. Proteins that can be engineered and reprogrammed to bind and cleave DNA do not exist in nature. However, it is possible to program a DNA-binding domain to bind to any user-defined site-specific sequence. This domain can be fused with another domain that can cleave the DNA specifically where it binds. These bimodular fusion proteins are the key to precise genome engineering because they can be programmed to bind to any user-selected sequence and generate a DSB. Such programmable site-specific binding proteins can carry other functional domains capable of effecting other genetic and genomic changes, including transcriptional regulation, epigenetic regulation, and even base editing without generation of DSBs (Komor et al., 2016; Puchta, 2016). The genome-engineering toolbox has three major platforms: zinc-finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and CRISPR/Cas systems. ZFNs and TALENs are protein-based and require protein engineering for every user-defined sequence. However, CRISPR/Cas is an RNA-guided system and can be easily engineered to bind to the DNA target (Belhaj et al., 2013; Osakabe and Osakabe, 2015; Quétier, 2016). TALENs and CRISPR/Cas9 have been used to produce many key agricultural innovations; therefore, we focus on these systems below.

TALEN-BASED SYSTEMS

In nature, the phytopathogen *Xanthomonas oryzae* (Xanthomonas) produces TAL effectors (TALEs), which enter the plant cell nucleus and reprogram the transcription machinery to benefit the pathogen (Doyle et al., 2013). They function as eukaryotic transcription factors by binding to the promoter region and activating gene expression. TALEs have unique structural features, including a central DNA-binding repeat that dictates DNA binding specificity through a one repeat to one base pair binding correspondence (Deng et al., 2012; Doyle et al., 2013). By engineering the number and type of these repeats, TALEs can be engineered to bind any DNA sequence (Li L. et al., 2012). Fusion of a TALE with a nuclease produces an enzyme that can generate site-specific DSBs *in vitro* and *in vivo* (Christian et al., 2010; Mahfouz et al., 2011).

The structural basis of TALE-DNA binding is amino acid 12 of the TALE repeat sequence, known as the repeat variable di-residue (RVD), which facilitates and stabilizes the contact, and amino acid 13, which confers binding specificity (Boch et al., 2009; Deng et al., 2012). The DNA-binding specificities of TALEs allows them to serve as DNA-binding modules for building synthetic transcriptional and epigenetic regulators. Several engineering platforms have been developed for TALEs. Moreover, researcher have interrogated genomes from microbes other than Xanthomonas and determined that another bacterium, *Ralstonia solanacearum* (Ralstonia), possesses Ralstonia TALE-like proteins (RTLs) with similar structure but completely

different repeats, along with enriched numbers of the RVDs that determine repeat specificity (Bogdanove et al., 2010).

Deciphering the code of RTL binding to DNA revealed that these RVDs provided a rich resource for TALEN-based engineering (Li L. et al., 2013). For example, the canonical TAL-binding RVD code (described using the single-letter amino acid code) is that the RVD HD binds to cytosine, NG binds to thymine, NN binds to adenine or guanine, and NS binds to any nucleotide. For RTLs, the DNA-binding code includes ND binding to cytosine, SH binding to guanine, NT binding to adenine, and HN binding to adenine or guanine, among others. These added binding specificities have provided diverse options and opportunities for TAL-based engineering (Li L. et al., 2013). Nonetheless, the requirement for engineering a specific protein for every target and the need for two TAL monomers to simultaneously bind the DNA strands makes TALEN-based genetic engineering time consuming and resource intensive (Joung and Sander, 2013; Nemudryi et al., 2014). Despite these challenges, many companies have chosen TALEN-mediated gene editing for its high precision and clear intellectual property landscape.

CRISPR/Cas SYSTEMS

In nature, bacterial and archaeal species fend off invading phages and foreign genetic elements through the use of clustered regularly interspaced palindromic repeats (CRISPR)/CRISPR-associated protein (Cas) adaptive immune systems. About 40% of bacteria and most archaea have with several CRISPR/Cas systems capable of targeting DNA, RNA, or both for degradation, thereby defending themselves against foreign genetic elements (Jansen et al., 2002; Sorek et al., 2008). When a phage infects a bacterium equipped with CRISPR, the bacterium acquires pieces of the phage DNA within the CRISPR array in what is called the adaptation phase. Acquisitions are ordered with most recent one closest to the leader sequence, which functions as a promoter. The CRISPR array is transcribed and generates mature RNAs (known as crRNAs) in the biogenesis phase. Cas9 uses these crRNAs as guides to target the phage genome during future invasions and thereby provide immunity to the bacterial cell, marking the interference or immunity phase (Barrangou et al., 2007; Rath et al., 2015). Cas9 usually cleaves a DNA region that is 3–4 nucleotides upstream of a three-nucleotide protospacer-adjacent motif (PAM), which is not found in the bacterial genome, thus allowing this adaptive immune system to specifically target invading phages (Jinek et al., 2012).

CRISPR systems are classified into two main groups, classes I and II (Makarova et al., 2011, 2015). In class I systems (subdivided into types I, III, and IV), the interference complex is a multicomponent system composed of multiple effectors. In class II systems (types II, V, and VI), the interference complex is a single-component system and the interference complex comprises a single effector guided by the crRNA (Makarova et al., 2011, 2015; Shmakov et al., 2017). The CRISPR/Cas9 system, which belongs to class II, is a two-component system composed of Cas9 and a single guide RNA (sgRNA) molecule. Recently, other class II systems have been discovered, including some based on the Cas12a enzyme (previously known as Cpf1), which generates DSBs with staggered ends and has a T-rich PAM, thereby enriching the options available for genetic engineering in repetitive T-rich genomic regions across eukaryotic species (Zetsche et al., 2015). Also in class II are the type VI systems, based on the enzyme Cas13a, which is capable of targeting the RNA of viral species, thereby providing a very effective machinery for RNA interference in both prokaryotic and eukaryotic species (Abudayyeh et al., 2016).

High-Efficiency Plant Genome Engineering Using CRISPR/Cas

For high-efficiency genome engineering in any eukaryotic cell, it is necessary to ensure that delivery of the genome-engineering reagents to the appropriate species be feasible and that editing of the target genome is both highly specific and efficient (**Figure 2**). Therefore, reagent delivery and editing specificity are key research areas for developing high-efficiency genome-engineering technologies. For plants, a current major focus is on developing delivery platforms for genome-engineering reagents, preferably for delivery into germline cells to bypass the need for tissue culture and regeneration after editing (Forner et al., 2015; Mao et al., 2016). Delivery platforms include bacterial and viral vectors, and physical delivery into different types of cells. Specificity research involves the identification of Cas9 variants that are inherently more specific than current enzymes and have optimized expression and sgRNA architectures, as well as the titration of sgRNA and Cas9 concentrations during the editing process. Editing research involves developing effective HDR technologies that provide ultimate control over the repair process and the genetic outcome, including the ability to generate gene fusions, targeted gene replacement and additions, and single-base substitutions (Ochiai, 2015; Vanden Bempt et al., 2016; Zhao et al., 2016). Efficient editing remains challenging in most eukaryotic cells, and several research efforts focused on improving gene editing are detailed below.

Efficient Delivery Vehicles for Genome-Engineering Reagents

Engineering of the CRISPR/Cas9 system currently means simply engineering the sgRNA molecule, which provides targeting specificity and can also include a template for HDR. Therefore, we sought to develop a system in which we could use a virus as the vehicle for sgRNA delivery into plants expressing Cas9 (Ali et al., 2015b). This approach involves the generation of a Cas9 overexpression line in a model plant species such as *Nicotiana benthamiana* or *Arabidopsis thaliana* and the subsequent delivery of sgRNAs via *Tobacco rattle mosaic virus* (TRV). After establishment of the TRV infection in the Cas9 overexpression line, we assayed for modification of the genomic target sequence. To further improve the efficiency of this approach and increase the recovery of seed progeny carrying the modification, we recently tested delivery with *Pea early browning virus* (PEBV), which is capable of infecting the germline (Ali et al., 2018a). When we compared the efficiencies of TRV and PEBV for

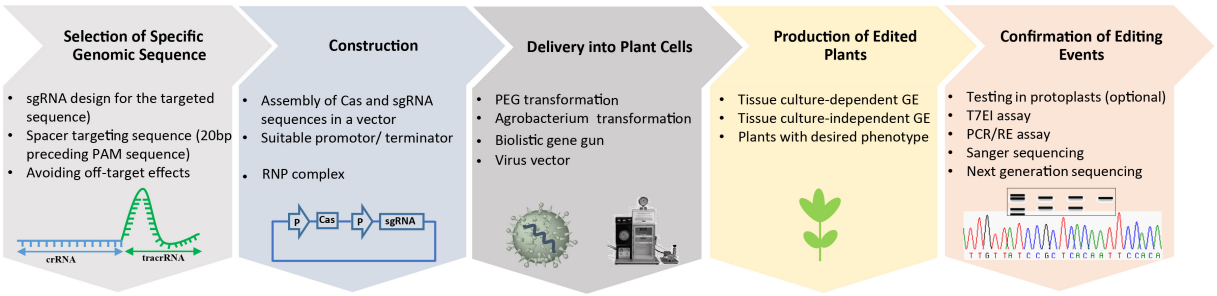

FIGURE 2 | Simplified workflow for CRISPR/Cas9-mediated plant genome editing. The production of edited plants with a desired phenotype starts from the design of a sgRNA for a specific target sequence and cloning of the sequence to express the sgRNA into a binary vector containing the Cas DNA sequence. Then the delivery of CRISPR/Cas materials into the plant cell, followed by assays to confirm the presence of the edited events, and regeneration of whole plants.

targeted mutagenesis of somatic tissues, we found that PEBV is highly efficient (Ali et al., 2018a).

The viral delivery system provides two options: (1) tissue-culture-free genome editing, in which the CRISPR/Cas9 machinery is active in the germline, and (2) tissue-culture-dependent genome engineering. Some RNA viruses are capable of infecting germline cells, albeit at low frequency, and this would enable the recovery of progeny carrying the intended genomic modification, as discussed in more detail below (Ali et al., 2018a). We can also start with leaf tissue, where the efficiency of our genome-engineering system is good, and regenerate whole plants, which we can then genotype for the presence of the modification (Ali et al., 2015b, 2018a; Aman et al., 2018). Therefore, the advantages of viral systems include the potential to perform tissue-culture-free genome editing, high-efficiency targeted mutagenesis, and also the possibility to do functional genomics experiments using a sgRNA library constructed in the viral vector, as detailed below.

Among prokaryotic vectors, Agrobacterium is a natural genetic engineer because of its ability to transfer a piece of its genome, the transfer DNA (T-DNA), into the plant genome (Nester, 2014). This intriguing interkingdom DNA transfer is facilitated by the virulence (vir) proteins, which are encoded by the Ti plasmid and facilitate DNA nicking, processing, transfer, and integration into the plant genome (Hoekema et al., 1983). The T-DNA is transferred through the type IV secretion system, along with many bacterial proteins, and eventually enters the cell nucleus where it integrates randomly into the plant genome. Some of these virulence proteins make the trip from the bacterial cell into the plant cell regardless of whether T-DNA transfer occurs. One intriguing possibility would be to use some of those proteins to deliver ribonucleoproteins (RNPs) from the bacterium into the plant cell nucleus, as this could make it possible to produce the CRISPR/Cas9 machinery in bacteria and then deliver it intact into plant cells, allowing researchers to recover seed progeny carrying the desired gene edits without the need for classical tissue culture.

Germline Engineering via CRISPR/Cas9

Current plant genome-engineering efforts are primarily conducted through classical transformation and tissue culture, as with transgenesis approaches. This limits the application of CRISPR/Cas technologies in crop species, especially those that are recalcitrant to Agrobacterium transformation or to regeneration. There is thus a pressing need to develop technologies that do not rely on classical transformation and regeneration of transformed cells. The ideal target cell types for this approach are the germline cells, where delivery of CRISPR/Cas9 machinery in DNA or protein form can permanently change the genotype. RNP-mediated engineering of germline cells would be ideal given the regulatory hurdles associated with DNA-based editing and the need to produce plants that are free of foreign DNA.

As mentioned above, some viral systems can deliver sgRNAs to germ cells. Several other approaches can be used, including direct delivery of the reagents via Agrobacterium and isolation of the germline cells for polyethylene glycol (PEG)-mediated transfection (Mao et al., 2016). Other approaches using biolistic gene guns, electroporation, optoporation, magnetofection, or microinjection are appropriate to some germline cells, depending on the plant species and developmental stage (Mohanty et al., 2016). Select nanoparticles can be used to deliver genome-engineering reagents in RNP form to target cells (Cunningham et al., 2018). Improving delivery methods would accelerate and expand the applications of plant genome engineering.

Single-Cell Genome Engineering

Because the CRISPR/Cas machinery is easy to engineer and has robust activity in plant cells, making individual cells with engineered genomes is quite efficient. However, producing whole plants from these cells remains challenging. For example, regeneration is often genotype dependent, and in most cases the cultivars used in laboratory experiments are not the elite germplasm used in agriculture (Altpeter et al., 2016). Moreover, with transformation methods generally use selectable markers like antibiotic- or herbicide-resistance genes.

Efficient single-cell regeneration will be a major achievement in plant biotechnology, and research in this area is ongoing on multiple fronts. Recent efforts have been made to deliver CRISPR/Cas9 in RNP form into the protoplasts of lettuce and tobacco, with subsequent editing and regeneration from single protoplast cells (Woo et al., 2015; Kim et al., 2017; Lee et al.,

2018). Regeneration from protoplasts is quite inefficient in most plant species, however, limiting the application of this technology and the ability to produce foreign-DNA-free edited plants.

Recently, morphogenic factors have been used to enhance regeneration frequency (Altpeter et al., 2016; Lowe et al., 2016). Other possible avenues of approach are applying transient expression of shoot-specifying transcription factors to protoplast single-cell transformation, identifying effective strategies to boost regeneration competence of edited cells, and/or comparing the germplasm of cells with high regeneration frequency with that of cells that are recalcitrant to regeneration, with the aim of identifying regeneration boosters. Any or all of these approaches may improve the ability to regenerate plants from single cell, a key requirement for harnessing the power of CRISPR/Cas for genome-engineering applications.

CRISPR-Mediated Genome-Wide Functional Genomics Screens

CRISPR/Cas systems offer the ability to produce a variety of genetic and epigenetic modifications that could be instrumental to testing gene functions and regulation in the genomic context. It is feasible to develop CRISPR genome-wide screens as a gene discovery platform whereby sgRNAs are used to generate mutations or epigenetic changes in single or multiple genes (Sharma and Petsalaki, 2018). In the CRISPR GWS genome-wide screen system, it might be possible to construct sgRNA libraries that target the entire genome (**Figure 3**). The sgRNA libraries would then be cloned into binary vectors for plant transformation. Once the CRISPR/Cas machinery is expressed and seed progeny with modifications are recovered, preferably in one generation to ensure homozygosity of the modification, they can be subjected to screening to identify interesting phenotypes like resistance to abiotic or biotic stress factors, virus resistance, architecture, flowering, yield, and other traits of interest.

Once plants with the desired phenotype are identified, the causal genes can be easily identified by cloning and sequencing the sgRNA. The results can then be confirmed by expressing the wild-type allele of the candidate causal gene. In the past, this required time-consuming and laborious mapping efforts, which are quite challenging in many crops that are key for food security. Not only is CRISPR much more efficient at generating mutations than older (e.g., chemical) mutagens, but the nature of the mutations is different, meaning that adding CRISPR to the plant breeders' toolbox will enrich plant populations and enhance the gene and trait discovery process.

These screens can be applied to either loss-of-function or gain-of-function platforms depending on which CRISPR/Cas system is used. Of particular utility would be the application of the CRISPR/Cas9 and CRISPR/Cas12a platforms, since they produce permanent changes in the genome and do not require the presence of the CRISPR/Cas9 machinery (Zetsche et al., 2015). An interesting modality would be the application of selective pressure during the expression of the CRISPR/Cas machinery, so as to force the generation of certain edits that could help plants resist those specific stress factors. Furthermore, for gene function analysis, researchers could use dCas9TF, Cas13a, or Cas13b along with base editors to transiently perturb key gene functions such as housekeeping and embryonic-lethal genes. These CRISPR systems are expected to allow very efficient gene and trait discovery not only in model species but in crop species as well, which will be crucial to improving crop yield and resilience under the unfavorable conditions of climate change.

CRISPR/Cas9 and TALEN Off-Target Activities

One major drawback to CRISPR/Cas9 systems is that they are prone to off-target activities (Zhang et al., 2015), owing to the ability of Cas9 to cut at other, unintended places in the genome in addition to the intended target sequence. This currently poses grave limitations on the use of CRISPR/Cas9 in gene therapy and genetic medicine. In contrast to CRISPR/Cas9, the TALEN system exhibit precision but delivery of TALENs is quite challenging.

Many approaches have been employed to reduce CRISPR/Cas9 off-target activity, including inducible systems to limit the availability window and concentration of Cas9, and different sgRNA architectures (Zhang et al., 2015; Cao et al., 2016). One strategy involves generating a chimeric fusion between a catalytically inactive Cas9 protein (dCas9) and the FokI catalytic domain. The inactive dCas9 is used as a targeting module to bring the FokI domain into close proximity and allow dimerization (Guilinger et al., 2014; Aouida et al., 2015), and the formation of homodimers with the right spacer sequence then allows the generation of DSBs. This dramatically increases the cutting specificity, because it requires 40 bp of unique sequence and a unique distance between the two monomers, thus limiting off-target activities (Yee, 2016). Several studies have indicated that off-target activities of Cas9 are not easily detected *in planta*, corroborating the general assumption that these off-target activities occur at very low levels in plants unlike a mammalian system where off-target activities is a serious problem (Ali et al., 2015b; Yee, 2016; Morgens et al., 2017).

Targeted Improvement of Crop Traits

Although genome engineering is relatively new, the technology has been efficiently adapted to a wide range of crops as a means to improve yield, quality and nutritional value, herbicide resistance, and biotic and abiotic stress tolerance (Wang et al., 2016) (**Table 1** and **Figure 4**). For identification of targets for genome editing, genetic studies have identified key yield-related loci and advanced sequencing technologies in crop species have produced key information on the sequence variation of trait-related genes. The identification of beneficial alleles that produce desirable phenotypes offers exciting possibilities for the use of genome engineering for accelerated and targeted trait improvement. Here, we provide highlights of key advances for improving crop traits using genome engineering and discuss the promise of these technologies for enhancing food security.

Improving Yield

Yield is one of the most important traits for crop plants. It is a quantitative trait, controlled by several genes (Xing and Zhang, 2010; Bai et al., 2012), and considerable research has been conducted to identify the quantitative trait loci (QTLs) controlling yield in various crop plants (Bai et al., 2012;

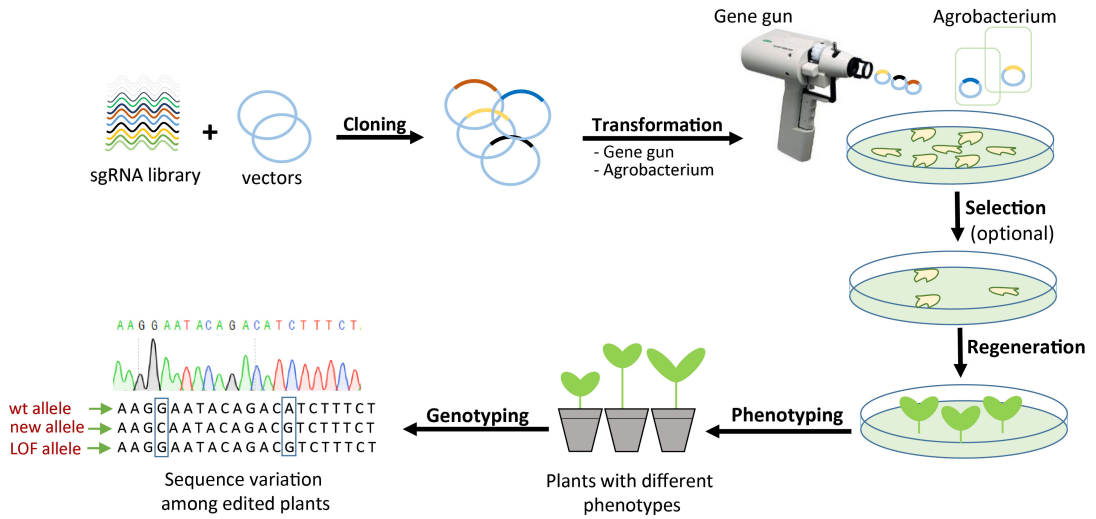

FIGURE 3 | CRISPR-mediated genome-wide screening. Schematic illustration of the procedure for generating a wide variety of new plant traits by targeting one or several genes using a pool of sgRNAs. After generation of edited plants, deep phenotyping and genotyping screening are required to discover the interesting traits and their genetic background. LOF, loss of function.

Jianru and Jiayang, 2014). Traditional breeding, the original method used to improve yield and develop plants able to survive in particular growth environments (Duvick, 1984), is a time-consuming process. Breeding relies on generating various combinations of QTLs and selecting the most promising ones for further breeding (Xufeng et al., 2012; Zuo and Li, 2014; Shen et al., 2018b). In addition, the introgression of QTLs between different varieties is not always easy, especially with closely linked loci.

Genome editing provides a promising tool to rapidly and specifically edit any genomic location. The most direct way of increasing yield is to knock out genes that negatively affect yield (Ma et al., 2016; Song et al., 2016) (**Table 1**). In one recent case, this was achieved by individually knocking out four negative regulators of yield (the genes *Gn1a*, *DEP1*, *GS3*, and *IPA1*) in the rice cultivar Zhonghua 11 by CRISPR/Cas9. Three of the resulting knockout mutations, *gn1a*, *dep1*, and *gs3*, showed enhanced yield parameters in the T_2 generation, resulting in improved grain number, dense, erect panicles, and larger grain size, respectively (Li M. et al., 2016). Similarly, Xu et al. (2016) simultaneously knocked out three major rice negative regulators of grain weight (*GW2*, *GW5*, and *TGW6*) using a CRISPR/Cas9-mediated multiplex genome-editing system. The resulting mutants showed a significant increase in thousand-grain weight. Zhang et al. (2016) targeted three homoalleles of *GASR7*, a negative regulator of kernel width and weight in bread wheat, by CRISPR/Cas9 and obtained an increase in the thousand-kernel weight. Similarly, using CRISPR/Cas9 to target a tomato *cis*-regulatory element in the *CLAVATA-WUSCHEL* stem cell circuit (*CLV-WUS*) that controls meristem size produced an edited tomato with an increased number of locules (seed compartments) and thus larger fruit size (Rodríguez-Leal et al., 2017). Moreover, CRISPR/Cas9 has been employed to generate functional knockouts of genes that indirectly contribute to the *improvement* of *yield* characteristics (Lawrenson et al., 2015; Soyk et al., 2016; Braatz et al., 2017; Li and Yang, 2017; Ma et al., 2018; Zhang et al., 2018).

Engineering Plant Disease Resistance

Plants are constantly infested by a variety of pathogens, including viruses, bacteria, and fungi (Taylor et al., 2004), that can cause significant losses of crop quality and yield (Savary et al., 2012). Considerable knowledge has been accumulated on the genetic basis of plant disease resistance, and genes related to disease resistance have been identified in different plant species, including Arabidopsis, rice, soybean, potato, tomato, and citrus (Michelmore, 1995; Hammond-Kosack and Jones, 1996).

Genome-engineering technologies have been widely harnessed to engineer plant resistance against pathogens (Ali et al., 2015b; Baltes et al., 2015; Ji et al., 2015; Iqbal et al., 2016) (**Table 1**). These technologies can be used to target host factors important for pathogen infection and replication, thus immunizing plants against various pathogens. For example, CRISPR/Cas9 was recently used to alter the promoter sequence of the canker susceptibility gene *CsLOB1* in citrus, leading to canker resistance and providing hope for generating disease resistance in citrus varieties (Jia et al., 2016; Peng et al., 2017).

Targeting homologs of *MILDEW-RESISTANCE LOCUS* (*MLO*) and other loci has improved resistance to fungal pathogens in several species. CRISPR/Cas9 and TALEN were successfully used to generate resistance to powdery mildew by simultaneously targeting the three homologs of the *MILDEW-RESISTANCE LOCUS* (*MLO*), *TaMLO-A*, *TaMLO-B*, and *TaMLO-D*, in wheat (Wang et al., 2014). In another example, the Tomelo transgene-free tomato, which is resistant to powdery mildew disease, was developed by targeting the *SlMlo1* gene using CRISPR/Cas9 (Nekrasov et al., 2017). Recently, Zhang et al. (2017) simultaneously modified the three homologs of the wheat *TaEDR1* gene to enhance resistance to powdery mildew

disease. In other efforts, knockout of the ethylene-responsive factor (ERF) gene *OsERF922*, a negative regulator of rice blast resistance, enhanced resistance to the blast fungal pathogen (Wang et al., 2016).

Modifications of sucrose transporters have proven successful for resistance against a devastating bacterial pathogen. Using TALENs, Li T. et al. (2012) induced site-specific mutations in the effector binding site of the promoter region of the rice sucrose-efflux transporter gene (*SWEET14*). These mutations affect the survival and virulence of the bacterial leaf blight pathogen *Xanthomonas oryzae* pv. *oryzae* (*Xoo*), resulting in resistant rice lines. CRISPR/Cas9 was also successfully implemented to create mutations in four rice *SWEET* type *S* genes (Zhou et al., 2014). These examples demonstrate the great potential of genome-engineering technologies for producing plant immunity to various pathogens.

CRISPR/Cas9-mediated interference against DNA viruses

Plant viruses can have disastrous effects on key staple crops, and the extreme economic impact of some plant virus epidemics

TABLE 1 | Application of genome editing tools in different plant species to improve yield, biotic, and abiotic stress resistance, and nutritional quality.

Target trait	Plant species	Targeted sequence(s)	Results	Method	Reference
Yield	*Oryza sativa*	*GS3*, *Gn1a*	Grain size and number increase	CRISPR/Cas9	Shen et al., 2018a
	Oryza sativa	*GW2*, *GW5*, *TGW6*	Grain weight increase	CRISPR/Cas9	Xu et al., 2016
	Oryza sativa	*Gn1a*, *DEP1*, *GS3*	Grain size and number increase and dense, erect panicles	CRISPR/Cas9	Li M. et al., 2016
Virus resistance	*Arabidopsis thaliana*	*eIF(iso)4E*	Potyvirus resistance	CRISPR/Cas9	Pyott et al., 2016
	Arabidopsis thaliana	BSCTV genome	Beet severe curly top virus resistance	CRISPR/Cas9	Ji et al., 2015
	Cucumis sativus	*eIF4E*[1]	Cucumber vein yellowing virus, zucchini yellow mosaic virus, and papaya ring spot mosaic virus-W resistance	CRISPR/Cas9	Chandrasekaran et al., 2016
	Nicotiana benthamiana	BSCTV genome	Beet severe curly top virus resistance	CRISPR/Cas9	Ji et al., 2015
	Nicotiana benthamiana	TYLCV genome	Tomato yellow leaf curl virus resistance	CRISPR/Cas9	Ali et al., 2015a
	Nicotiana benthamiana	*AGO2*	Virus resistance	CRISPR/Cas9	Ludman et al., 2017
Fungus resistance	*Oryza sativa*	*OsERF922*	Rice blast resistance	CRISPR/Cas9	Wang et al., 2016
	Solanum lycopersicum	*SlMlo*	Powdery mildew resistance	CRISPR/Cas9	Nekrasov et al., 2017
	Triticum aestivum	*TaMLO-A1*	Powdery mildew resistance	CRISPR/Cas9 TALEN	Wang et al., 2014
Bacterial resistance	*Citrus sinensis* Osbeck	*CsLOB1*	Canker resistance	CRISPR/Cas9	Peng et al., 2017
	Oryza sativa	*OsSWEET13*	Bacterial blight resistance	CRISPR/Cas9	Zhou et al., 2015
	Oryza sativa	*Os11N3* (*OsSWEET14*)	Bacterial blight resistance	TALEN	Li T. et al., 2012
Drought tolerance	*Arabidopsis*	*mir169a*	Improved drought tolerance	CRISPR/Cas9	Zhao et al., 2016
	Zea mays	*ARGOS8*	Improved grain yield under field drought stress conditions	CRISPR/Cas9	Shi et al., 2017
Salt tolerance	*Oryza sativa*	*OsRAV2*	Salt stress tolerance	CRISPR/Cas9	Duan et al., 2016
Herbicide tolerance	*Linum usitatissimum*	*EPSPS*	Glyphosate tolerance	CRISPR/Cas9	Sauer et al., 2016
	Nicotiana tabacum	*MEL1*	Herbicide tolerance	ZFN	Cai et al., 2009
	Nicotiana tabacum	*ALS*	Resistance to imidazolinone and sulfonylurea herbicides	TALEN	Zhang et al., 2013
	Oryza sativa	*ALS*	Chlorsulfuron and bispyribac sodium tolerance	CRISPR/Cas9	Sun et al., 2016
	Oryza sativa	*EPSPS*	Glyphosate tolerance	CRISPR/Cas9	Li J. et al., 2016
	Solanum tuberosum	*ALS1*	Chlorsulfuron and bispyribac sodium tolerance	CRISPR/Cas9	Butler et al., 2016
	Zea mays	*IPK1*	Herbicide tolerance	ZFN	Shukla et al., 2009
Nutritional improvement	*Camelina sativa*	*FAD2*	Enhancement of seed oil	CRISPR/Cas9	Jiang et al., 2017
	Oryza sativa	*SBEI*, *SBEIIb*	High amylose content	CRISPR/Cas9	Sun et al., 2017
	Oryza sativa	*OsBADH2*[2]	Increased fragrance content	TALEN	Shan et al., 2015
	Solanum tuberosum	*GBSS*	High-amylopectin starch	CRISPR/Cas9	Andersson et al., 2017
	Zea mays	*ZmIPK*	Reduced phytic acid content	CRISPR/Cas9 TALEN	Liang et al., 2014

[1] *eIF4E, eukaryotic translation initiation factor 4E.* [2] *BADH, betaine aldehyde dehydrogenase.*

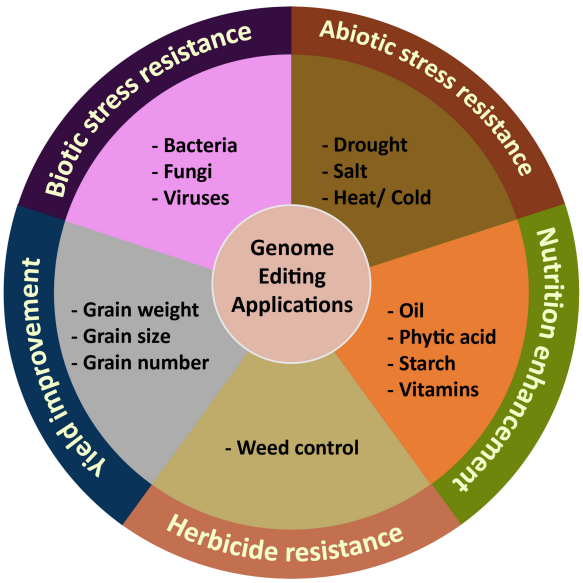

FIGURE 4 | Application of plant genome editing for targeted trait improvement.

and outbreaks is well documented (Legg and Thresh, 2000; Anderson et al., 2004; Sasaya et al., 2014). Genome-engineering technologies can be employed to target viral genomes directly. We and others have recently shown that CRISPR/Cas9 can be harnessed to engineer plant immunity against various DNA geminiviruses, including *Tomato yellow leaf curl virus* (TYLCV), *Beet curly top virus* (BCTV), *Merremia mosaic virus* (MeMV), *Bean yellow dwarf virus* (BeYDV), and *Beet severe curly top virus* (BSCTV) (Ali et al., 2015a, 2016; Baltes et al., 2015; Ji et al., 2015). Interestingly, we found that a single gRNA targeting a conserved region in multiple geminiviruses can mediate interference against multiple viruses, illustrating the great potential of CRISPR/Cas9 as an effective strategy against plant DNA viruses (Ali et al., 2015a).

CRISPR/Cas13a-mediated interference against RNA viruses

RNA viruses represent the majority of plant pathogenic viruses, and engineering plant immunity to RNA viruses is increasingly important. We have employed CRISPR/LshCas13a, an RNA-targeting CRISPR/Cas system (Abudayyeh et al., 2016; East-Seletsky et al., 2016; Liu et al., 2017), to engineer interference with an RNA virus, *Turnip mosaic virus* (TuMV), in plants, and thus demonstrated that Cas13a can mediate plant immunity to RNA viruses (Aman et al., 2018). Despite the modest activity of Cas13a against the TuMV-GFP virus, this study highlighted the exciting potential of CRISPR/Cas13 as an antiviral strategy, and it should encourage the identification and development of more robust and effective RNA-targeting CRISPR systems. These will be useful not only for RNA virus interference but also for a variety of RNA targeting and manipulation strategies in plants (Mahas et al., 2017; Ali et al., 2018b).

Enhancing Plant Abiotic Stress Tolerance

Abiotic stresses such as drought, salinity, and extreme temperature significantly limit crop yields worldwide by reducing plant growth and development (Pandey et al., 2017). The conditions predicted to result from global climate change will worsen many of these stresses, potentially causing an enormous drop in global crop productivity. Plants withstand various abiotic stresses through elegant response mechanisms that generally involve the expression of multiple stress-inducible genes (Kuzuoglu-Ozturk et al., 2012; Golldack et al., 2014). In particular, transcription factors are keystones in gene regulatory networks that control the expression of many genes involved in stress responses (Singh et al., 2002). Advances in genetics and genomics have improved our understanding of the complex nature of abiotic stresses and the interactions between signaling, regulatory, and metabolic pathway components (Nakashima et al., 2009; Takashi and Kazuo, 2010; Garg et al., 2014; Mickelbart et al., 2015). Numerous potential candidate genes have been identified and transformed by classical genetic engineering methods to improve abiotic stress tolerance in both model plants and agriculturally important crop plants (Bidhan et al., 2011; Gong and Liu, 2013).

Owing to the complex nature of abiotic stress, fewer genome-editing studies have so far been done in this area than in the field of pathogen resistance (**Table 1**). In one recent study, DuPont scientists successfully modified a gene encoding maize negative regulator of ethylene responses, *ARGOS8*, using CRISPR/Cas9 (Shi et al., 2017). They used the HDR pathway to insert the maize native *GOS2* promoter into the 5′ untranslated region of *ARGOS8*, which resulted in drought-tolerant maize that survives and has better yield under water-deficit conditions. Another group used CRISPR/Cas9 to induce a mutation in the Arabidopsis *OST2* gene; the mutation resulted in an altered stomatal closing pattern in response to environmental conditions, enhancing the plants' tolerance of drought stress (Osakabe et al., 2016). Recent studies have used CRISPR/Cas9 and validated the involvement of rice *NCED3* and *RAV2* and tomato *MAPK3* in conferring adaptive abiotic stress responses (Duan et al., 2016; Wang et al., 2017; Huang et al., 2018). A recent trial in wheat protoplasts by Kim et al. (2018) targeting two abiotic-stress-responsive transcription factor genes encoding dehydration responsive element binding protein 2 (*TaDREB2*) and ethylene responsive factor 3 (*TaERF3*), further confirmed that CRISPR/Cas9 can be used to manipulate abiotic stress genes for future crop improvement.

Enhancing Plant Herbicide Resistance

Weeds compete with crop plants for resources such as water, nutrients, light, and space, causing considerable reductions in yield. Numerous techniques have been used for weed management, especially chemical herbicides and genetic engineering approaches. Herbicides usually target a vital step in a plant metabolic pathway, and therefore completely kill weeds and may cause considerable damage to crop plants as well. The herbicides bring economic benefits by increasing the food supply worldwide, but they can endanger human and animal health and have negative impacts on the environment. The advent of

biotechnology has revolutionized farming practices by making it possible to transfer a specific herbicide-resistance gene to multiple crops (Lombardo et al., 2016) (**Table 1**), allowing the herbicide to selectively kill the weeds without causing damage to the herbicide-tolerant transgenic crops. This approach has greatly reduced the cost of weed control and also somewhat reduced the deleterious effects of these chemicals.

Recently, scientists have begun to use genome editing to knock out endogenous genes, such as *EPSPS* and *ALS*, to produce herbicide-tolerant plants (Lombardo et al., 2016). *ALS* encodes acetolactate synthase, a key enzyme that catalyzes the first step in the biosynthesis of branched-chain amino acids such as valine, leucine, and isoleucine (Lee et al., 1988; Chipman et al., 1998). Its enzymatic activity is inhibited by certain classes of common herbicides, including the sulfonylureas, imidazolinones, triazolopyrimidines, pyrimidinylthio (or oxy) benzoates, and sulfonylamino-carbonyl-triazolinones (Mazur et al., 1987; Zhou et al., 2007). Genome-editing-based gene replacement has been used to introduce precise alterations in the conserved region of *ALS* to prevent its inhibition by these herbicides. The resulting modified plants are able to grow in the presence of herbicide. In 2009, ZFN-mediated gene targeting was first used to introduce specific mutations in the tobacco *ALS* gene to confer resistance to sulfonylurea herbicides (Cai et al., 2009; Shukla et al., 2009; Townsend et al., 2009). The same gene has been targeted in several other crops, using TALENs and CRISPR/Cas9, to obtain herbicide-resistant potato, rice, maize, and soybean varieties (Butler et al., 2015; Svitashev et al., 2015; Li J. et al., 2016; Sun et al., 2016).

EPSPS encodes 5-enolpyruvylshikimate-3-phosphate synthase, an enzyme in the shikimate pathway, which is involved in the biosynthesis of essential plant aromatic amino acids (Ganesh and Dilip, 1988). In plants, EPSPS is a target of glyphosate, a widely used herbicide that binds to and inhibits its enzymatic activity (Ganesh and Dilip, 1988; Schönbrunn et al., 2001). CRISPR/Cas9 has been used to substitute two nucleotides in the *EPSPS* glyphosate-binding site in the presence of single-stranded oligo DNA repair templates in *Linum usitatissimum* (flax), resulting in genotypes with elevated glyphosate tolerance (Sauer et al., 2016). A similar approach has been used to produce glyphosate-resistant rice (Li J. et al., 2016).

Improving Food Crop Quality

Genome editing can also enhance crop nutritional properties to produce healthier foods. Several studies have proposed potential applications of genome editing in the modification of plant components. For example, phytate, which exists in many crops, is usually regarded as an anti-nutrient due to its ability to form complexes with proteins and minerals, reducing their digestive availability (Zhou and Erdman, 1995; Feil, 2001). TALENs and CRISPR/Cas9 have both been used to reduce phytate content in maize by knocking out *ZmIPK*, a gene involved in phytate biosynthesis (Liang et al., 2014). Another application targeted acrylamide, a potential carcinogen produced by the reaction of reducing sugars (e.g., glucose and fructose) with free amino acids (e.g., in asparagine) in starchy foods, such as potato, under high heat. Clasen et al. (2016) used TALEN to knock out *VInv*, the gene encoding vacuolar invertase, which catalyzes the breakdown of sucrose into glucose and fructose, and thereby produced acrylamide-free potatoes. CRISPR/Cas9 has been used to develop wheat with hypoimmunogenic gluten and tomato with enhanced lycopene content through the generation of functional knockout mutants of α-gliadin genes and several genes involved in carotenoid biosynthesis, respectively (Li et al., 2018; Sánchez-León et al., 2018).

The development of an improved waxy potato is another example of food quality improvement through genome editing. CRISPR/Cas9 was used to knock out the four alleles of the granule-bound starch synthase (*GBSS*) gene in potato. The edited potato produces only amylopectin and lacks amylose-containing starch (Andersson et al., 2017). A similar concept underlies a waxy maize developed by DuPont Pioneer by disrupting the amylose biosynthesis gene (*Wx1*) through CRISPR/Cas9 (Waltz, 2016a). Conversely, a high-amylose rice was generated by knocking out the starch branching enzyme genes *SBEI* and *SBEIIb* using CRISPR/Cas9 (Sun et al., 2017).

Genome editing has also been used to modify seed oil content to produce healthier food oils, as well as biofuels. This approach was made possible by increased knowledge of the metabolic pathways and the genes encoding enzymes related to fatty acid biosynthesis (Wu et al., 2005; Damude and Kinney, 2008). Seed oil content can be modified by increasing and decreasing the levels of particular fatty acids or by incorporating additional fatty acids of nutritional importance. For example, high levels of polyunsaturated fatty acids such as linolenic acid in food oils are undesirable because of their poor oxidative and frying stability. It is now feasible to change fatty acid compositions by targeting the genes encoding fatty acid desaturase (FAD). TALENs have been used to knock out *FAD2-1A* and *FAD2-1B* in soybean, increasing the oleic acid level by almost four times as compared to wild type (Haun et al., 2014). Two independent groups have recently used CRISPR/Cas9 to simultaneously knock out all three *FAD2* homeologs in the allohexaploid oilseed crop *Camelina sativa*, resulting in reduced levels of the less desirable polyunsaturated fatty acids and a significant enhancement of the oleic acid level (Jiang et al., 2017; Morineau et al., 2017).

REGULATION OF GENOME-EDITED CROPS

Genome-editing tools have been used to effect precise modifications in many plant genomes. They have had a great influence on basic research as well as crop improvement. A primary advantage of these technologies is that the transgenes initially used to induce genetic alterations can be easily removed from the genome by genetic segregation, making the resulting plants typically indistinguishable from naturally occurring genetic variants. More recent modification methods, especially CRISPR/Cas, have improved the robustness of this process by allowing genetic changes to be accomplished without any integration of foreign DNA, through transient expression of a site-specific nuclease within the plant cell (Weeks et al., 2016). The transient nature of the expression often results from the

degradation of nuclease-encoding DNA constructs after they have done their job and before they can be integrated into the plant's genome. This can be achieved by using viral vectors to deliver the site-specific nuclease in the form of either mRNA, which is unstable and quickly degrades, or protein, which is not transmitted from parent to offspring (Marton et al., 2010; Baltes et al., 2014; Ali et al., 2015b; Ilardi and Tavazza, 2015; Yin et al., 2015). In these cases, we argue that the edited plants should not be regulated in the same way as those generated by classical genetic engineering methods (Sauer et al., 2016).

Scientists, policymakers, and regulatory authorities have extensively debated the regulation of genome-edited plants (European Food Safety Authority Panel on Genetically Modified Organisms, 2012; Lusser and Davies, 2013; Podevin et al., 2013; Pauwels et al., 2014). Among the numerous issues discussed are such questions as whether genome-edited plants should be regulated under the existing frameworks for GMOs. Should regulations consider process-based regulation, which considers the procedures and techniques used to create the crop, or product-based regulation, which considers the possible risk of the final crop products? Should they deal with edited plants on a case-by-case basis according to parameters such as (1) the tool and repair pathway employed (NHEJ versus HDR), (2) the characteristics of the developed or modified trait, and (3) the possibility of off-target effects (Araki et al., 2014; Hartung and Schiemann, 2014; Araki and Ishii, 2015; Wolt et al., 2016)?

The United States Department of Agriculture (USDA) stated in 2012 that plants edited with ZFNs and meganucleases using the NHEJ pathway should not be considered as, or regulated as, GMOs (Waltz, 2012). The USDA has followed this product-based distinction in later judgments and recently allowed the cultivation and commercialization of CRISPR-edited mushrooms and waxy corn without passing them through the existing GMO regulation (Waltz, 2016b). DuPont Pioneer is planning to release the waxy corn variety as the first commercialized genome-edited crop in 2020. The European Union (EU) regulations are mainly process based. Nonetheless, various anti-GMO forces consider genome-edited plants to be unnatural products and are attempting to have them banned under the GMO regulatory scheme. These arguments are illogical, however, given that the EU previously approved several older crops created by the even more imprecise conventional methods of chemical and radiation mutagenesis. Very recently, however, a ruling by the European Court of Justice (ECJ) included CRISPR-edited crops within the GMO category, complicating commercialization efforts and severely undercutting CRISPR-based efforts for crop trait improvements in Europe and other markets with intensive agricultural trade relations with European countries (Urnov et al., 2018). We certainly hope that this decision will be revisited and that a science-based and informed decision is made on this matter. This decision should take into consideration the opportunities to use this technology to address agricultural challenges and enhance food security globally (Urnov et al., 2018).

In practical terms, genome-editing technologies offer a great chance for improving crops and ensuring global food security. We should grasp this opportunity to increase crop productivity and potentially save the lives of millions of people around the world, particularly in developing nations. Treating genome-edited crops like those produced naturally or by older artificial mutagenesis will have a number of positive impacts on global food security, including (1) reducing the time and cost of regulatory scrutiny, which will encourage more small biotechnology companies to adopt genome editing; (2) increasing the number of researchers using these tools and encouraging them to improve the system's efficiency and develop more robust techniques; and (3) allowing the technology to be applied to more crops, including food and horticultural species. As a result, revolutionary changes in crop improvement can be expected in the near future to help meet the increasing demand for food and ensure global food security.

CONCLUSION

CRISPR/Cas systems have revolutionized plant genome engineering and democratized their application through their high efficiency, facile engineering, and robustness. The current state of this technology enables many applications suitable for improving plant productivity, disease resistance, and resilience to climate change. Various technological improvements are still needed, especially precise editing and delivery of genome-engineering reagents to germline cells to bypass the need for tissue culture. In addition, regulatory and ethical considerations may limit the wide applications of these technologies. We must learn from past experience and improve the technology to avoid regulatory hurdles and ensure that its fruits are within reach for the poor and for subsistence farmers. Genome-editing technologies are poised to reshape the future of plant agriculture and food security to feed the world's burgeoning population.

AUTHOR CONTRIBUTIONS

KS, AM, and MM wrote the manuscript. KS, AM, and MM prepared the figures and table. KS and MM edited and finalized the manuscript.

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Plants Developed by New Genetic Modification Techniques—Comparison of Existing Regulatory Frameworks in the EU and Non-EU Countries

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The development of new genetic modification techniques (nGMs), also referred to as "new (breeding) techniques" in other sources, has raised worldwide discussions regarding their regulation. Different existing regulatory frameworks for genetically modified organisms (GMO) cover nGMs to varying degrees. Coverage of nGMs depends mostly on the regulatory trigger. In general two different trigger systems can be distinguished, taking into account either the process applied during development or the characteristics of the resulting product. A key question is whether regulatory frameworks either based on process- or product-oriented triggers are more advantageous for the regulation of nGM applications. We analyzed regulatory frameworks for GMO from different countries covering both trigger systems with a focus on their applicability to plants developed by various nGMs. The study is based on a literature analysis and qualitative interviews with regulatory experts and risk assessors of GMO in the respective countries. The applied principles of risk assessment are very similar in all investigated countries independent of the applied trigger for regulation. Even though the regulatory trigger is either process- or product-oriented, both triggers systems show features of the respective other in practice. In addition our analysis shows that both trigger systems have a number of generic advantages and disadvantages, but neither system can be regarded as superior at a general level. More decisive for the regulation of organisms or products, especially nGM applications, are the variable criteria and exceptions used to implement the triggers in the different regulatory frameworks. There are discussions and consultations in some countries about whether changes in legislation are necessary to establish a desired level of regulation of nGMs. We identified five strategies for countries that desire to regulate nGM applications for biosafety–ranging from applying existing biosafety frameworks without further amendments to establishing new stand-alone legislation. Due to varying degrees of nGM regulation, international harmonization will supposedly not be achieved

in the near future. In the context of international trade, transparency of the regulatory status of individual nGM products is a crucial issue. We therefore propose to introduce an international public registry listing all biotechnology products commercially used in agriculture.

Keywords: new genetic modification techniques, nGM, genome editing, regulation, biosafety, risk assessment, regulatory trigger

INTRODUCTION

Genetically modified (GM) crop plants developed by recombinant DNA (rDNA) technology are regulated in most countries by biosafety frameworks established by specific legislation. These biosafety frameworks typically build on the fundamental principles for food and feed safety and the environmental risk assessment of crops produced by modern biotechnology developed e.g., by international bodies like the FAO/WHO and the OECD (Jones, 2015a). Particularly important for the development and international harmonization of biosafety frameworks is the Cartagena Protocol on Biosafety (CPB), established under the Convention on Biological Diversity. The Parties to the CPB, currently 171 countries, have the obligation to follow the provisions laid down in the Protocol, when developing their biosafety regulations.

In parallel to classic GM technology a wide range of "new genetic modification techniques" (nGMs) was developed for the (genetic) modification of organisms, including plants, for research purposes or for the development of crops for agricultural use. These nGMs are also referred to as "new techniques" or "new breeding techniques" in other sources (Lusser et al., 2012; Vogel, 2016; SAM, 2017). For the purpose of clarification and to avoid the possible misconception on the part of non-experts that these technologies are just variants of conventional cross-breeding methods we do not use these terms in this paper.

The range of nGMs addressed in this paper includes the following techniques:

- Genome editing with site-directed nucleases (SDNs), e.g., using clustered regularly interspaced short palindromic repeat (CRISPR)-directed nucleases, transcription activator-like effector nuclease (TALENs), zinc-finger nucleases (ZFNs) or meganucleases. SDN-based techniques can also be applied for multiplex genome editing and "base editing" as well as for modification of transcriptional regulation.
- Genome editing directed by synthetic oligonucleotides, also referred to as oligonucleotide-directed mutagenesis (ODM)
- RNA directed DNA methylation, an approach for modifying epigenetic regulation of gene expression
- Cisgenesis and intragenesis
- Transgrafting, in particular the use of GM-rootstocks in grafting
- Agro-infiltration
- Haploid induction and accelerated breeding, i.e., examples of techniques developed to assist complex breeding schemes

Currently particular focus is placed on nGM applications for genome editing, which involve the use of SDNs, like CRISPR/Cas9 (Wolt et al., 2016a). These genome editing approaches are deemed relevant for the future development of crop plants due to their practical advantages, like the wide range of potential applications concerning different plant species and traits (Voytas and Gao, 2014; Bortesi and Fischer, 2015). Genome editing by SDNs may be used in different ways and to achieve different objectives (Schiml and Puchta, 2016). Generally there are three types of SDN-approaches: (i) applications to introduce random changes to the genomic DNA sequence at specific locations, which are created by error-prone repair of double-strand breaks introduced by a particular SDN (SDN-1 type applications); (ii) applications based on homology-dependent repair of site-specific double-strand breaks to introduce small specific sequence changes at genomic targets instructed by DNA-template sequences which are supplied *in trans* (SDN-2 type applications); (iii) applications based on homology-dependent introduction of larger-sized DNA elements of heterologous origin into the recipient genome at specific locations (SDN-3 type applications).

Besides these basic types of genome editing a number of additional approaches were developed recently. At the one hand CRISPR-based systems directed by multiple guide RNAs can be used for simultaneous modification of different genomic target loci ("multiplex genome editing"). On the other hand modified SDNs with a disabled nuclease function can be employed to introducing specific sequence changes via directed chemical modification of nucleobases in an intact strand of DNA ("base editing") and to modifying the transcriptional regulation of gene expression ("epigenome editing") (Puchta, 2016; Tycko et al., 2017; Rees and Liu, 2018).

Currently a lively discussion is underway in many countries concerning the regulatory approach toward crops generated by nGMs and in particular for applications of genome editing (Jones, 2015b; Huang et al., 2016; Wolt et al., 2016b). The debate is fuelled on the one hand by a significant interest of the research and development community in these technologies and their wide range of application in plant development. Furthermore, widespread public interest is focused on nGMs and genome editing because the application of these biotechniques in plant development is challenging existing regulatory paradigms for plants produced by biotechnology (Wolt and Wolf, 2018). Discussed in these respect are similarities and differences of nGMs from either classic GM-technology or conventional breeding approaches. Such debates are conducted at both the national and international levels, including the CPB or the

OECD (OECD, 2016, 2018), and involve a wide range of stakeholders, including regulators, scientists, industry and non-governmental organizations.

The main question addressed in these discussions is whether products generated by different nGMs should be subject to existing biosafety frameworks. A closely related issue relevant for all regulatory frameworks is which risk assessment and risk management approaches are deemed appropriate for nGM applications (Wolt, 2017). This question is relevant for all countries.

The ongoing general discussion also addresses other related issues: Which monitoring, labeling and traceability requirements should nGM products be subject to? How can coexistence be ensured between biotechnology and non-biotechnology plants? Should a broader assessment of sustainability and socio-economic issues, e.g., as conducted in some countries for GMOs, be implemented for nGM applications? Such questions are highly relevant for regulatory frameworks, which implement these requirements for GMOs, among them the EU. Because these issues are not directly connected with premarket risk assessment and not all countries implement such requirements, we do not focus on these questions in this publication. We, however, want to underline that these issues merit in-depth consideration in their own right and need to be further addressed.

In relation to risk assessment regulatory challenges associated with the application of nGMs arise as a result of their specific methodological characteristics and the broad range of different products which may be developed using these techniques:

- nGMs may be used to generate different types of genetic and epigenetic modifications. Different qualities and quantities of modifications need to be taken into account, ranging from random or directed changes of the DNA sequence at specific genomic locations (SDN-1 and SDN-2, respectively), to insertion of larger-sized DNA elements of heterologous origin into the recipient genome at specific or random locations (SDN-3 and cisgenesis/intragenesis respectively), and transient or heritable changes of epigenetic modifications (epigenome editing).
- nGMs are associated with a different potential to introduce unintended modifications which are either linked to characteristics of the particular nGM method or to other biotechnological techniques used throughout the development process (e.g., methods for *in vitro* plant cell cultivation, the generation and use of plant protoplasts and the regeneration of viable plants from single cells or tissue cultures) (Zheng and Gu, 2015; Bortesi et al., 2016). Depending on the phenotypical outcomes of such unintended modifications, they may lead to adverse phenotypical effects.
- nGMs may be used to create a variety of different traits. Those traits might already be present in natural populations and/or agronomically used plant varieties or may be novel in terms of agricultural use (HCB, 2017).

Thus, different sources of potential hazards need to be considered to assess whether applications developed by specific nGM approaches are associated with relevant risks (Eckerstorfer et al., 2014). Such hazards may be associated directly with specific new trait(s) e.g., herbicide resistance traits which are associated with adverse environmental impacts (Schütte et al., 2017). Hazards can also be indirectly associated with the intended modifications if these changes have additional unintended phenotypes. An example are crops developed by genome editing with increased disease resistance due to the knockout of certain (*mlo*) disease susceptibility genes, which are also involved in other physiological functions in addition to their role in fungal pathogenicity (Kusch and Panstruga, 2017). Another source of potential hazards are unintended changes introduced throughout the process of developing a final product by a particular nGM or a combination of biotechnological methods, e.g., nGMs developed by genome editing may be associated with adverse effects if off-target modifications at genomic sequences other than the targeted loci result in significantly negative phenotypical changes (Zhao and Wolt, 2017). The possibilities that hazards may be associated with nGM applications and particularly with genome editing applications are discussed in more detail by Eckerstorfer et al. (2017). Therefore, the regulatory frameworks in different countries need to provide appropriate and workable procedures for regulation and risk assessment to address a diverse range of risk issues which may be associated with certain nGM applications. The question which regulatory trigger is implemented in a particular biosafety framework is a matter of crucial importance in this context.

Existing biosafety frameworks for the regulation of GMO use different regulatory triggers, i.e., definitions specifying the products covered by the regulatory frameworks. Such regulatory triggers either refer to specific characteristics of regulated products and the newly developed traits (product-oriented regulatory triggers) or the use of certain technologies in the generation of regulated products (process-oriented regulatory triggers). What both regulatory regimes have in common is that the risk associated with the regulated product, i.e., the modified organism, needs to be evaluated. A key question is if process- or product-oriented regulatory triggers might be better suited for the regulation of biotechnological products in general and of nGM applications in particular (Voytas and Gao, 2014; Kuzma, 2016b; McHughen, 2016; Sprink et al., 2016a). To address this question we analyzed some features of the different regulatory frameworks currently implemented in European and non-European countries with the aim to inform the further discussion on the subject in the EU.

ANALYSIS AND COMPARISON OF DIFFERENT EUROPEAN AND NON-EUROPEAN BIOSAFETY FRAMEWORKS

Our study investigates the differences and similarities of regulatory frameworks for biosafety in Argentina, Australia, Brazil, Canada, the EU, New Zealand, Norway, South Africa, Switzerland, and the USA. In particular we examine how nGM applications are covered and regulated by these frameworks, including the general requirements for risk assessment. Furthermore, we analyse current reviews of these systems and

proposed amendments, in particular those which are developed to better address nGM applications.

Our comparison of the different frameworks is based on available literature analyzing and explaining the existing legislation related to regulation of biotechnology products in general and nGM applications in particular. To update and complement this information we conducted interviews with regulators and/or experts involved in risk assessment according to existing biosafety legislation. The interview partners answered our questions in a personal capacity based on the understanding that no transcripts of the interviews would be published and that no direct quotes from the interviews would be attributed to specific persons. The information from the interviews provided a background against which previously published information was checked for correctness and validity (September 2017) (**Supplementary Material**).

Contrary to previous analyses (Schuttelaar, 2015; NAS, 2016; Sprink et al., 2016a; Academy of Science of South Africa, 2017) we did not specifically focus on the regulatory status of emerging nGM applications (i.e., whether specific nGM applications are subject to a particular biosafety legislation framework or not), but on the experience with existing regulatory approaches and their procedures for risk assessment as well as on possible implications for nGM applications.

The studied biosafety frameworks are embedded in different legislative environments and all of the respective countries have actively been implementing these regulations for many years. The USA, Canada, Argentina, Brazil, South Africa, and Australia are among the main producers and exporters of agricultural GM products (ISAAA, 2016). In all of the selected countries an active discussion on how to deal with future regulation of nGM applications is underway at the national level.

Most of the surveyed regulatory biosafety frameworks were introduced in the 1980s and 1990s with the aim to regulate biotech products, in particular products generated by GM-technology (see overview in **Table 1**).

The majority of the countries (Argentina, Australia, Brazil, New Zealand, Norway, and South Africa, Switzerland) and the EU established new sectoral legislation for applications of biotechnology, along with specific regulations for implementation. The biosafety laws or Gene Technology Acts were informed by early work at the international level, e.g., work undertaken by the OECD (OECD, 1986) or work on drafting the CPB. The biosafety framework adopted by the EU in 1990 was also influential in the subsequent adoption of biosafety laws in other European and non-European countries, e.g., South Africa.

The respective laws define the scope of the regulations and provide definitions of products or organisms, which, in the opinion of regulators, stakeholders, and literature, are considered to be mostly process-oriented. The EU definition is widely considered to be process-oriented especially by a number of legal experts (Krämer, 2015; Spranger, 2015). Other experts are of the opinion that the EU-trigger is both process- and product-oriented (Kahrmann et al., 2017).

On an international level the CPB includes a definition for "living modified organisms," which is also widely considered to be a process-oriented regulatory trigger. The CPB trigger is based on a slightly different wording compared to the definitions used in the EU and other mentioned countries. The USA, Australia, Argentina and Canada are not Parties to the Protocol. Therefore, full compatibility with the CPB is not an issue for these countries. However, the trigger definition for the biosafety law in Argentina is very similar to the trigger of the CPB. All other countries included in this study and the EU are Parties to the CPB and their legislation has either been compatible with the CPB since it entered into force in 2003, or respective changes have been introduced later to establish compatibility, e.g., 2005 in Brazil.

The USA and Canada have used (and updated) existing national legislation to establish regulatory frameworks for biotechnology applications. These countries use product-oriented triggers to define regulated products; however, different triggers have been adopted in the USA and in Canada.

General Similarities of and Differences Between the Biosafety Frameworks

The analyzed biosafety frameworks were established against different national legislative backgrounds. One of the main differences was the decision on whether to use and adapt existing legislation for biosafety regulation, as in the USA and in Canada, or to establish new sectoral biosafety legislation. On a global level most countries, including the other analyzed countries and the EU, have taken the latter approach.

Both approaches have specific consequences, particularly for developers and regulators. When using existing legislation and established regulatory authorities, the administrative system for regulating biotech products is typically better aligned with existing procedures and statutory responsibilities for non-biotech products. This can result in higher consistency concerning the risk assessment of biotech and non-biotech products as e.g., in Canada. On the other hand the developers need to deal with a number of different statutory authorities which are responsible for different regulatory issues, e.g., the environmental release of modified plants and animals or placing on the market of biotech foods and feeds, e.g., as in the USA and Canada. In both countries several different authorities are involved in the regulation of products.

In the USA the "Coordinated Framework for the Regulation of Biotechnology" has been established 1986 by the White House Office of Science and Technology Policy and updated in 1992 and in 2017 (NAS, 2016; EOP, 2017). It was introduced with the aim of coordinating the regulatory responsibilities of several federal agencies under their existing statutes (Wolt and Wolf, 2018): USDA-APHIS is responsible for applications related to the import, interstate movement, as well as environmental release for field trials and unrestricted cultivation, US-EPA is responsible for products with plant-incorporated protectants and GM microbial pesticides and FDA covers food safety issues and the safety of biotechnological products for medical use.

In Canada the Canadian Food Inspection Agency (CFIA) is responsible for the environmental release of 'Plants with Novel Traits' (PNTs), including PNTs developed with biotechnology methods, and the use of feedstuffs derived from PNTs.

TABLE 1 | Regulatory frameworks for biotechnology analyzed in this study—Legal foundations, characteristics and regulatory requirements for unconfined release e.g., for commercial cultivation or the marketing of regulated biotechnology products.

Country	Main biosafety legislation	Framework or specific law (for env. release)	Regulatory trigger*	Regulatory requirements for unconfined environmental release	Authorisation period (for marketing)
European Union	Biosafety Directives and Regulations (food/feed, env. release) (1990, updated 2001/2003)	Dir 2001/18/EC, supplemented by implementing regulations and GM food and feed regulation (2003)	Process-oriented**	Risk assessment, risk management, coexistence, monitoring, labeling, detection methods	10 years, renewable
Argentina	Regulation Framework for Agricultural Biotechnology (1991)	Supplementary Resolution for release of GMOs	Process-oriented	Risk assessment, socio economic considerations	Not limited (possibility of revocation)
Australia	Gene Technology Act (2000), Food Standards Australia New Zealand Act (1991)	Supplementary Regulations e.g., Gene Technology Regulation (2001)	Process-oriented	Risk assessment, risk management and monitoring	Not limited (possibility of revocation)
Brazil	Biosafety law (1995; updated 2005)	Biosafety Law supplemented by implementing Resolutions	Process-oriented	Risk assessment, coexistence, monitoring, labeling, optional socio economic considerations	Not limited (possibility of revocation)
Canada	Regulatory Framework for Biotechnology (1993)	Framework includes regulations for plants with novel traits and novel foods and feeds	Product-oriented (novelty- and risk-based)	Risk assessment, stewardship (risk management)	Not limited (possibility of revocation)
Norway	Gene Technology Act (1993)	Regulations for risk assessment	Process-oriented	Risk assessment, risk management, monitoring, labeling, detection methods, socio-economic/sustainability assessment	10 years, renewable
New Zealand	Hazardous Substances and New Organisms Act (1996), Food Standards Australia New Zealand Act (1991)	Supplementary Regulations (1998, 2003) and Methodology Order (1998)	Process-oriented	Risk assessment, risk management, monitoring (for conditional releases)	Not limited (possibility of revocation)
South Africa	GMO Act (1997)	GMO Regulations (amended in 2010)	Process-oriented	Risk assessment, monitoring, labeling, detection methods, optional socio economic considerations	Not limited (possibility of revocation)
Switzerland	Gene Technology Act (2003)	Release Ordinance (2008)	Process-oriented	Risk assessment, monitoring, labeling, detection methods	10 years, renewable
USA	Coordinated framework for the regulation of biotechnology (1986)	Framework refers to relevant sectoral legislation (e.g., Plant Protection Act, Federal Insecticide, Fungicide, and Rodenticide Act, Toxic Substances Control Act)	Product-oriented (risk-based)	Risk assessment	Not limited (possibility of revocation)

Classification of the regulatory triggers is not based on legal determination, but according to an assessment by the authors, based on information in the literature and information gathered from interviews with regulatory experts.

***Classification is disputed, some sources claim that the trigger is both process- and product-oriented (BVL, 2015; Kahrmann et al., 2017).*

Environment Canada is responsible for GM microorganisms and Health Canada for the safety of novel (biotech) foods, respectively (Shearer, 2014; Smyth, 2017).

It was noted that split responsibilities may create complex regulatory pathways and may result in difficulties for product developers to navigate the system (Kuzma, 2016a). For example in the USA US-FDA is providing regulatory oversight for a range of modified animals (as "New Animal Drugs"), that are developed for purposes comprising environmental release. This includes e.g., GM salmon (AquAdvantage), which was authorized for land-based production in 2018, GM mosquito products intended to reduce the vectoring capacity for viruses or other pathogens of these insects or their pathogen load and organisms which qualify as "animals with intentionally altered genomic DNA" under FDA's "Veterinary Innovation Program" (FDA, 2018). In contrast GM mosquito products modified for population suppression are regulated by US-EPA since October 2017[1]. To ensure consistent regulation and to avoid duplication of efforts, close coordination between the involved authorities is necessary. Procedures to address these issues have been implemented in Canada and the USA (EOP, 1992, 2017; Shearer, 2014; NAS, 2016), they include e.g., the possibility for developers to engage in pre-submission consultations with the authorities to address questions regarding the regulatory status of specific products, as well as regulatory and information requirements for risk assessment (Shearer, 2014).

Introduction of new sectoral legislation for biosafety regulation is typically coupled with the establishment of specific lead authorities with consolidated responsibility for all types of biotech products. According to the opinion of some interviewed regulatory experts this might be less confusing for applicants as far as the specific biosafety requirements are concerned, e.g., regarding risk assessment. However, this does not necessarily grant an easier route to quicker decision making on applications submitted for authorization, the EU regulatory framework being an example in point. Decision making in the EU is based on a highly complex and time-consuming procedure involving the European Commission and all Member States, once the risk assessment has been conducted under the lead of the European Food Safety Authority (EFSA) (Hartung and Schiemann, 2014).

All Investigated Regulatory Frameworks Are Risk-Oriented

All analyzed biosafety frameworks establish a risk-oriented regulatory approach. A mandatory risk assessment is conducted for all regulated products prior to authorization for environmental release or food and feed use with the general intention to ensure environmental and health safety (McHughen, 2016).

[1]Department of Health and Human Services, Food and Drug Administration [Docket No. FDA-2016-D-4482]. *Clarification of the Food and Drug Administration and Environmental Protection Agency Jurisdiction Over Mosquito-Related Products; Guidance for Industry*. Federal Register Vol. 82. Available online at: https://www.govinfo.gov/content/pkg/FR-2017-10-05/pdf/2017-21494.pdf

Consequently the regulatory triggers of the different regulatory frameworks relate to risks in direct or indirect ways:

- The triggers used in the USA are directly related to specific risk issues: A modified organism is subject to regulatory oversight and risk assessment by the respective statutory authorities if the recipient organism or the source organism of the introduced genetic elements or traits is known to display risky and/or unwanted characteristics (e.g., plant pathogenicity, weediness, toxicity or pesticidal effects or compositional differences that are not generally recognized as safe).
- The product-oriented regulatory trigger used in Canada can also be considered risk-oriented, although in a different way: novelty of the product indicates a lack of an existing history of safe use, whereas the other trigger component, i.e., the general plausibility of risks related to protection goals as addressed by the biosafety framework, indicates that relevant unwanted risks may exist, which cannot be discounted without a scientific assessment.
- Frameworks which implement process-oriented triggers refer in an indirect way to risks associated with the products established by genetic modification techniques. As stated in Directive 2001/18/EC only products created with techniques, which "*have conventionally been used in a number of applications and have a long safety record*" should be exempt from the Directive. In line with a precautionary approach the wider range of genetic modifications which may be introduced by GM-technology, the limitations of existing knowledge concerning the potential effects of a GMO and the difficulties to address the complex interactions of modified organisms with the environment are important reasons for conducting a risk assessment prior to use.

In all analyzed regulatory frameworks decisions to determine the regulatory status of particular products are typically based on legal and/or technical interpretations of the definitions of regulated products and the scope of exemptions included in the respective legislation. An evaluation of the specific hazards associated with a particular application is not conducted at this stage, but only during the risk assessment of applications which are subject to a specific biosafety framework.

Implementation and Interpretation of Different Trigger Definitions Results in Heterogenous Regulatory Scopes

Relevant differences can be seen in both classes of triggers (process- and product-oriented). As a result different ranges of organisms and products are being regulated by the different national biosafety frameworks.

The product-oriented regulatory triggers in the USA and Canada biosafety legislation differ significantly from each other:

- The US trigger for the regulation of environmental release applications relates to specific risk issues as outlined in the Coordinated Framework for the Regulation of Biotechnology, e.g., plant pathogenicity, the risks of creating a modified

variety of a noxious weed, environmental toxicity of plant protectants (EOP, 2017). The intended focus is on different product-related risks. However, this is not achieved with full consistency in practice. Kuzma (2016b) notes that for the majority of products regulated by USDA-APHIS the process of the employed GM technology, i.e. *Agrobacterium*-mediated transformation, triggers regulation. This results in de facto process-based decisions (Wolt, 2017). The US system is described as "a strange patchwork of rules and exceptions" (Strauss and Sax, 2016) and considered to be a hybrid of process- and product-oriented reasoning (McHughen, 2016; Strauss and Sax, 2016).

- The regulatory trigger implemented by Canada is based on novelty in combination with a given plausibility that these products may have adverse (environmental) effects (Shearer, 2014). The technology used for the generation of a modified organism is therefore irrelevant for the determination of the regulatory status. Since the introduction of the biosafety framework all GM plants have been considered to contain novel traits and have been assessed for environmental safety in Canada (NAS 2016). According to the expert interview this was still the case in late 2017, however this policy could change in the future based on the outcome of an ongoing national review of implementation of the biosafety framework. The scope of the Canadian regulations also covers novel plants derived by non-GM breeding methods like classical mutagenesis. In the USA such plants are only regulated if a trait is associated with one of the specific risk factors mentioned above. In all other analyzed countries plants derived by conventional breeding methods are not subject to the respective biosafety laws.

Process-oriented regulatory triggers are based on country-specific definitions. Differences exist concerning the specific references made to certain techniques in the definitions of regulated products as well as in the exemptions according to the respective biosafety laws and regulations. The scope and specificity of such exemptions decisively influence the overall range of regulated products. Exemptions can be defined quite specifically as e.g., in New Zealand or more general, e.g., as in the EU biosafety framework. The uncertainty concerning the scope of the exemption of mutagenesis according to the EU Directive 2001/18/EC was only by a ruling of the European Court of Justice (ECJ), which determined that products developed by mutagenesis induced by genome editing are covered by the EU trigger definition. Only products of mutagenesis induced by chemical mutagens or ionizing radiation which have a long safety record are exempt from regulatory oversight according to the ECJ decision (ECJ, 2018). In some countries, the presence or absence of a foreign DNA sequence in the final product is a crucial characteristic which determines if the products are subject to regulation or not. For example in Argentina biotechnology applications which retain no transgenic modifications in the final product ("null segregants") are not regulated. This is an indication that some process-oriented triggers include features of product-orientation. In summary both systems using either product- or process-triggers show features of the respective other system. This explains the difficulties to unequivocally classify some regulatory frameworks as either product- or process-oriented. As a result the classifications of existing regulatory frameworks according to different authors vary, e.g., as exemplified by the diverging classifications provided by Ishii and Araki (2017) as compared with other analyses, including the study at hands.

Risk Assessment Is Trigger-Independent, but Takes Into Account the Process of Development

The risk assessments conducted under all legislations address similar general goals, i.e., to identify adverse effects on human and animal health as well as on the environment. All frameworks require that a case-specific problem formulation is conducted to identify specific risk hypotheses for the individual products/organisms. The problem formulation needs to address relevant risk issues, associated with the characteristics of the regulated products or organisms, i.e., the new trait(s), the modified organism as a whole, and its interaction with the receiving environment. None of the analyzed regulatory frameworks, including frameworks with process-oriented triggers, is specifically focusing the risk assessment on technology-related issues. However, all frameworks, including the ones with product-oriented triggers, consider technology-related issues in the course of the risk assessment process. Most frameworks, including the ones in Canada and the USA, require specific information on methods applied during development, usually in the context of molecular characterization of the assessed products. Canadian authorities also initiated and conducted research projects aimed at elucidating and characterizing method-related unintended effects relevant for risk assessment (Ladics et al., 2015).

Thus, we cannot make a clear distinction between systems with either product-oriented or process-oriented regulatory triggers regarding their general approaches to risk assessment. Therefore, we argue that the terms "process-oriented" and "product-oriented" only apply to the type of regulatory triggers. In our opinion these terms should not be used otherwise, e.g., in relation to approaches used for risk assessment as implied in some previous publications (Schuttelaar, 2015; McHughen, 2016; Ricroch et al., 2016), since risk assessment in the different biosafety frameworks is conducted independent of the particular nature of the regulatory trigger (Kuzma, 2016b).

While the general principles and approaches to risk assessment applied in the analyzed countries are comparable and independent of the implemented regulatory triggers, the specific (data) requirements and the extent of the risk assessment requirements vary between legislations. e.g., not all biosafety frameworks mandate a comprehensive assessment of indirect and long-term effects similar to the approach implemented in the EU. Likewise additional regulatory requirements, which are implemented in correspondence to the results of risk-assessment, like risk management (e.g., conditions for use to address identified risks) and monitoring (including general surveillance for unanticipated effects) are applied to different

degrees in the countries analyzed in this study (see **Table 1**). For countries which decided to implement a comprehensive set of requirements for applications subject to the respective biosafety frameworks it does therefore matter very much if a particular application is found to be covered by the biosafety framework or not. Therefore, the extent of consistency regarding the level of scrutiny which is provided for individual applications is closely tied to the particular details of the regulatory trigger which is applied in a given country.

APPROACHES TO REGULATE nGM APPLICATIONS

The countries analyzed in this study have different levels of regulatory experience concerning applications developed with nGMs (**Table 2**). In all countries nGM approaches and particularly genome editing are used in basic and applied research. However, most nGM applications are still being developed in confined facilities. In a number of countries, including the EU, field testing of different nGM applications (e.g., products developed by genome editing, cisgenesis and null-segregant technology) is under way.

Technological developments are rapidly expanding the range of available nGMs, e.g., of methods increasing the range of possible traits and the speed of development. This means that the regulatory bodies will be confronted with a growing range of nGM applications and products with different characteristics (OECD, 2018).

At the present time, the available practical experience with the regulation of nGM applications and the determination of the regulatory status of individual nGM applications is still quite limited. For the time being most of the requests to determine the regulatory status of nGM applications were received by the authorities in the USA, Argentina and Canada.

Determination of the Regulatory Status for nGM Applications

Differences exist between the countries regarding the determination of the regulatory status of an application, (i.e., the initial decision on whether a particular product, e.g., an nGM application, is covered by the respective biosafety legislation or not).

Only a few countries, e.g., Argentina, Brazil, Canada, New Zealand, and the USA, include provisions that lay down specific procedures for the determination of the regulatory status of applications in their biosafety legislation. In other regulatory frameworks, particularly in the EU, a significant level of uncertainty remained about the regulatory status of nGM applications (Jones, 2015b; Sprink et al., 2016b; Wolt et al., 2016a). In the absence of a specific policy to address this uncertainty the developers have to consult the respective competent authority about the status of individual products or request that these authorities determine a status of regulation. Some authorities, e.g., in Australia and in Canada, actively recommend that developers address any unclear issues during pre-submission consultations.

In some EU Member States, e.g., Germany, UK, the Netherlands, and Sweden, developers have approached the authorities with requests to determine the status of different plants developed by genome editing (BVL, 2015; Jansson, 2018). These decisions, e.g., concerning herbicide resistant oilseed rape lines developed by ODM, were based on an interpretation of the GMO definition given in Article 2 of Directive 2001/18/EC which argues that the expression "… *organism, …, in which the genetic material has been altered in a way that does not occur naturally* …" refers to the characteristics of the genetic modifications in the final product rather than to the methods used for genetic modification (BVL, 2015; Sprink et al., 2016b; Kahrmann et al., 2017). However, these decisions were taken prior to the ECJ ruling on applications of directed mutagenesis published in July 2018. The ECJ determined in its ruling that applications of directed mutagenesis are covered by the regulatory trigger implemented by Directive 2001/18/EC in the EU and also provided the interpretation that they are not exempted according to Article 3, Para 1 and Annex 1B of the directive. The court concluded that the exemption of mutagenesis methods referred to in Annex 1B does not apply to the introduction of genetic modifications by nGMs like genome editing, since the risks linked to the use of those new genetic modification techniques/methods of mutagenesis might prove to be similar to those which result from the production and release of a GMO through transgenesis (ECJ, 2018). The ruling confirmed that a general exemption of new methods for mutagenesis would not be in line with obligations for regulatory oversight and risk assessment in accordance with the precautionary principle enshrined in European legislation. Consequently any previous decisions taken by authorities of EU member states have to be reviewed and repealed when not in line with the ECJ ruling.

According to the different regulatory triggers employed in other legislations, some countries, e.g., the USA, have decided otherwise when dealing with applications developed by genome editing and other nGMs. One of the US authorities, USDA-APHIS, operates a service dedicated to answer inquiries about the regulatory status of specific products according to Title 7 CFR part 340. The application letters and results of the "Am I regulated?"-process are made available to the public via a dedicated website (USDA-APHIS, 2018). More than 30 "Am I regulated?"-inquiries were submitted between 2011 and May 2018 for products developed with different nGMs including genome editing, cisgenesis (or offspring from cisgenic plants), and null segregants (developed for epigenetic engineering, accelerated breeding and chromosome elimination purposes). 16 applications of genome editing were evaluated, mostly SDN-1 applications. 7 of these applications were developed with CRISPR-methods. The inquiries concerned applications for a variety of intended traits (disease-resistance, compositional modification, drought tolerance, salt tolerance, and modified developmental characteristics such as delayed flowering) in major crops (including maize, wheat, soybean, rice, and potato) as well as in plants like tomato, tobacco, alfalfa and wild foxtail millet, apple trees, and plum trees. As also noted by Waltz (2018) most of these applications are not subject to regulation by USDA-APHIS, since no sequences derived from plant pathogens are introduced

TABLE 2 | Regulatory aspects related to nGM applications.

Country	Current regulatory approach	Policy development regarding nGMs	Focus of policy amendments	Current experiences with nGM applications
European Union	Determination if specific types of nGMs are subject to GMO legislation	No amendment of Directive 2001/18/EC proposed by Europ. Commission, but Europ. Court of Justice ruled that directed mutagenesis is subject to GMO legislation (ECJ 2018)	–	No experience on European level with applications for unconfined release and placing on the market; however field trials with some nGM applications are conducted (SAM, 2017)
Argentina	Determination if nGM product is subject to GMO legislation	Supplementary resolution adopted 2015 providing criteria for case-by-case decisions (Resolution No. 173/2015)	–	Until June 2018 12 requests were evaluated according to Resolution No. 173/2015, incl. 10 applications of genome editing, mostly in plants, mostly not regulated (OECD, 2018)
Australia	Determination if nGM process is subject to GMO legislation	OGTR proposed technical amendments to legislation, consultation in progress	Genome editing (SDN-1)	No applications for unconfined release; field trials with some nGM applications are conducted
Brazil	Determination if nGM product is subject to GMO legislation	Supplementary resolution adopted in January 2018 (Normative Resolution No 16) comparable to supplementary regulation in Argentina)	–	Use of nGMs in contained use facilities; two yeast lines modified by genome editing were evaluated according to Resolution No 16 (not regulated)
Canada	Determination if individual nGM product is novel	Review of risk assessment requirements initiated	–	Several applications authorized (e.g., cisgenic potato, genome edited oilseed rape)
New Zealand	GMO legislation is currently applied for all nGMs	Government adopted policy to direct technical ruling by NZ-EPA, no immediate policy changes foreseen	GMO legislation only exempts chemical or radiation induced mutagenesis	Use of nGMs for research and development activities; some genome editing determined to be regulated
Norway	Determination if specific types of nGMs are subject to GMO legislation	Technical discussions to inform further steps (following EU approach)	–	No applications for unconfined release submitted; use of nGMs in contained use facilities
South Africa	GMO legislation is currently applied for all nGMs	Discussion on policy amendment ongoing	–	No applications for unconfined release submitted; use of nGMs in contained use facilities
Switzerland	Determination if specific types of nGMs are subject to GMO legislation	Stakeholder discussions to inform future policy	–	No applications for unconfined release; field trials with some nGM applications are conducted
USA	Determination if individual nGM product is regulated	Consultations on policy to deregulate certain techniques (e.g., cisgenesis)	–	Several decisions to exempt nGM applications from regulation; a number of nGM applications in regulatory review

during their development and the modified plant species are themselves not known to be plant pathogens or noxious weeds. Therefore, no regulatory oversight or risk assessment will be provided by USDA-APHIS for these applications (Waltz, 2018). Only one application (a cisgenic scab-resistant apple) has been found to be subject to regulation by USDA-APHIS so far, due to the fact that *Agrobacterium tumefaciens*, a known plant pathogen, was used as a vector agent for transformation.

However, the inquiries addressed to USDA-APHIS do not necessarily indicate that the above mentioned products can be expected to be commercialized in the near future. Rather they only indicate that the developers are interested in further development of these products, including field testing. Of better predictive value for commercialization in the near future are the statements by the US Food and Drug Administration (FDA) concerning the results of the (voluntary) consultations of developers with FDA concerning the food safety of their products. However, as of July 2018 only a few respective FDA statements have been published, mostly addressing potato lines with increased disease resistance and altered composition (FDA, 2018).

Thus, only limited experience is available so far related to regulatory oversight for nGM applications according to the existing biosafety frameworks and in particular with case-specific risk assessment of such applications.

Transparency Concerning the Status of Regulation of nGM Products Is a Crucial Issue

Transparency in decision-making is an important issue for all regulatory frameworks which implement process- or product-oriented regulatory triggers. This is acknowledged by regulators from all countries that have been investigated for this study. Most of the biosafety frameworks do not provide the means for ensuring transparency. Only the regulatory decisions taken by USDA-APHIS in the USA in response to the inquiries for the status of regulation are made publicly available irrespective

of whether to the products were found to be subject to regulation under Title 7 CFR part 340 or not. In other countries, including Canada, transparency is typically provided only for those applications which are covered by the respective biosafety legislation, e.g., the PNT regulation.

However, informing the public about the regulatory status of biotech applications and in particular of nGM applications is regarded as a matter of crucial importance. Even experts calling for decreasing the level of regulatory oversight of biotechnology applications in the USA support that a registry of all applications should be established and maintained (Strauss and Sax, 2016). Such a registry should also include applications which have differing regulatory status in varies countries (e.g., SDN-1 in Argentina, Brazil and the USA compared to the EU and New Zealand). With a view to international trade and the varying regulatory status of comparable nGM applications, access to this information will be highly important.

Regulatory Approaches Addressing nGM Applications

The countries investigated in this study including the EU have not implemented specific regulations for nGM applications, which are independent from the existing regulatory biosafety frameworks for GMOs.

Only Argentina and Brazil have passed supplementary legislation (Normative Resolution No. 173/2015 and Normative Resolution No. 6/2018, respectively) to better address regulatory issues associated with nGM applications (Whelan and Lema, 2015; OECD, 2018). These resolutions outline procedures and criteria for the determination of the regulatory status, which can be applied by the competent authority to decision making on submissions of individual nGM applications. Until June 2018 12 requests concerning the regulatory status of different nGM applications mostly for modified plants were evaluated in Argentina according to Resolution No. 173/2015, including 10 applications of genome editing, Most of these applications were found not to be subject to the Argentinian biosafety law.

In the absence of a general policy on nGM applications, other countries which implement process-oriented regulatory triggers are also facing the challenge to make decisions on the regulatory status of individual nGM applications. The determination of the regulatory status may be initiated by a specific inquiry about an individual nGM application addressed to the respective competent authority, as happened for herbicide-resistant oilseed rape produced by ODM in several EU member states like Germany and Sweden. The case also led to the recent ECJ proceedings and the ECJ ruling provided a binding legal interpretation of the current EU legislation which determined that all genome editing applications are subject to Directive 2001/18/EC and thus the EU biosafety framework.

In order to better define the status of nGM applications and to amend or change legislation accordingly, various countries, among them the EU, USA, and Australia, have performed general and nGM-specific policy reviews of existing regulatory approaches or started to conduct such reviews, e.g., as in Australia (LGFGT, 2018). National discussions on nGM applications involving policy makers, regulatory bodies, technical expert groups, scientific academies, and a wide range of other stakeholders and public consultations are also conducted in other countries.

The discussions concerning general or technical amendments of existing legislation are currently at different stages in the countries included in this study. Australia is a good example to illustrate that this process can be associated with some challenges. Different Australian institutions are currently conducting several parallel reviews of different elements of its biosafety framework:

- The Australian Office of the Gene Technology Regulator (OGTR), which oversees the environmental release of GMOs, has proposed technical amendments to the existing definitions of the GMO regulations to better address nGM applications. According to the proposed amendment SDN-1 type genome editing applications, should not be regulated in the same way as GMOs (OGTR, 2018).
- Food Standards Australia and New Zealand (FSANZ), a shared authority between Australia and New Zealand, is currently conducting a parallel consultation process to gather input from scientists, developers and the public concerning the regulation of foods derived using different nGMs (FSANZ, 2018a). The aim is to determine if a concrete proposal should be developed to amend the Food Standards Code and to determine which changes would be reasonable and acceptable for the various stakeholders (FSANZ, 2018b).
- Further general amendments to the overall framework in Australia might be proposed in the course of the ongoing third review of the national gene technology regulatory framework (LGFGT, 2018).

An important issue recognized by FSANZ (2018b) is the importance that any amendments addressing different elements of the regulatory framework should be aligned to achieve coherence of what is regulated as GMO for environmental release purposes and what is regulated as food produced using gene technology in Australia and New Zealand. However, this will not be easy to achieve. First of all a different range of technologies is addressed by OGTR in Australia, by NZ-EPA in New Zealand and by FSANZ at the binational level. Secondly OGTR in Australia and NZ-EPA in New Zealand are likely to implement different regulatory approaches vis a vis applications of various types of genome editing (NZ-EPA regulating all applications of genome editing, while OGTR may only regulate SDN-2, SDN-3, and ODM applications). Therefore, overall consistency between specific regulations for applications for environmental release and for food safety can hardly be achieved.

Furthermore, the issue remains whether the future amendments in Australia will be able to ensure that products with similar characteristics will be subject to similar regulatory requirements. The OGTR states that the proposed technical revision "best supports the effectiveness of the legislative framework"(OGTR, 2018). However, an implementation of such a proposal will not achieve that plants with comparable genetic modifications and traits are consistently addressed by similar risk assessment: according to the proposal all SDN-2 and ODM applications would be subject to risk assessment according to

the Australian Gene Technology Act and Regulations, whereas all SDN-1 applications would not. The two product classes would thus be regulated differently even if certain applications from either class would contain similar genetic modifications or traits.

New Zealand introduced a clarification of the law as a response to a court decision on specific applications of genome editing (Kershen, 2015): Only products developed by chemical or radiation induced mutagenesis are exempted from regulation. The New Zealand government has also decided that for the time being all nGM applications are regulated according to the national biosafety framework. In the EU the recent ruling of the ECJ has clarified the current regulatory status of nGMs for genome editing in a similar way (ECJ, 2018).

Taking decisions on individual applications (i.e., case-by-case) is the default for determining the regulatory status in biosafety frameworks implementing product-oriented regulatory triggers, i.e., the USA and Canada.

Overall it can be concluded that all of the analyzed countries are discussing similar questions and that most of them face comparable challenges, which are including but not limited to:

- Ambiguities of the trigger definitions (process and product) (all countries)
- Inconsistent coverage of products associated with comparable risks (EU, other countries with process-oriented regulation, USA)
- Lack of suitable criteria for the determination of the regulatory status (most countries).

Concerning the regulation of applications developed by genome editing (in particular for SDN-1, SDN-2, SDN-3, or ODM-based techniques) different approaches are or may be taken in the future in the countries covered in this study:

- No biosafety oversight of genome editing applications, if no genetic elements from pathogenic species or pesticidal traits are introduced: USA (applications for environmental release).
- Regulation of SDN-3 applications involving recombinant DNA constructs, but not of SDN-1, SDN-2 or ODM applications (that do not contain any recombinant DNA): Argentina, Brazil.
- Regulation of ODM and SDN-2, but not of SDN-1 applications: Australia (according to the proposed revision of the Gene Technology Regulations).
- Coverage of all types of genome editing if they are used to develop plants with novel traits: Canada.
- Coverage of all types of genome editing approaches: EU, New Zealand.

A whole spectrum of different approaches is used in the analyzed countries. At the one end of the spectrum most genome editing applications, i.e., all applications which do not contain genetic elements from pathogenic species or pesticidal traits, are excluded from biosafety oversight in the USA. At the other end of the spectrum, all types of genome editing are covered by the existing biosafety framework, either irrespective of the nature of the traits developed by genome editing (EU, New Zealand), or for all applications containing novel traits (Canada).

Other countries have introduced (Argentina, Brasil) or have proposed to introduce specific criteria (Australia) to determine different types of genome editing applications are or will be covered by the biosafety frameworks of these countries. These criteria are mostly aimed at improving regulatory certainty for authorities and applicants. This is done either by providing further clarifications to the trigger definitions included in the respective biosafety laws, e.g., using the presence or absence of recombinant DNA constructs to clarify if a "novel combination of genetic material" was established (in Argentina) or if "genetic engineering technique(s)" were used (in Brasil) or by introducing a clear way to distinguish between different types of genome editing applications(e.g., SDN-1 applications without the use of nucleic acid sequences supplied as repair template(s) *in trans* and SDN-2, SDN-3 and ODM applications which use such template DNA(s) to direct genetic modifications). However, these criteria are not aimed specifically at distinguishing between applications with a different level of associated risk.

REGULATORY OPTIONS FOR nGM APPLICATIONS

In summary our analysis indicates that the following approaches are used or may be used when countries wish to provide regulatory oversight for nGM applications:

1. Existing regulatory framework for GMOs is applied to nGM applications
   (a) For all nGMs (South Africa) or for certain types of nGMs (EU)
   (b) Based on case-by-case decisions on individual nGM applications (USA, Canada)
2. Technical revision of existing regulations (definitions and exemptions) (New Zealand, Australia)
3. Implementation of supplementary legislation supporting the existing framework to clarify aspects related to regulation of nGM applications (Argentina, Brazil)
4. New stand-alone legislation for nGM applications, in addition to existing legislation for GMOs (option, no example as yet)
5. "New" overall framework for all biotechnology applications (option, no example as yet).

So far, most countries have not introduced specific legal instruments for nGM applications and have been using the existing regulatory framework to deal with them. In countries with product-oriented triggers individual applications are evaluated at a technical level to determine whether they are covered by the criteria included in the respective legislation (option 1b). Authorities and courts from countries (and the EU) which implement process-oriented regulatory triggers have to provide legal interpretations of the existing laws to determine the regulatory status of categories or types of nGM applications (option 1a).

A few countries have introduced amendments to the existing regulatory framework, either technical revisions of existing definitions of regulated products and exemptions included in the

current legislation (option 2) or supplementary regulations to introduce procedures and criteria for the determination of the regulatory status of nGM applications (option 3).

Options 4 and 5 have not been used in practice yet. A general new biosafety framework for all biotechnological applications (also including nGM applications) is discussed in Switzerland, however no proposal has as yet been developed.

Advantages and Disadvantages of Process-Oriented or Product-Oriented Regulatory Triggers for the Regulation of nGM Applications

Are regulatory systems based on either product- or process-oriented regulatory triggers more advantageous for the regulation of nGM applications (Sprink et al., 2016a)? We analyzed the available information and interviewed regulatory experts concerning their views. A non-exhaustive overview on the perceived general advantages and disadvantages of both systems is presented in **Table 3**.

The analysis shows that both trigger systems have a number of generic advantages and disadvantages. Experience in the analyzed countries demonstrates how important the specific details of implementation of the basic concepts are for the workability of both regulatory approaches. Thus, as noted by Kuzma (2016b) neither system can be regarded as superior at a general level.

However, systems based on product-oriented triggers are considered more flexible when it comes to products developed with newly emerging technologies, without the need to repeatedly adapt existing legislation. Frameworks based on product-oriented triggers may strengthen consistency in the regulation of products with comparable characteristics. This however depends on whether a particular system indeed achieves consistent coverage of products associated with comparable possible risks. The US regulatory framework shows that specific product-oriented trigger definitions can result in an inconsistent range of regulated products: e.g., *Agrobacterium*-mediated transformation results in regulation by USDA, while transformation with similar transgenic constructs of non-plant pathogenic origin by particle bombardment does not (NAS, 2016). The current distribution of responsibilities in the USA between existing authorities also results in emerging biotech products being regulated by authorities that have an inadequate regulatory focus for such products, resulting in particular challenges in addressing issues of greatest concern during risk assessment (Kuzma, 2016a). Product-oriented triggers require a separate determination of the regulatory status for each specific application, which is considered by the interviewed regulatory experts to be more laborious and complex for involved authorities.

A main advantage of frameworks based on process-oriented regulatory triggers is that they provide a clear and straightforward means to establish the regulatory status of classic GMOs both for developers and authorities. The establishment of specific authorities with a consolidated responsibility for all matters of sectoral biosafety legislation can provide a better framework to prevent regulatory gaps and to ensure that a comprehensive risk assessment approach is implemented. These systems however are significantly challenged by several types of nGM applications, particularly products developed by genome editing, if existing definitions are ambiguous. Without concrete policy and appropriate criteria for interpretation, lengthy legal disputes e.g., as in New Zealand and the EU can occur, delaying decisions on individual applications as well as policy development.

An adaptation of process-oriented triggers to ongoing technical developments typically requires the repeated introduction of specific amendments in response to technological developments. Such amendments may need considerable time for their introduction, e.g., for consultation and implementation, and this might cause a temporal regulatory gap for the respective nGM applications. Trigger definitions covering a very broad scope of applications might potentially be flexible enough to avoid the development of regulation gaps, however at the expense of a higher number of applications which need to be assessed for risks by the competent authorities.

Our analysis indicates that the specific details of a particular trigger are more important than the general choice of either a product-oriented or a process-oriented system. The respective differences of implementation result in

- significantly different ranges of regulated products, particularly of nGM products,
- different levels of regulatory uncertainties to determine the status of regulation of specific (nGM) products,
- different levels of consistency to address comparable risks of products developed by different technologies (including GM technology, nGMs and conventional breeding).

Further discussions should therefore not only focus on the question whether a system is based on a process- or product-oriented trigger. The implications of the specific details of existing or proposed trigger definitions on the range of regulated articles also should be taken into account when judging the advantages or disadvantages of a particular system.

It is noted that only some product-oriented systems, like the Canadian Plant with Novel Traits-regulations, implement a similar regulatory approach for all novel products irrespective of the methods used for their development and consistently regulate novel biotech crops as wells as novel plants produced by conventional breeding methods.

Would Sectoral Regulation Outside the Biosafety Frameworks be Sufficient for nGM Applications to Ensure a Suitable Risk Assessment?

For nGM applications which are subject to any of the biosafety frameworks, the same regulatory requirements, e.g., regarding risk assessment or other obligations, apply as for any other regulated products. However, the USA and Canada currently consider specifying different risk assessment requirements for applications belonging to different risk classes.

Generally biotech products which do not to fall under the provisions of the respective biosafety frameworks are still subject to other regulations addressing agricultural products (e.g., seed and plant propagating materials, animal and plant health, food and feed safety, nature conservation). Our analysis indicates that the general requirements according to such

TABLE 3 | General analysis of the advantages and disadvantages associated with product- and process-oriented regulatory triggers as well as the associated challenges concerning implementation of such systems [as particularly relevant for nGM applications (nGMs) or GM applications (GMO)].

Advantages	Disadvantages	Challenges
PRODUCT-ORIENTED REGULATORY TRIGGERS		
High flexibility to accommodate products of emerging technologies without need for legislation change (nGMs)	Some product-oriented triggers may result in inconsistent coverage of products with comparable traits (USA: nGMs and GMOs)	Different competent authorities may be involved, if a broad scope of use is intended (env. release and food/feed use)—split responsibilities, need for coordination
Existing regulatory structures can be used for comparable products	Individual applications may need to be reviewed for regulatory status	Criteria and guidance required for decision making on regulatory status
Similar regulatory approach for comparable products developed by different techniques	Process to determine regulatory status considered more complicated and less predictable compared with process-related triggers (GMOs)	Limited compatibility with regulatory systems based on process-oriented triggers regarding the scope of regulated articles
Consistent risk assessment perspective for products irrespective of the method of production	The typical remit of existing authorities may be ill-suited to address risk assessment challenges of emerging applications	
PROCESS-ORIENTED REGULATORY TRIGGERS		
Typically new sectoral legislation is introduced and implemented by a specific authority	Limited flexibility to accommodate products of emerging technologies—possible need for legislation change in reaction to technological developments (nGMs)	Severe challenges of trigger interpretation regarding some nGMs if specific guidance is not available
Newly introduced sectoral regulations address all relevant risk assessment requirements	Regulation gaps until newly emerging technologies are addressed by trigger amendments (nGMs)	Ambiguous trigger definitions may lead to interpretation conflicts that have to be settled by administrative and/or court proceedings (nGMs in particular)
Process-oriented triggers considered easier to implement and more predictable (GMOs)	Trigger specifics (exemptions) may result in inconsistent coverage of products with comparable risk (nGMs)	Limited compatibility with regulatory systems based on product-oriented triggers regarding the scope of regulated articles

legislation in the different countries are broadly comparable. The following examples of such requirements apply to products of genome editing or other nGM applications in case it is found that these products are not subject to existing biosafety legislation:

- Variety registration regimes are implemented in all countries included in this study as well as globally to ensure seed quality, the distinctiveness and stability of traits, as well as the uniformity of seed lots and a number of safety parameters for certain plant species. These issues are assessed according to international standards (UPOV, 2002).
- The general provisions of food and feed safety legislation in the different countries are also applicable to biotech products. In some countries specific products may also be covered by legislation addressing novel foods, such as Regulation (EU) 2015/2283 in the European Union.
- All of the investigated countries implement phytosanitary measures according to the WTO Sanitary and Phytosanitary (SPS)-Agreement. According to this agreement requirements for pest risk assessment can be implemented based on standards developed by the International Plant Protection Convention (IPPC), as well as weed risk assessments which may be required for newly imported plant propagating material.
- Some countries, in particular Australia and New Zealand, implement quarantine and assessment requirements for organisms which are newly introduced into the respective countries.

A recently published legal opinion analyzed whether existing EU legislation e.g., for seeds, food and feed, pesticides and nature conservation, would provide a suitable framework for the assessment of nGM applications outside the biotechnology legislation for risks to human and animal health and to the environment: Spranger (2017) concluded that such sectoral legislation will not provide a suitable framework for an assessment of nGM applications. A premarket assessment of products is either not generally required (e.g., according to the food law) or the required assessments are unsuitable for replacing the comprehensive risk assessment required by the EU biosafety framework (e.g., for novel food laws, pesticide regulations). This conclusion is supported by the results of another recent study conducted by Voigt and Klima (2017).

Furthermore, some general requirements according to regulations for quarantine, phytosanitary measures and invasive alien species only apply to organisms or species, which are newly introduced into a country.

The information gathered from regulatory experts from non-EU countries indicates that the general conclusion drawn by Spranger (2017) for the EU also applies to all other regulatory systems: The general requirements applicable to the agricultural use of plants in the different countries do not ensure a risk assessment comparable to that according to the respective national biosafety frameworks. This outcome is independent of the type of regulatory trigger implemented in a respective framework and can also affect systems with particular product-oriented triggers like the USA (Kuzma, 2016b; Zetterberg and Edvardsson Björnberg, 2017).

CONCLUSIONS

Our analysis investigated how regulatory systems determine the regulatory status of biotechnology applications. In general two categories of regulatory triggers can be distinguished: process-oriented and product-oriented. The overarching question was which trigger would generally be better suited to address new developments in the field of biotechnology, including different nGMs.

Our review of available scientific literature and the results of the interviews conducted with regulatory experts allows us to draw the general conclusion that in practice neither trigger system can be generally regarded as superior when addressing the challenges posed by nGMs. We note that all existing triggers have generic advantages and disadvantages and that the specific trigger definitions and their implementation are more important when defining the range of covered products than an initial choice of a either a process- or a product-oriented trigger system. On the one hand none of the existing trigger systems allows for a straightforward, unambiguous denomination of regulated articles. In process-triggered systems administrative, legislative or court decisions (like in the EU or New Zealand) are necessary to clarify which categories of nGM applications fall under the respective legislation/GMO definition. In frameworks based on product-oriented triggers nGM applications are scrutinized individually to assign their regulatory status.

On the other hand most of the existing biosafety frameworks do not address newly developed products in a fully consistent manner. What all biosafety frameworks have in common is that they aim to identify and assess environmental and health risks associated with a given product generated by biotechnology. Ideally those frameworks should aim to regulate products with comparable risks in a similar manner. In practice many examples can be identified where products with comparable characteristics are subject to very different requirements. In frameworks with process-oriented triggers products generated with GM-technology need to be assessed for biosafety, whereas comparable products developed with conventional approaches are not required to undergo a similar premarket risk assessment. In the product-oriented framework operated in the USA similar products developed with different transformation methods are treated differently irrespective of similar characteristics of the final product. A higher degree of consistency is currently only achieved in the Canadian framework, which is based on a product-oriented trigger focusing on the novelty of products.

With the advent of nGMs coherent regulation of novel biotechnology products becomes even more challenging. The information gathered in our study indicates that sectoral regulation which applies for all agricultural- and food-products does not provide for a comparable breadth and standard of risk assessment as compared with the requirements according to the respective biosafety frameworks. The decision as to whether certain nGM applications should fall under the respective biosafety frameworks is therefore critical for the scope and the quality of risk assessment which is provided for these applications. This decision is ultimately a political one. With that in mind legislators have different options to regulate nGMs for biosafety purposes, if desired. Those options range from applying and/or adapting existing rules to developing a new overall framework for all biotechnology applications or additional biosafety regulations for nGM applications. The latter would amount to substantial changes of the existing frameworks, specifically for frameworks based on process-oriented triggers. According to the information collated in our study such major legislative changes are not likely to be implemented in any of the investigated countries.

The regulatory status of nGM applications is in the process of being resolved in a growing number of countries by administrative or judicial decisions based on the existing biosafety laws and by introducing supplementary regulations specifying concrete criteria for such decisions. However, the lack of harmonization at the global level concerning such approaches will lead to situations that identical biotechnological applications/products are assigned opposing different regulatory status in different jurisdictions. This will result in a serious challenge for international trade between such countries. To address this challenge transparency in decision-making for nGM applications is a crucial issue acknowledged by regulatory experts from all investigated frameworks. We consider a public international registry which includes all biotech products that are placed on the market, among them (nGM) applications exempted in certain countries from regulatory oversight and risk assessment prior to commercial use, to be essential. This would ensure that all countries are enabled to identify products developed by nGMs, if their respective legislation requires them to do so. Non-registered and undescribed products developed by certain nGMs, e.g., SDN-1 type genome editing, can be difficult to detect and keep track of. Shipment of agricultural products suspected to be of uncertain composition, i.e., containing nGM products, could provoke unwanted disruptions of international trade.

We note that the Biosafety Clearing House (BCH) according to the CPB is an existing registry for GMO applications at the international level that also contains information voluntarily submitted by non-parties to the Protocol. It may also provide an appropriate framework for the purpose of sharing relevant information on nGM applications. We are, however, aware of the fact that it will be a challenge to establish and maintain a registry including nGM applications, which are not subject to regulation according to some national biosafety frameworks, since active voluntary cooperation of country administrations and developers is required. Nevertheless stakeholders from all countries should be aware that sharing information on nGM products will be vital, since global harmonization of regulatory approaches toward applications of genome editing and other nGMs will not be easily achieved in the near future.

AUTHOR CONTRIBUTIONS

MFE conducted the study and drafted the manuscript. ME, AH, SS, and HT contributed to the study design and edited the manuscript. All authors have read and approved the manuscript for publication.

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An EU Perspective on Biosafety Considerations for Plants Developed by Genome Editing and Other New Genetic Modification Techniques (nGMs)

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The question whether new genetic modification techniques (nGM) in plant development might result in non-negligible negative effects for the environment and/or health is significant for the discussion concerning their regulation. However, current knowledge to address this issue is limited for most nGMs, particularly for recently developed nGMs, like genome editing, and their newly emerging variations, e.g., base editing. This leads to uncertainties regarding the risk/safety-status of plants which are developed with a broad range of different nGMs, especially genome editing, and other nGMs such as cisgenesis, transgrafting, haploid induction or reverse breeding. A literature survey was conducted to identify plants developed by nGMs which are relevant for future agricultural use. Such nGM plants were analyzed for hazards associated either (i) with their developed traits and their use or (ii) with unintended changes resulting from the nGMs or other methods applied during breeding. Several traits are likely to become particularly relevant in the future for nGM plants, namely herbicide resistance (HR), resistance to different plant pathogens as well as modified composition, morphology, fitness (e.g., increased resistance to cold/frost, drought, or salinity) or modified reproductive characteristics. Some traits such as resistance to certain herbicides are already known from existing GM crops and their previous assessments identified issues of concern and/or risks, such as the development of herbicide resistant weeds. Other traits in nGM plants are novel; meaning they are not present in agricultural plants currently cultivated with a history of safe use, and their underlying physiological mechanisms are not yet sufficiently elucidated. Characteristics of some genome editing applications, e.g., the small extent of genomic sequence change and their higher targeting efficiency, i.e., precision, cannot be considered an indication of safety *per se*, especially in relation to novel traits created by such modifications. All nGMs considered here can result in unintended changes of different types and frequencies. However, the rapid development of nGM

plants can compromise the detection and elimination of unintended effects. Thus, a case-specific premarket risk assessment should be conducted for nGM plants, including an appropriate molecular characterization to identify unintended changes and/or confirm the absence of unwanted transgenic sequences.

Keywords: new genetic modification techniques (nGM), genome editing, CRISPR/Cas, plant modification, biosafety, risk assessment

INTRODUCTION

A wide range of new genetic modification techniques (nGM), which are also collectively referred to as "new techniques" or NTs in short, has been developed to modify plants for research purposes or for the development of crops (Lusser et al., 2012; Vogel, 2016; SAM, 2017). nGMs and genome editing in particular are different from conventional breeding methods and from classic genetic engineering technology and are used to produce plants with traits or a combination of traits suitable for agricultural use (Songstad et al., 2017). In recent years a number of different genome editing approaches were developed to introduce either random or directed genetic changes at specific genomic locations, particularly methods based on site-directed nucleases, e.g., CRISPR-based systems (Puchta and Fauser, 2014; Voytas and Gao, 2014; Weeks et al., 2016; Zhang et al., 2017a). Genome editing, especially approaches based on CRISPR/Cas-technology, rapidly gained prominence due to their versatility, simplicity, speed, and typically low costs. Other nGM approaches which were used to develop crop plants comprise cisgenesis, transgrafting, and approaches to support and accelerate crossbreeding schemes, such as accelerated breeding, haploid induction or reverse breeding. The latter involve genetic modification (GM) technology in intermediate steps resulting in final products that are non-transgenic, i.e., they no longer contain the inserted transgenes (Ricroch and Hénard-Damave, 2016; Schaart et al., 2016; SAM, 2017). Another motivation for plant breeders to apply such methods is that some of them, including certain types of genome editing, are not or may not be covered by biosafety legislation in certain countries (Wolt et al., 2016; Eckerstorfer et al., 2019).

Currently only limited biosafety information is available for most of the plants developed with different nGMs from risk assessment conducted for these applications. The question of whether the agricultural use of nGM plants might pose risks for the environment and/or human and animal health is mostly based on available experience with plants obtained by classic mutagenesis (particular in relation to applications of genome editing) and with transgenic plants developed by standard GM technology (e.g., in relation to cisgenesis, transgrafting, and genome editing applications aimed to integrate recombinant DNA constructs at certain genomic locations). However, the traits and unintended changes in nGM applications may differ significantly from modifications present in existing conventional or transgenic plants. Therefore, the available experience and knowledge may only be of limited value for the assessment of novel nGM plants. The availability or lack of robust biosafety information for certain nGM plants is a significant issue in the ongoing discussion concerning the regulation of nGM applications by existing biosafety frameworks, initially introduced for products developed by GM technology (Jones, 2015a; Sprink et al., 2016; Wolt, 2017) or by other legislation applicable to nGM plants used for agricultural purposes (Eckerstorfer et al., 2019).

OVERVIEW ON nGMS COVERED IN THIS STUDY AND ON THEIR CHARACTERISTICS

Due to new developments, the spectrum of nGMs and variations thereof are increasing at a high speed (EPRS, 2016). The nGMs addressed in this study were selected based on an early and a more recent EU-level report on nGMs (Lusser et al., 2012; SAM, 2017). The following nGMs were addressed in this (see also **Table 1**):

- Genome editing with site-directed nucleases (SDNs), e.g., using clustered regularly interspaced short palindromic repeat (CRISPR)-directed nucleases, Transcription activator-like effector nucleases (TALENs), zinc-finger-directed nucleases (ZFNs), or meganucleases. Such SDN-based techniques can also be applied for multiplex genome editing. Other approaches were developed for base editing as well as for modification of transcriptional regulation.
- Genome editing directed by synthetic oligonucleotides, also referred to as oligonucleotide-directed mutagenesis (ODM)
- RNA dependent DNA methylation, an approach for modifying epigenetic regulation of gene expression
- Cisgenesis and intragenesis
- Transgrafting, in particular the use of GM-rootstocks in grafting
- Agro-infiltration
- Haploid induction and reverse breeding, i.e., examples of techniques developed to assist complex breeding schemes.

Thus, very different approaches are used to introduce genetic and phenotypic variation in plants for the development of traits of agricultural interest (van de Wiel et al., 2017). As discussed in more detail below the modifications introduced by these nGMs vary significantly from each other. We also note that these nGMs or rather the resulting plant products differ significantly from each other regarding their applicability in agriculture, as well as the associated safety issues.

Genome editing, cisgenesis, and intragenesis have in common that they introduce genetic modifications which are meant to be present in the final plant products and passed on to

TABLE 1 | Overview of the nGMs addressed in this study and strategy employed for literature search.

Type of nGM	nGM	Search terms used for literature searches
Genome editing with site-directed nucleases (SDN)	CRISPR-based systems for genome editing (CRISPR)	(crispr OR cpf1) AND (plant OR plants OR plant* OR "plant breeding" OR crop* OR tree*); (crispr OR cpf1) AND tree* NOT (plant OR plants OR plant* OR "plant breeding" OR crop*)
	TALE-directed Nuclease systems for genome editing (TALEN)	("transcription activated-like nuclease*" OR TALEN OR "transcription activator-like effector nuclease*") AND (plant* OR crop*)
	Zinc-Finger-directed Nuclease systems for genome editing (ZFN)	("zinc finger nuclease" OR ZFN) AND (plant* OR crop*)
Genome editing directed by oligonucleotides	Oligonucleotide-directed Mutagenesis (ODM)	(oligonucleotid* OR "oligonucleotide directed mutagenesis" OR ODM OR "chimeric oligonucleotid*" OR "chimeric RNA/DNA oligonucleotid*" OR chimeraplasty OR "site-directed mutagenesis" OR "gene targeting") AND (plant* OR crop*)
	Multiplex Automated Genomic Engineering (MAGE)	"multiplex automated genomic engineering"
Modification of gene expression	RNA-directed DNA Methylation (RdDM)	(TGS) AND (plant* OR crop*); (RDDM OR RNA*directed DNA methylation) AND (plant* OR crop*)
Variants of GM technology	Cisgenesis (CG) / Intragenesis (IG)	(cisgen* OR intragen* OR "all native DNA transformation" OR "all-native DNA transformation") AND (plant* OR crop*)
	Transgrafting (TG)	(graft* AND (transg* OR transform* OR GM graft OR GM scion) AND (plant* OR crop* OR tree*); transgrafting applications involving GM rootstocks: (graft* OR transgraft* OR trans-graft*) AND ("GM rootstock*" OR "transgen* rootstock")
	Agro-infiltration (AI)	(agroinfiltr* OR agroinocul* OR agroinfect*) AND (plant* OR crop*)
Breeding support techniques	Haploid Induction (HI)	(CENH3 OR "haploid induction" OR "genome elimination" OR haploids) AND (plant* OR crop*)
	Reverse Breeding (RB)	("reverse breeding" OR "crossover control") AND (plant* OR crop*) AND (plant* OR crop*)

offspring during sexual reproduction (Holme et al., 2013). As regards genome editing a variety of different approaches are employed to achieve different types of desired modifications (Tycko et al., 2017). Approaches using SDNs and ODM are applied to introduce random (SDN-1) or directed sequence changes (SDN-2 and ODM) at specific, predefined genomic loci (Podevin et al., 2013; Sauer et al., 2016a). These approaches do not necessarily require the stable introduction of recombinant constructs into the plant genome. ODM for example is directed by small-sized synthetic oligonucleotides, which are transiently introduced into the recipient plant cells and supposed to be degraded by the cellular metabolism (Sauer et al., 2016a). SDNs which facilitate genome editing can either be inserted into the genome of the target cell as a transgene, or introduced into target cells as functional (ribonucleo-) proteins (Kanchiswamy, 2016) or expressed from transiently introduced DNA constructs (Butler et al., 2016). Some approaches for genome editing, commonly referred to as SDN-3, facilitate the insertion of transgenic constructs at specific genomic locations (Petolino and Kumar, 2016). The respective transgenic insertions are present in the final breeding product (plant or plant product) and are heritable.

Besides these basic types of genome editing a number of additional approaches, e.g., for base editing, were developed recently. Base editing uses modified SDNs, typically CRISPR variants, to modify certain DNA bases in a deliberate way (C to T or A to G) (Matsoukas, 2018; Rees and Liu, 2018).

nGMs like agro-infiltration (Vaghchhipawala et al., 2011) and transgrafting (Schaart and Visser, 2009) are typically used to modify somatic tissues or to produce chimeric plants, e.g., GM rootstocks fused to non-GM scions by transgrafting (SAM, 2017). Typically the genetic modifications introduced by these approaches are not passed on by sexual reproduction. However, the whole plant may be affected, i.e. in the above mentioned case effector substances produced in the GM rootstock may reach the upper non-GM scion and influence its phenotype (Stegemann and Bock, 2009).

RNA-directed DNA methylation (RdDM) is used to modify the expression of endogenous genes not by changing its DNA sequence, but rather through introducing epigenetic modifications which may be passed on for some generations (Mahfouz, 2010).

nGMs like haploid induction (Ravi and Chan, 2010; Britt and Kuppu, 2016) or reverse breeding (Dirks et al., 2009; Wijnker et al., 2012) are predominantly used to enable and/or speed up specific breeding schemes. They involve transgenic insertions intended to be present only at intermediate steps. Therefore, the respective transgenic modifications must be verifiably absent from the final breeding products (SAM, 2017).

LITERATURE SURVEY TO IDENTIFY APPLICATIONS OF nGMS WITH RELEVANCE FOR RISK ASSESSMENT

To identify nGM applications which may be relevant from a risk assessment point of view the following approach was used: different sources were screened for research on and development

of plants developed by nGMs, hereinafter referred to as nGM plants, for potential future use in agriculture.

The sources included previously published reports addressing the nGMs in question, which contain information on relevant nGM plants as well as their state of development (Vogel, 2016; Hilscher et al., 2017b). Also scientific reviews addressing the use of genome editing or other nGM approaches for the development of crops for agriculture were screened for relevant information (Khatodia et al., 2016; Paul III and Qi, 2016; Hilscher et al., 2017a; van de Wiel et al., 2017). In addition the recent scientific literature was screened to identify publications addressing the use of nGMs, that were not already included in previous reviews.

The general timeframe for the literature search covered the period from January 2011 until June 2017. The searches addressing genome editing by CRISPR-based methods were limited to the period from January 2016 until June 2017, with a view to the availability of reviews covering previous years (e.g., Hilscher et al., 2017b). Relevant scientific publications from peer reviewed journals were retrieved using the databases Scopus, ProQuest Natural Science Collection, the Web of Science, and PubMed. Searches were conducted with a set of keywords relating to the individual nGMs, combined with search terms or filters to exclude applications other than plant biotechnology (see **Table 1**). The titles and abstracts of the references were manually screened for relevance.

The objective of the literature searches was to establish a non-exhaustive overview on recent usage of the respective nGMs. The search was not intended to establish a comprehensive collection of the whole scientific literature on nGM applications, but rather to identify the focus of current nGM approaches, the modified plant species and the developed traits. Systematic reviews of the available literature might be helpful for a more detailed discussion of specific techniques. Such a systematic review is underway for applications of genome editing in plant breeding (Modrzejewski et al., 2018). We consider that our results nevertheless broadly illustrate the significance of the different approaches in plant breeding. Our literature search also covered publications of nGM applications that are near commercialization or already commercialized in some countries, such as a herbicide-resistant oilseed rape variety developed by ODM (Gocal et al., 2015). As seen in Sovova et al. (2017) information on patents did not add significant information in terms of application and the potential for commercialization. We are thus confident that the sources we have considered sufficiently serve the purpose of this work.

In total 172 research publications addressing work in plants with all listed nGM were retrieved for the period January 2016–June 2017 (**Table 2**). Most of them reported the application of genome editing in different species, among them model species for research (such as *Arabidopsis* and tobacco), as well as different crop and tree species. The majority of publications (114) applied CRISPR-based approaches for genome editing. A significant focus was on the further development and adaption of CRISPR-based methods for different plant species (72).

This supports prior findings that CRISPR-based genome editing quickly established itself as the most important tool in genome editing (Hilscher et al., 2017a). Further variants of CRISPR-technology are continuously being developed. A small number of publications addressed the use of emerging variants of CRISPR-based systems, e.g., the use of modified or alternative CRISPR-type nucleases like Cpf1 (4) (Kim et al., 2017; Tang et al., 2017; Xu et al., 2017; Yin et al., 2017), as well as the use of modified Cas9 nucleases, e.g., as single strand nickases (2) (Schiml et al., 2014; Mikami et al., 2016) or for targeted base-editing (4) (e.g., Shimatani et al., 2017; Zong et al., 2017). This underlines the interest in the development of variants of CRISPR-based systems with increased specificity of targeting or approaches for introducing specific types of mutations at specific genomic locations, e.g., via chemical modification of specific nucleotides present in the targeted genomic sequences (Komor et al., 2016, 2017; Schaeffer and Nakata, 2016; Arora and Narula, 2017).

Significantly fewer publications addressed applications of other SDN-based genome editing methods, involving TALENs (10), ZFNs (17), and meganucleases (5). Only a single publication could be retrieved for the application of ODM between January 2016 and June 2017 (Sauer et al., 2016b). However, according to other sources these methods are actively used for the development of modified crop plants for (future) agricultural use, e.g., by companies like KeyGene and CIBUS (Abbott, 2015) as well as Calyxt in case of TALEN (Gelinsky, 2017).

SDN-1 applications clearly dominated the field of genome editing applications employing SDNs (108/130); they are applied to modify (mostly to knockout) all alleles of specific genes present in a plant line. Only 16 publications described the use of SDN-2 and SDN-3 applications; TALEN- and ZFN-based genome editing was more frequently used for SDN-3 applications (3/8 and 3/7) in comparison with CRISPR-based systems (4/114). Some of these publications describe approaches for integration and stacking of transgenes at specific, pre-modified genomic locations ("trait landing pads") by commercial developers (Ainley et al., 2013; Kumar et al., 2015, 2016). Also the relative number of publications addressing the development of traits for agricultural use was higher between January 2016 and June 2017 for TALEN (6/8) and ZFN (3/7) when compared to the respective CRISPR applications (20/114).

When compared to genome editing (131), other nGMs were covered significantly less often in papers published between January 2016 and June 2017 (41), with transgrafting being the most prominent technique in this group (23). In that period only a few publications described approaches based on RdDM, cisgenesis, and intragenesis. However, more work using these technologies with relevance for the development of agricultural plants was published between 2011 and 2017. Publications on agro-infiltration during that period focused on its use for basic research.

The numbers in **Table 2** for publications on TALEN and ZFN before 2016 correspond to the ones reported by Hilscher et al. (2017a); from January 2016 onwards a low but continuous interest remained in TALEN- and ZFN-approaches (8 and 7, respectively). Meganuclease-based systems were used less often (5 publications by the end of 2015, 1 in the subsequent period) due to the technical challenges to target different genomic sequences with this method.

TABLE 2 | Research publications between 2011 and 2017 covering several applications for different nGMs.

Applications \ nGMs	Genome editing					RdDM	CG	IG	TG*	nGMs to support breeding	
	CRISPR*	TALEN	ZFN	MN	ODM					AI	HI
JAN. 2011–DEC. 2015											
Total number	**n.a.**	**10**	**17**	**5**	**1**	**6**	**7**	**4**	**n.a.**	**14**	**9**
JAN. 2016–JUNE 2017*											
Total number (172)	**114**	**8**	**7**	**1**	**1**	**1**	**2**	**4**	**23**	**4**	**7**
SDN−1	99	5	4	–	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
SDN−2	5	–	–	–	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
SDN−3	4	3	3	1	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Base editing	4	–	–	–	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Other types of genome editing	2	–	–	–	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
OBJECTIVE OF APPLICATIONS (JAN. 2016–JUNE 2017)											
Method development	72	1	2	1	–	–	1	1	6	–	3
Basic research	22	1	2	–	–	–	–	–	7	4	1
Applied development	20	6	3	–	1	1	1	3	10	–	2

SDN, site-directed nuclease; CRISPR, CRISPR (Clustered regularly interspaced short palindromic repeat)-directed nuclease; TALEN, Transcription activator-like effector nuclease; ZFN, Zinc-Finger-directed nuclease; MN, Meganucleases; ODM, Oligonucleotide-directed mutagenesis; RdDM, RNA dependent DNA methylation; CG, Cisgenesis; IG, Intragenesis; TG, Transgrafting; AI, Agro-infiltration; HI, Haploid induction; Other types of genome editing: different variants of CRISPR-based genome editing, including use of nickases; n.a.: not applicable.

**For the use of CRISPR-based systems for genome editing and transgrafting literature was only screened for the time period Jan. 2016-June 2017.*

Bold values indicate total numbers of publications for individual nGMs for the indicated time periods.

The analysed literature on nGM applications in plants demonstrates that an extremely wide range of species was used in relevant research and development projects: The range includes model species for research (like *Arabidopsis* and tobacco), most crop species including important crops such as maize, rice, wheat and other cereals, soybean, potato and other plants for oilseed production as well as a broad range of vegetable and spice plants and perennial plants including fruit trees and forest trees as well as lower plants, e.g., moss species.

RISK ASSESSMENT CONSIDERATIONS

General Approach of Risk Assessment

An important aspect in the overall discussion on nGMs is whether specific biosafety issues may be associated with their plant products. To address this question two main issues have to be determined: (a) whether plant development with a particular nGM approach can lead to unintended genetic or epigenetic changes and whether they may be associated with adverse effects on human and animal health as well as the environment; (b) whether the intended use of the nGM plants may result in adverse effects related to the newly developed traits (Mahfouz et al., 2016; Bujnicki, 2017).

Considerations Regarding Unintended Effects Associated With nGM Applications

As with GM technology or other biotechnological methods, the presently available nGMs are not sufficiently specific to introduce only the intended molecular changes into plants. Thus, a range of unintended molecular changes may be introduced by a particular nGM method and these molecular changes may lead to phenotypic effects affecting the properties of the modified plant (SAM, 2017).

In general several types of unintended effects can be distinguished (Agapito-Tenfen et al., 2018):

- Unintended changes at genomic locations other than the genomic target site(s) for intended modifications; i.e., modifications which are usually not genetically linked to the desired trait(s)
- Unintended molecular changes in the vicinity of the intended site of modification; i.e., changes different from the intended modifications, but tightly linked to the desired trait(s)
- Unintended effects different to the desired trait(s) which are due to the modifications at the genomic target; i.e., pleiotropic effects of the intended modification(s) linked to the desired trait(s).

Unintended changes may modify the expression of endogenous genes and impact the plant's metabolism and phenotype. According to the nature of the particular phenotypic effects, these unintended changes may be considered either harmless or adverse in terms of human health and the environment.

Method-related unintended molecular changes may be associated with different aspects of the overall development process of nGM products. They depend either on the mechanisms of the particular nGM or on the characteristics of further methods required for the overall development of a particular nGM plant, such as methods for *in vitro* cultivation of plant cells and tissues, methods to facilitate the uptake of nGM components (e.g., protoplast transfection methods)or methods for the regeneration of plants from cultivated cells or tissues.

Typically exogenous effector molecules need to be introduced into recipient plant cells to initiate nGM processes, such as (i)

recombinant DNA constructs for stable genetic transformation of plant cells, e.g., to express nucleases for genome editing or other molecular tools required for a particular nGM; (ii) recombinant DNA constructs for transient expression of nGM-related components (RNA or proteins required for the respective nGMs); (iii) specific DNA, RNA or ribonucleoprotein complexes. Unintended genetic or epigenetic changes can be introduced as a side effect of transformation or the transfer of method-related components into the recipient cells (Latham et al., 2006; Mehrotra and Goyal, 2012).

Unintended changes may also result from the integration of genetic constructs into the recipient genome of plant cells for nGM approaches that involve the use of GM techniques. This relates to e.g., cisgenesis/intragenesis, the transformation of rootstocks for transgrafting and genome editing approaches that are based on the expression of SDN components from transgenic constructs. It is typically a random process and thus can result in unintended genetic changes, e.g., by the disruption of functionally important genomic sequences or due to the integration of other unrelated DNA sequences (SAM, 2017). Untargeted integration of non-endogenous sequences can also modify the expression of endogenous genes located in the vicinity of the integration site(s) (Ladics et al., 2015).

It should also be noted that genetic constructs that are only transiently introduced into plant cells to express method-related components may integrate into the genome of the recipient cells. If transgenic constructs should only be present during intermediate steps it is important to assess whether all such modifications are indeed fully removed and absent from the final product. This relates to any inserts of the constructs for expression of method components as well as to secondary inserts, e.g., of vector backbone sequences. Braatz et al. (2017) for example found by way of whole-genome sequencing that transformation of oilseed rape with an CRISPR-Cas9 expression construct resulted in at least five independent insertions of vector backbone sequences in the genome of the modified plant.

Unintended genetic and epigenetic changes may also result from the respective particular nGM mechanism. Well-known examples are off-target modifications associated with approaches for genome editing. They typically happen in genomic sequences that share a sufficiently high degree of similarity with the target loci and thus can associate with the molecular editing tools leading to off-target edits (Kanchiswamy et al., 2016; Yee, 2016). Off-target activity can also be associated with other nGM, e.g., RdDM approaches. In such cases not only the target site(s) are epigenetically modified, but also other genomic loci (Galonska et al., 2018).

The frequency of off-target effects as well as their extent and distribution in the genome are different for the various genome editing approaches and depend on both the targeting characteristics of the particular approach and on the specific method used for genome editing (HCB, 2017; Wolt, 2017), including the exact experimental protocol (Yee, 2016).

From a risk assessment point of view it is relevant to assess whether the respective unintended molecular changes are leading to phenotypic changes of an adverse nature (SAM, 2017). Off-target modifications, which result in readily detectable phenotypic changes, can be identified and possibly eliminated during downstream breeding when generating elite lines (Zhao and Wolt, 2017). Significant alterations of important agronomic parameters, such as yield, fitness, growth, and reproduction may be detected quite readily. However, not all induced phenotypic changes can be easily detected. Subtle changes e.g., in composition are more difficult to detect, however they may impact the nutritional quality or may be associated with allergenic or toxic effects. Also, some unintended changes may be genetically tightly linked to the desired trait(s) while others are not. That does influence how easily they can be removed, if at all. The probability that unintended changes are indeed removed depends critically on the number of breeding steps involved to establish a final breeding product. While this is less of a concern with annual crop plants which are typically subjected to a sufficient number of breeding cycles, this constraint is relevant for plants like trees, which do not undergo the same number of breeding cycles for practical reasons, as well as for plants which are mostly propagated vegetatively. On the other hand nGMs like genome editing may be used for direct modification of elite lines to speed up breeding processes, according to information presented at a recent conference (OECD, 2018). However, faster ways of plant breeding may negatively impact the ability to safely remove any unwanted unintended modifications. Thus, strategies to minimize off-target activity and to identify unintended modifications should be implemented for the use of genome editing approaches to produce modified plants (SAM, 2017).

Most nGM approaches require the use of further techniques to cultivate cells or explanted tissues (embryogenic or somatic tissues used for callus transformation or plant cells treated to yield protoplasts to facilitate transfection of genetic material or other method-related components), and methods to regenerate modified plants from single cells. A fair number of the genome edited plants reported in Bortesi and Fischer (2015) as well as Schaeffer and Nakata (2016) involved protoplast transfection which was used to deliver the genetic constructs for the expression of SDN-reagents. Plant protoplast technology is also involved in DNA-free methods for genome editing. For such approaches functional site-directed nucleases, mostly CRISPR-ribonucleoproteins, are introduced into protoplasts to initiate editing (Malnoy et al., 2016; Kim et al., 2017). These approaches are currently considered and promoted as alternative to genome editing applications involving the delivery of DNA (Kanchiswamy, 2016; Ran et al., 2017). However, it is known that techniques such as protoplast technology, *in vitro* cultivation of cells and regeneration of plants from cells and tissues are associated with unintended genetic changes (Filipecki and Malepszy, 2006; Bairu et al., 2011; Ladics et al., 2015; HCB, 2017). These techniques can induce somaclonal variation which adds to the range of random genetic changes introduced by nGMs. While somaclonal variation is not a specific feature of nGM approaches, but can also happen in conventional breeding involving cell and tissue cultivation steps, some nGM methods dependent on methods known to promote somaclonal variation. It should thus be ensured that such changes are eliminated during subsequent steps of the breeding process.

Some types of genetic modification can also give rise to pleiotropic effects, i.e., unintended secondary phenotypes which are also determined by the modified gene(s) and which are expressed along with the desired trait (SAM, 2017). Pleiotropic effects can occur with traits developed by all types of breeding approaches, including nGMs. Pleiotropic effects will be present in the final breeding products, since they are tied to the desired trait(s). An example are nGM plants which were modified for increased disease resistance due to the inactivation of susceptibility genes, namely the *mlo* genes conferring broad-spectrum resistance against powdery mildew fungi (Kusch and Panstruga, 2017). A range of pleiotropic effects was found to be associated with the inactivation of certain *mlo* genes, including yield decrease and increased susceptibility to other fungal pathogens as well as effects on mycorrhizal development in roots (Brown and Rant, 2013). Data gathered in the course of screening for unexpected effects during the development process of nGM plants can support the risk assessment of unintended pleiotropic effects conducted in accordance with guidance established by EFSA (EFSA, 2010).

Unintended effects may also be based on modifications/alterations, in particular disruption, of endogenous genomic sequences in proximity to integration sites of DNA introduced to develop plants by certain nGMs. Applications of cisgenesis, intragenesis, or SDN-3 applications may be associated with such effects, depending on the characteristics of the integration site. Due to the genomic proximity of the integrated genetic elements and the altered genomic sequences flanking these elements, such unintended modifications cannot be removed by segregation during further breeding steps. Provided that their functions are understood the molecular characterization of the genomic sequences altered during the integration can provide indications as to whether unintended effects may arise. It may even be possible to predict the phenotype that may result from the modification.

For the purpose of a comprehensive risk assessment of nGM plants unintended effects associated with all technical interventions involved in the process to develop a specific nGM plant have to be considered. A particular focus should be on unintended effects that may be predicted based on the specific characteristics of certain nGMs, such as off-target effects associated with a particular approach for genome editing. This can be addressed through an appropriate molecular characterization of the nGM application taking into account all procedures that were used to establish the nGM application in question. Information from the molecular characterization can then be used to address the question of whether the identified molecular changes may be tied to potential effects at the phenotypic level that should be further assessed.

Considerations Regarding Traits Developed by nGMs

For a comprehensive assessment the risks associated with the newly developed nGM plants and their use in particular (agricultural) environments need to be considered. The new traits generated by an nGM can influence the species-specific characteristics of modified plants and are thus highly important for the assessment of overall risks.

nGMs and genome editing in particular can be used to introduce traits already present in wild populations or related species in a fast and straightforward way. Some of these traits may alternatively be introduced with either conventional breeding or GM technology, though, nGMs in many cases have technical advantages, e.g., providing a simpler, faster, and less costly approach (HCB, 2017). However, many of these traits developed with genome editing and other nGMs need to be considered novel concerning their use in crop plants. Such traits are not present in stable, cultivated populations of the plant species at significant levels (HCB, 2017). For plants with such novel traits typically only limited knowledge and experience concerning their (environmental) effects are available and no history of safe use. In regulatory frameworks which are based on novelty as a product-oriented regulatory trigger, i.e., in Canada, this aspect is crucial for the denomination of products which are subject to oversight for biosafety, e.g., according to the "Plants with novel traits (PNT)"-Regulations (Shearer, 2014). Canada also regulates PNTs which are generated by conventional plant breeding approaches for biosafety (Eckerstorfer et al., 2019).

The available literature on nGM plants provides indications, regarding what traits are currently developed with different nGMs. For a discussion of the associated risks they are grouped into the following classes:

(1) Herbicide resistance (HR)
(2) Disease resistance (to viral, bacterial, and fungal plant pathogens)
(3) Altered composition
(4) Enhanced fitness against environmental stressors and alteration of morphological or reproductive plant characteristics.

In the following sections examples of nGM plants for each trait class are presented. Where available, applications with an advanced stage of development are included. The examples are not meant to be exhaustive, but rather to highlight that a case-specific assessment of applications of the respective class is warranted. A significant number of these traits are developed using different types of genome editing. However, for technical, legal or other reasons also other nGM approaches are used to generate plants with traits from all of the four classes.

Safety considerations associated with applications of genome editing or other nGMs, like transgrafting or cisgenesis/intragenesis, should be based on the characteristics of the particular application. Due to their different modes of action the particular issues for risk assessment can be very different.

nGM Plants With Herbicide Resistance

nGMs are used to develop resistance to a number of different herbicides in several agricultural crops:

- Resistance to acetolactate synthase (ALS) inhibiting herbicides was established via ODM in oilseed rape (Gocal et al., 2015), by SDN-1 technology in potato with TALEN (Nicolia et al., 2015; Butler et al., 2016) and CRISPR/Cas9 (Butler et al., 2016),

in rice with TALEN (Li et al., 2016a) and in tobacco with ZNF (Townsend et al., 2009). In Chinese cabbage this trait was introduced by cisgenesis (Konagaya et al., 2013).

- Resistance to glyphosate-based herbicides was developed by intragenesis in strawberries (Carvalho and Folta, 2017) and in flax by ODM (Abbott, 2015), as well as by a combination of ODM and CRISPR/Cas9 (Sauer et al., 2016b).
- Furthermore genome editing approaches based on SDN-2 and SDN-3 technology were developed for the targeted introduction of (multiple) herbicide resistance genes: resistance to glufosinate ammonium and 2,4 D herbicides in maize with ZFN (Ainley et al., 2013), resistance to glyphosate-based herbicides in cassava using CRISPR/Cas9 (Chauhan et al., 2017), in cotton with meganucleases (D'Halluin et al., 2013) and in maize using ZFN (Kumar et al., 2016). Resistance to bialaphos was developed in tobacco using ZFN (Schneider et al., 2016). Resistance to ALS-inhibiting herbicides via SDN-2 CRISPR/Cas9 technology was developed in maize (Svitashev et al., 2015, 2016) as well as via SDN-3 in rice (Sun et al., 2016) and soybean (Li et al., 2015).

Experience with effects resulting from these traits is available from existing risk assessments of herbicide resistant GM plants. Of particular relevance are indirect effects on biodiversity resulting from the changes in weed management, and the development of herbicide resistant weeds (EFSA, 2010; Schütte et al., 2017). For herbicide resistant oilseed rape, experience is available from comparable conventional HR crops, indicating a number of concerns, e.g., dispersal and persistence of HR volunteers (Expertgroup, 2014; Huang et al., 2016). As noted by Ishii and Araki (2017) ALS-resistant rice which was cultivated in Italy and the USA hybridized with related wild species and HR resistant weeds emerged from these outcrossing events. This underlines the fact that the assessment of the herbicide resistance trait is important independent of the method or technology that was used to produce the crops.

It has been shown recently in *Arabidopsis* that elevated expression levels of modified EPSPS can lead to pleiotropic effects, like elevated auxin content and increased fecundity of the modified plants (Fang et al., 2018). To ensure food and feed safety the absence of unintended effects on composition should be confirmed for respective HR nGM crops.

Some GM crops, in particular soybean, have been made resistant to multiple herbicides, including glyphosate, glufosinate ammonium, dicamba and others (see e.g., http://bch.cbd.int/database/lmo-registry/). Such crops can be expected to contain cocktail mixes of pesticide residues. After methods to assess the cumulative and synergistic effects of pesticides were developed (EFSA, 2013), they have to be taken into account for risk assessment and potential human health impacts (Regulations (EC) No. 396/2005 and No. 1107/2009). A report by the European Food Safety Authority (EFSA) on how to consider effects of pesticide cocktails on the nervous system is about to be finalized (see information at: http://www.efsa.europa.eu/en/consultations/call/180508-0). As mentioned above, maize with two HR genes was already developed using ZFN. Therefore, cocktail mixes of pesticide residues can be expected to become a relevant risk assessment issue for nGM crops as well.

The herbicide resistant oilseed rape from Cibus developed using ODM is the only HR-nGM plant which is actually cultivated so far, but other crops with similar traits are in the commercial pipeline. It can be expected that herbicide resistant nGM crops will continue to be an important objective for future commercial plant development (KASKEY, 2018).

nGM Plants With Disease Resistance

A number of different approaches were developed for increased resistance of plants against different viral, bacterial and fungal pathogens. Approaches included

- Knockout by genome editing approaches of plant susceptibility factors for bacterial and fungal pathogens in grapefruit, wheat, tomato, grapevine, apple, and rice (Wang et al., 2014, 2016; Jia et al., 2016, 2017; Malnoy et al., 2016; Blanvillain-Baufum et al., 2017; Nekrasov et al., 2017; Zhang et al., 2017b) or knockout of viral host factors in *Arabidopsis* (Pyott et al., 2016) and cucumber (Chandrasekaran et al., 2016). Most of these applications were developed with SDN-1 approaches using CRISPR-based methods (Jia et al., 2016, 2017; Wang et al., 2016; Zhang et al., 2017b) or TALENs (Wang et al., 2014; Blanvillain-Baufum et al., 2017).
- Expression of resistance genes in apple, potato, and grapevine (Vanblaere et al., 2011, 2014; Haverkort et al., 2016) and antimicrobial substances against fungal pathogens (Rubio et al., 2015) by transgrafting and cisgenesis applications.

Resistance to powdery mildew, a fungal disease, was established by knocking out plant susceptibility genes by genome editing. However, a number of pleiotropic effects such as reduced plant size or premature senescence were described (Kusch and Panstruga, 2017) most likely because the knocked out plant genes may have several other functions as well. Also knockout or silencing of members of the *mlo* gene family that are not involved in pathogen susceptibility by off-target activity may lead to unintended effects on physiology, development or composition with implications for food, feed and environmental safety (Pessina, 2016).

Other aspects have to be considered for applications to induce virus resistance by transgrafting. Lemgo et al. (2013) identified several concerns, that should be addressed during risk assessment: These include pleiotropic silencing effects, effects of the transgenic rootstock on non-target organisms, e.g., on soil organisms, gene transfer of virus resistance to wild type plants resulting in increased fitness and invasiveness, potential development of novel viral strains and food safety effects. For transgrafting applications in general the potential mobility of the transgenic product across graft junctions influences the likelihood for environmental or food safety risks (Schaart and Visser, 2009; Song et al., 2015).

nGM Plants With Compositional Changes

A variety of nGM plants with changed composition were developed mostly by genome editing approaches and some

by cisgenesis/intragenesis. Examples of targeted traits were among others:

- CRISPR/Cas-mediated (SDN-1) changes of sugar and starch content in potato and rice (Andersson et al., 2017; Sun et al., 2017).
- CRISPR/Cas-mediated SDN-1 knockouts of genes resulting in altered lipid composition, e.g., in *Camelina* (Jiang et al., 2017; Morineau et al., 2017) and soybean (Haun et al., 2014; Demorest et al., 2016; Kim et al., 2017).
- CRISPR/Cas-mediated SDN-1 genome editing to reduce browning in mushrooms (Waltz, 2016), TALEN-mediated reduction of lignin in sugarcane (Jung and Altpeter, 2016), ZFN-based reduction of phytate in maize (Shukla et al., 2009) and TALEN-mediated reduction of components which reduce the storage capacity and processing quality of potatoes (Clasen et al., 2016) and rice grain (Ma et al., 2015).
- CRISPR/Cas-mediated silencing of several different α-gliadins in wheat, resulting in a reduction in the content of anti-nutritional gluten (Sanchez-Leon et al., 2018).
- TALEN-mediated SDN-1 knockout of genes modifying the content of substances increasing fragrance in rice (Shan et al., 2015).
- Cisgenic modification to increase the anthocyanin content in apple (Schaart et al., 2016) and to reduce the acrylamide-forming potential of potato tubers during processing (Chawla et al., 2012).

Based on experience with problem formulation for the risk assessment of GM plants (EFSA, 2011) a number of potential risk issues as regards food and feed safety and environmental effects should be addressed in the risk assessment of nGM plants from this class, particularly any toxic or allergenic effect resulting from proteins with modified sequence, or any anti-nutritive effect of newly produced compounds. Compositional changes can furthermore result in environmental effects due to altered interactions with herbivorous animals, e.g., for nGM plants with increased sugar content, or by effects on morphological characteristics, like stability, e.g., for nGM plants with reduced lignin content.

nGM Plants With Enhanced Fitness Against Environmental Stressors and Alteration of Morphological or Reproductive Plant Characteristics

Several approaches including genome editing applications and transgrafting were used to establish a variety of different traits with environmental/ecological relevance:

- Transgrafting in tomato (Nakamura et al., 2016) and genome editing in *Arabidopsis* to improve abiotic stress response e.g., to cold, drought, salinity (Osakabe et al., 2016; Zhao and Zhu, 2016).
- CRISPR/Cas9-mediated SDN-1 knockout of two ALCATRAZ genes for increased seed shatter resistance of oilseed rape (Braatz et al., 2017).
- CRISPR/Cas9-mediated SDN-1 knockout in tomato of a factor (SlAGL6) influencing early maturation and facultative parthenocarpy for fruit production under climate (heat) stress (Klap et al., 2017) and of a flowering repressor (SP5G) for early flowering (Soyk et al., 2017).
- CRISPR/Cas9-mediated SDN-1 type alterations of the SlCLV3 promoter in tomato to generate larger fruit and increased numbers of flower buds (Rodríguez-Leal et al., 2017).
- CRISPR/Cas9-mediated SDN-1 editing of several genes in rice to generate early-maturing cultivars (Li et al., 2017), and in genes acting as regulators of grain number, panicle architecture, grain size, and plant architecture to create mutants showing enhanced grain number, dense erect panicles, and larger grain size (Li et al., 2016b) as well as modification of three negative regulators of grain size in a multiplex approach (Xu et al., 2016).

Traits related to enhanced fitness can result in adverse effects due to an increased potential for invasiveness or weediness in the modified plants or sexually compatible species following introgression of such traits. However, depending on the modified trait and the wild relative, effects of outcrossing can be adverse for different reasons: in the case of related valued species a decrease in reproduction or fitness would be regarded as adverse, similar as an increase of reproductive fitness in case of the weedy relatives.

Two recent publications (Li et al., 2018; Zsögön et al., 2018) indicate the potential of genome editing for an approach called *de novo* domestication, i.e., to rapidly develop crop lines from wild forms with desired properties like strong resistance toward pathogens or salt tolerance. In both cases characteristics associated with domesticated tomato plants were established in different lines of *Solanum pimpinellifolium* by simultaneously editing only 4 or 6 genomic loci, respectively, while maintaining the desired resistances present in the wild lines. Among the introduced domestic characteristics were increased fruit number, size, shape and nutrient content of fruits as well as plant architecture and growth characteristics. The authors regard their approach as a viable route for the direct development of new crop varieties from wild plants in order to exploit their genetic diversity and thus as a fast and simple alternative to classic breeding programs. However, also any potential hazards associated with the agricultural use of such novel crops with wildtype genetic backgrounds need to be carefully assessed.

nGM Characteristics Relevant for Risk Assessment Considerations

Combination of Biotechnological and Conventional Methods

The scientific literature considered in this study demonstrates that in most cases specific nGMs are not used in isolation, but various biotechnological methods are combined in the different breeding processes to establish nGM applications. The following examples of the combined application of different methods for the development of nGM applications illustrate the various relationships.

In many approaches GM technology is used at some point to establish intermediate or final products containing transgenic insertions. Typically such approaches are used to transfer and express the molecular tools necessary for the development of a variety of nGM applications. This includes

e.g., expression of site-directed nuclease components for genome editing approaches, expression of transgenes in the modified rootstocks (or other parts) of plants established by transgrafting or during intermediate steps in the development of plants utilizing nGM approaches to speed up breeding cycles, e.g., accelerated breeding (Zhang et al., 2010). For haploid induction, reverse breeding and accelerated breeding as well as for most products developed by SDN approaches for genome editing, the recombinant components are first integrated into the genome of the plant to be modified and then removed by segregation during later steps to derive the final breeding products.

Likewise nGMs may be used as technical tools to support the application of another nGM category. For instance genome editing can be used to knockout specific endogenous plant genes, e.g., to initiate early flowering as a tool for developing products by accelerated breeding (Zhang et al., 2010), or to suppress meiotic recombination in plants which are used in reverse breeding applications (Dirks et al., 2009). CRISPR-based systems in combination with DNA methyltransferases can be utilized for targeted modification of genomic methylation patterns to change the expression of targeted genetic elements (Guha et al., 2017).

Genome editing of type SDN-3 is used to support the targeted insertion of transgenes at specific chromosomal loci and for molecular stacking of multiple transgenes (Ainley et al., 2013; Kumar et al., 2015). Such approaches may be similarly used for targeted insertion of cisgenic or intragenic constructs (AGES, 2013).

Sauer et al. (2016b) and Rivera-Torres and Kmiec (2016) point out that ODM may be simultaneously applied with SDN-techniques to make genome editing applications more efficient. Typically, the rate of sequence change by ODM is quite low, but is substantially increased, when double-strand breaks are introduced in close vicinity to the ODM target site.

Other nGMs, such as agro-infiltration (Vogel, 2012) and/or the use of viral vectors for gene transfer and expression of method related components (Butler et al., 2016; Lozano-Duran, 2016), are used as tools for transient gene expression in plant cells for two different purposes: (i) as a tool to study the effects of expression of a specific gene or genetic construct in a target crop, or (ii) as a tool to express molecules like dsRNAs or site specific nucleases which then initiate the further biotechnological modification of the respective crops, e.g., by RdDM or genome editing. Examples for (i) are e.g., the use of agro-infiltration to study the effects of transgenes involved in fatty acid metabolism (Grimberg et al., 2015), other examples are provided in Vogel (2016). Examples for (ii) are e.g., approaches for the expression of site specific nucleases as well as of donor DNA constructs required for SDN-2 and SDN-3 applications to initiate genome editing in the target plants (Baltes et al., 2014). Currently methods are developed to use viral vectors for plant modification in the environment, relying on insects to disseminate the viral vectors in the field (DARPA, 2016).

nGMs such as CENH3-mediated haploid induction (HI) were developed for the fast production of homozygous lines from a heterozygous parent without the need for lengthy back-crossing cycles. The method induces the *in vivo* production of haploid offspring from crosses between a haploid inducer line and a wildtype parent. Double-haploid plants containing two identical sets of chromosomes can then be generated from the haploid lines in a second step. Haploid induction can be used to e.g., produce homozygous plant lines from genome edited plants (Gurushidze et al., 2017). However, CENH3-mediated haploid induction could be applied as a general tool to speed up all breeding activities by substituting time-consuming back-crossing steps with the faster HI approach.

As already mentioned, conventional methods are typically used in all nGM approaches. Particular methods, e.g., *in vitro* culturing of isolated plant cells or tissues or protoplast technology, are associated with a different potential for inducing unintended modifications, especially the introduction of random genetic changes unrelated to the intended modifications (Filipecki and Malepszy, 2006).

Specificity of Genome Editing vs. Off-Target Effects

Any method for altering the genetic make up of plants, including conventional breeding, which is not sufficiently specific to induce only the desired genomic modifications is associated with unintended effects (Ladics et al., 2015). nGM are no exception to this rule, even if some of them, e.g., genome editing, are significantly more specific compared to other methods including GM technology and classical mutagenesis. Recent technical reviews note that different nGM approaches achieve different levels of precision, i.e., specificity of targeting (Agapito-Tenfen and Wikmark, 2015; Hilscher et al., 2017b; SAM, 2017).

Likewise the various types of genome editing are dissimilar in terms of the number of unintended effects due to off-target activity. Some factors which influence the level of off-target activity and thus the precision or rather the efficiency of the particular approach were identified (Yee, 2016; Zhao and Wolt, 2017). According to Yee (2016) off-target activity depends on

(1) the frequency of homologous sequences in the genome
(2) the characteristics of the specific nuclease type
(3) the expression level of the nuclease
(4) the time span for which the nuclease is present in the target cell
(5) the accessibility of the homologous sequence and of any potential off-target sequences in the chromatin.

The accessibility of DNA genomic regions to some nucleases used in genome editing, especially to MNs, ZFNs, and TALENs, depends e.g., on their specific methylation pattern (Guha et al., 2017). Other factors influencing off-target activity are explained in the following.

In recent years CRISPR-nuclease variants with enhanced specificity were developed to reduce off-target activity, such as a modified, high-fidelity Cas9 or nucleases from other bacteria with an intrinsically higher specificity, e.g., Cpf1 (Kleinstiver et al., 2016; Zhao and Wolt, 2017). Unwanted off-target activity could be reduced through transient expression of nuclease components and by expression at reduced levels and in specific cell-types or developmental stages (Yee, 2016). Also various other methods are developed to limit the activity of SDNs in target cells, including the use of inducer or repressor molecules to control the expression or activity of the respective nucleases (Pawluk et al.,

2016). Furthermore, fewer off-target changes occurred when functional nuclease molecules were preassembled and directly introduced into recipient cells, instead of delivering SDN-components as genetic constructs (Guha et al., 2017; Hilscher et al., 2017b; Liang et al., 2017).

Different approaches may be used to limit the off-target activity of SDN-mediated genome editing. First developers can select and apply suitable methods with a high level of specificity taking into account the above mentioned factors. Furthermore, off-target activity can also be influenced by the choice of the specific genomic target sequence, e.g., by selecting target sequences which display a low homology to other genomic sequences, in order to limit the number of unintended binding sites throughout the respective plant genome.

Bioinformatic tools and special software help to predict genomic target sites and design suitable SDNs described in Kanchiswamy et al. (2016) and Zhao and Wolt (2017). There are concerns, however, that such *in silico* screening/identification for off-target sites may not reliably identify all *in vivo* off-target sites. Thus, for genome editing of animal cells new approaches have been suggested (see e.g., Akcakaya et al., 2018), which may be also employed for genome editing to modify plants. In addition calls have been issued to also consider and investigate potential target sites with lower cutting probabilities (Chakraborty, 2018).

A suite of *in vitro* methods is available to identify sites of potential off-target activity in the genome; some of them, including Genome-wide, Unbiased Identification of DSBs Enabled by Sequencing (GUIDE-seq), High-Throughput Genomic Translocation Sequencing (HTGTS), Breaks Labeling, Enrichments on Streptavidin and Next-Generation Sequencing (BLESS), and Digested Genome Sequencing (Digenome-seq), can provide unbiased whole genome screens for such sites (Kanchiswamy et al., 2016; Zischewski et al., 2017). Additionally the final genome edited plants can be checked with whole genome sequencing and biochemical methods for potential off-target modifications (Zischewski et al., 2017). However, testing by whole genome sequencing may be constrained by technical limitations, e.g., if sequence information from repetitive sequences cannot be obtained (SAM, 2017). If adequate reference genomes are not available additional efforts to generate whole genome data from the parental line are required to conduct the comparison to identify unintended sequence changes.

In recent years a number of genome editing applications in plants were checked for off-target changes. Hilscher et al. (2017b) concluded that overall levels of untargeted mutational changes throughout the plant genome were not elevated. However, their review included several reports that identified off-target edits at genomic locations which were very similar to the target sequence (see Hilscher et al., 2017b). Another report noted unexpectedly high off-target activity (Zhang et al., 2016b). Furthermore, recent research has shown that assumptions regarding the level of specificity associated with a particular SDN may not always hold true. In a specific case a modified Cas9 nuclease with less stringent requirements for matching a specific protospacer adjacent motive (PAM) unexpectedly displayed a higher overall specificity (Hu et al., 2018). Recent reports from genome editing experiments in mammalian cells indicate that significant numbers of larger deletions were caused by CRISPR/Cas9-mediated genome editing using different methods, including stable transformation with SDN-expression constructs, transient expression of CRISPR/Cas and transfection with functional CRISPR-Ribonucleoprotein complexes (Kosicki et al., 2018). In addition to genetic modifications at target sequences different kinds of secondary modifications (point mutations, indels, deletions and insertions) were found at distant genomic loci (Kosicki et al., 2018). It needs to be seen whether these results are also relevant for plant systems. However, it illustrates that assumptions regarding the high degree of specificity of genome editing approaches may not hold true as a general rule. It also underlines that current knowledge concerning prediction and detection of off-target modifications associated with genome editing is still limited and needs to be improved (Wolt, 2017).

Uncertainties that remain regarding the occurrence of unintended effects cannot sufficiently be addressed by a rational design of the methods for genome editing at the time being. Rather developers still have to resort to empirical testing of the efficiency and specificity of different method variants approaches to select methods with a good ratio of on-target efficacy vs. off-target activity, e.g., as described by Kleinstiver et al. (2016). Similarly appropriate approaches for the molecular characterization of nGM plants should be implemented to identify unintended effects during risk assessment. The results can then be addressed by a targeted phenotypical assessment to determine the significance of the unintended effects identified. The existing principles for risk assessment established for GMOs provide a general framework for this. However, specific guidance for this approach is needed, but not yet available.

Depth of Intervention

Genome editing applications of SDN-1 type introduce small sized, random sequence changes or even point mutations at targeted genomic locations. Due to the characteristics of the changes introduced by SDN-1 applications, they were compared with plants carrying spontaneous mutations or plants produced by classical mutagenesis (Pauwels et al., 2014). However, spontaneous mutations and classical mutagenesis are neither directed nor targeted. Both widen the genetic diversity of plants in the first step and then breeders select plants with desired phenotypical modifications in a second step. As outlined below, certain SDN-1 applications, particularly applications to introduce multiple modifications at different genomic targets, can result in substantial metabolic reprogramming; this is generally overlooked when SDN-1 applications are merely judged by the small extent of genetic change introduced at single target sites.

Analysis of current developments show that several SDN-1 type applications aim to simultaneously introduce modifications (i) into multiple alleles, (ii) into all members of a gene family or (iii) into different functional genes (Khatodia et al., 2016; Paul III and Qi, 2016). This is also called multiplexing (Khatodia et al., 2016; Paul III and Qi, 2016). In particular CRISPR-based systems for genome editing provide a platform to achieve fast and efficient multiplexing in plants or other organisms (Lowder et al., 2015; Qi et al., 2016; Zhang et al., 2016b; Zetsche et al., 2017).

Proof-of-concept studies for multiplexed approaches with different site-directed nucleases were conducted in various crops, including maize (Qi et al., 2016), rice (Xu et al., 2016) and wheat (Wang et al., 2014; Gil-Humanes et al., 2017). In rice up to 21 different target genes were modified in a single step (Liang et al., 2016). In a recent study in wheat 35 different alpha-gliadin genes out of the 45 genes present in a wildtype line were knocked out using a multiplexed approach (Sanchez-Leon et al., 2018). Sanchez-Leon et al. (2018) suggest that multiplexed genome editing approaches can provide a route to develop low gluten wheat, something which has not been achieved by traditional plant breeding and mutagenesis approaches so far.

In the initial phase most genome editing applications addressed single genomic targets, i.e., single genes or all alleles of single genes. However, modifying complex polygenetic traits, like the gliadin content in wheat, requires simultaneous modification of multiple different genomic targets. For a significant number of multiplexed genome editing approaches no comparable products exist, that were developed by other approaches. Conventional approaches were used for such purposes only in few cases, such as a TILLING approach to introduce multigenic powdery mildew resistance (Acevedo-Garcia et al., 2017). Therefore, mostly no history of safe use is available for products of multiplexed applications of genome editing.

Further examples of multiplexed genome editing approaches address environmental stress response, plant development and composition:

- Knockout of transcription factors CBF1/2/3, that directly regulate cold responsive genes in *Arabidopsis* (Zhao et al., 2016; Shi et al., 2017b)
- Targeting of six of the 14 PYL ABA receptor genes in *Arabidopsis* to assess their functional importance e.g., for root elongation and plant growth (Zhang et al., 2016b)
- Knockout of two ALCATRAZ (ALC) homoeologs involved in regulation of seed shattering of mature fruits in oilseed rape (Braatz et al., 2017)
- Knockout of four closely related rice MPK genes essential for rice development (Minkenberg et al., 2017)
- Knockout of three flowering suppressor genes that negatively control the heading date of rice varieties (Li et al., 2017)
- Targeted mutagenesis of the three delta-12-desaturase (FAD2) genes to modify oil composition in *Camelina* (Jiang et al., 2017; Morineau et al., 2017)
- Targeted mutagenesis of the FAD2-1A and FAD2-1B genes to establish soybean varieties low in polyunsaturated fatty acids (Haun et al., 2014).

Chari and Church (2017) assume that the current approaches are only a first step to future large scale engineering of metabolic pathways and improved resistance to disease and environmental stress. They envision the application of extensive, but highly specific multiplexed genome editing in target organisms with the help of template DNAs, either fully synthetic or extensively remodeled by MAGE ("multiplexed automated genome engineering") in a prior step (Wang and Church, 2011). Until now MAGE was not applied directly to plants.

The phenotypic outcomes of complex multiplexed interventions may not be fully predictable based on currently available information. In those cases further information and testing is necessary, e.g., based on the existing framework of GMO risk assessment. In addition presentations at a recent conference (OECD, 2018) indicated that the overall efficacy of multiplexed editing approaches is still quite low. Low efficacy of approaches however could compromise their specificity and the low relative frequency of unintended changes. The removal of unintended modifications through crossbreeding is more difficult to achieve for multiplexed approaches, since several different modified genes need to be retained in the final breeding product. Thus, a sufficient molecular and phenotypic characterization is required to assess the effects of the genetic modifications on physiological functions. These considerations are not specific for multiplexed genome editing, but apply likewise to all nGM approaches resulting in complex and novel types of outcomes, e.g., modifications that result in manifold changes of gene expression in the respective plants or approaches for *de novo* domestication (see nGM plants with enhanced fitness against environmental stressors and alteration of 491 morphological or reproductive plant characteristics).

Risk Assessment for nGM Crops According to the EU Regulatory Framework

Until the recent ruling of the Court of Justice of the European Union (ECJ, 2018) considerable legal uncertainty remained concerning the regulatory status of nGM applications, genome editing in particular (Jones, 2015b). Consequently it was also unclear whether risk assessment requirements for GMOs according to Directive 2001/18/EC would apply for nGM plants or not.

The ECJ ruled that organisms obtained by mutagenesis are GMOs and in principle subject to the obligations of Directive 2001/18/EC (ECJ, 2018). The Court considered that the risks of the use of new techniques of mutagenesis might prove to be similar to those resulting from the release of GMOs developed by transgenesis. Indeed, many of the risk hypotheses e.g., considered by EFSA for GM plants (EFSA, 2010, 2011) are also relevant for nGM plants with traits directed to increase environmental fitness to abiotic stress, diseases or pests, as well as traits for changed composition and herbicide resistance. The ECJ also referred to the novelty of nGMs, i.e., their lack of a long safety record, and their potential to produce GMOs at a significantly faster rate compared with methods of conventional mutagenesis. The Court's ruling is based on a legal analysis of the current regulatory framework in the EU, i.e., Directive 2001/18/EC. It concludes that applications of genome editing should undergo a premarket risk assessment and be subject to risk management as appropriate.

The court ruling was met with quite some astonishment and policy makers were called to amend Directive 2001/18/EC to exclude genome editing applications from regulation (Purnhagen et al., 2018; Urnov et al., 2018). Preliminary proposals toward this have already been submitted by the Netherlands, but have been met with mixed enthusiasm and support. Therefore, it remains to be seen whether amending Directive 2001/18/EC will happen

in the near future. From a risk assessment point of view excluding any genome editing approach from biosafety regulation right now would have significant consequences for the standard and quality of assessment which is provided for these applications: Other sectoral EU regulations which apply to all agricultural and food products, among others the EU Novel Food Regulation No. (EU) 2015/2283 or the regulatory requirements for registration of plant varieties in EU or national catalogues, fail to provide for a breadth and standard of risk assessment comparable with the requirements according to the respective biosafety frameworks (Spranger, 2017; Eckerstorfer et al., 2019).

Toward a Case-Specific Framing of Risk Assessment

At present risk assessors and regulators face a number of challenges when considering which specific biosafety issues need to be addressed for nGM applications.

One major challenge is that the fields of nGMs in general and genome editing in particular are complex and rapidly developing. The overall range of such nGMs is very broad and is expanding rapidly. The various methodologies used for crop modification aim at different breeding objectives and thus result in products with significantly different traits and characteristics. A common risk assessment framework for all nGM plants therefore needs to take into account the range of methods used and the range of traits introduced. Not all plants developed by a particular nGM approach will be associated with a similar level of risk. Consequently potential risks of a nGM plant have to be considered in a case-specific manner, taking into account the characteristics of a particular nGM approach and the nature of the developed traits (SAM, 2017).

Certain nGMs such as reverse breeding are applicable to a limited range of plant species only and help to exploit the genetic diversity available rather than to generate genetic variability (Schaart et al., 2016). Other nGMs like genome editing can be applied very broadly to all major annual crops and forest trees, and their respective genomes can be specifically targeted to introduce a variety of different traits. At present, the range of possible new traits and the crops that can be targeted seem to be constrained mostly by the limited knowledge of functional genomics and crop biology (Scheben et al., 2017).

The level of risk associated with a certain nGM plant depends significantly, but not exclusively on the effects of the modified trait(s) on the overall characteristics of the modified plant species (Duensing et al., 2018). With regards to the effects of the modified traits the risk assessment needs to consider intended effects, as well as any unintended or unforeseen consequence of the expression of these modified trait(s). Three categories of nGM plants can be distinguished with respect to the target traits:

(1) nGM plants with trait(s) which are related to traits occurring in crops produced by conventional approaches and are used without adverse effects for comparable purposes. Typically these nGM plants will not contain non-native genes or genomic changes, that are not yet present in cultivated populations of the plant species (Schaart et al., 2016). Several examples for this category are available, including herbicide resistant plants, plants with altered composition and plants resistant to e.g., fungal pathogens. The experience available with conventional plants harboring comparable traits can be used to judge whether plausible risks due to the specific traits may be expected.

(2) nGM plants with traits similar to those established in GM plants, e.g., herbicide resistance, disease resistance or insecticidal traits. For this category of nGM plants similar approaches for risk assessment to those implemented for the respective GMOs should be applied. Previous experiences with the assessment of such GMOs should be taken into account for the development of risk assessment approaches specifically adapted to the characteristics of nGM plants.

(3) nGM plants with traits which could not yet be established by conventional or other biotechnological methods. This category contains only novel, i.e., new and untried, traits developed by nGMs, e.g., through multiplexed approaches of genome editing resulting in complex physiological changes.

Our review of the available literature indicates that a wide range of nGM plants with novel traits is currently being developed for future agricultural use. Typically prior knowledge regarding safe use of these nGM plants is insufficient and the available information related to physiological functions of the modified genes and the effect of the specific modification(s) may be very limited.

Some of the novel traits will be based on multiple genetic modifications with possible complex impacts on metabolism and phenotype. Emerging methods, e.g., for multiplexed genome editing, simplify the rapid and simultaneous modification of multiple genome targets. Multiplexing increases the range of phenotypic changes that can be achieved at once, but also the depth (i.e., the extent) of molecular and physiological intervention. The present capacity of other biotechnological or conventional methods to achieve similar outcomes is limited. Typically no history of safe use is available for nGM applications and that increases uncertainty as to whether unintended effects may be associated with a particular application. Thus, the novelty status of traits developed with nGMs is a crucial factor regarding the risk assessment of nGM plants (HCB, 2017).

However, possible risks are not restricted to nGM plants with novel traits. Experience with either conventional HR plants or GM plants indicate that plausible risk hypotheses may also apply to many of the nGM plants currently being developed to express traits that are not novel. Two examples illustrate the range of environmental risks: (i) In the case of resistance of nGM plants to abiotic stress, e.g., drought (Zhang et al., 2016a; Shi et al., 2017a) or salinity (Duan et al., 2016), possible environmental risks related to the outcrossing of such traits into related species need to be addressed; (ii) In the case of HR nGM plants compositional changes through herbicide application as well as cocktail mixes of pesticide residues need to be assessed for food and feed safety while indirect risks related to e.g., changes in weed management need to be addressed in terms of environmental safety.

The following aspects should be considered for the case-specific framing of a risk assessment of nGM plants, no matter whether the trait is novel or known: (i) the

knowledge available for the targeted genomic locus and the impact, (ii) of the (genetic) modification, and (iii) of the expression of the modified trait on the physiology and phenology of the nGM plant. Our findings indicate that very diverse cellular mechanisms and functional pathways are involved in different groups of nGM applications: (1) HR plants, (2) plants with resistance to diseases, (3) plants with changed composition, and (4) plants with increased resistance to environmental stressors and altered morphology or reproduction. Significant differences concerning relevant risk issues also exist between individual applications in those groups. The level of new information required to assess the respective issues should consider the extent of scientific knowledge and experience available for the specific nGM plants and traits.

It is doubtful, that the overall experience with traits derived from classical mutagenesis can provide a safe history of use for all novel traits developed e.g., by SDN-1 applications. It is reassuring that in the past no plant safety issues emerged for the mutants developed by classical mutagenesis (Duensing et al., 2018). However, this conclusion cannot simply be extrapolated to all SDN-1 traits, because, on the one hand, a fair number of these traits are novel, and on the other hand, adverse effects may not always be selected out during further crossbreeding steps and selection—steps which are indispensable in applications of classical mutagenesis. Without having analyzed possible effects caused by a particular genetic change a general assumption of safety for all SDN-1 applications lacks a robust scientific basis.

Novel traits may be developed in a very specific manner, e.g., by genome editing approaches. However, it should be noted that the level of specificity of an nGM approach *per se* does not provide an adequate measure of the level of risk associated with the respective trait.

On the other hand the level of specificity should be considered during the assessment of unintended effects related to the methods employed. Again, the specific characteristics of the respective nGM methods (i.e., how they work and at which stage they are used) as well as their level of specificity have to be considered in a case-specific manner. The need for such an approach is illustrated by the spectrum of available methods for genome editing, including ODM and the many different applications of the CRISPR-system. As mentioned, these methods introduce different modifications including (i) small random mutations at specific genomic loci (SDN-1), (ii) directed, but typically small sequence changes at specific genomic locations (SDN-2 and base-editing), and (iii) targeted insertion of exogenous genetic constructs and transgenes (SDN-3). In addition, specific epigenetic changes can be achieved by modifying the methylation pattern. Different levels of off-target activity and different outcomes are associated with the different approaches. Even if the number of off-target mutations may be lower for genome editing approaches compared to some approaches for random mutagenesis, especially when disregarding subsequent screening and breeding steps, they should not be neglected. A case-specific analysis of off-target activity can provide useful indications whether potential adverse outcomes may be expected (Zhao and Wolt, 2017). This approach should not just rely on predictions by bioinformatics, since these tools might not be robust enough yet (Cameron et al., 2017; Zischewski et al., 2017). Additional analytical testing is required and a range of approaches is available for focused as well as unbiased genome-wide assessment (Agapito-Tenfen et al., 2018).

Schemes to develop nGM plants typically involve a combination of different technologies. Most nGM approaches also involve GM technology at certain (intermediate) steps and/or techniques of cell and tissue cultivation and regeneration, e.g., protoplast technology, which cause an elevated level of random genetic change (Wolt, 2017). Therefore, genome editing approaches should not be solely judged by the specificity of their mechanism, e.g., the characteristics of the used type of site-directed nuclease. On the contrary, a comprehensive view is required to consider the potential of the overall development process to either induce unintended genetic changes or to remove unwanted mutations during downstream steps. Some nGMs like genome editing can speed up breeding processes significantly, e.g., by direct modification of elite lines, which in turn can impair the likelihood to detect and remove those unintended genetic changes, which are not genetically linked to the intended modification, when the final product is established.

In our opinion a general assessment framework should be implemented for nGM plants, which is addressing the characteristics of each particular nGM plant, its traits and the consequences of unintended effects. It would incorporate the following elements, some of which are recommended to be used in a case-specific way by other authors as well (Huang et al., 2016; Ricroch et al., 2016; HCB, 2017):

- Case-specific risk assessment requirements, which take into consideration the nature of the developed traits, unintended consequences of the introduced modifications, the available experience with comparable products and relevant protection goals specified by the respective countries.
- Appropriate molecular characterization, to assess among other things whether any transgenic inserts are unintentionally present in final nGM products and to determine the presence of off-target modifications and other unintended genetic changes, which might result in adverse phenotypic effects.
- Phenotypic characterization to specifically test parameters related to plausible risk issues associated with particular nGM plants, that are not covered by other existing legislation applicable to nGM plants [e.g., plant variety registration, food safety, and others (see Spranger, 2017)].

For a robust characterization of unintended effects in nGM plants we recommend that risk assessors apply a 10 step approach as proposed and outlined in **Box 1**. The outlined steps are based on considerations discussed in more detail throughout this study.

The existing regulatory framework in the EU for GMOs includes requirements for a scientific risk assessment conducted by EFSA (Agapito-Tenfen et al., 2018). The currently applied assessment approach is based on a case-specific problem formulation according to the principles and the general process laid out in Directive 2001/18/EC (EFSA, 2010).

BOX 1 | Proposal for a 10 step approach to characterize nGM plants regarding unintended effects.

Steps 4–6 are specific for genome editing applications; the other steps are relevant for all nGM applications.
For the development of concrete criteria for the risk assessment of nGM plants these points need to be further elaborated based on the emerging knowledge and state of the art of analytical methods.

(1) Consider the specific characteristics of the applied nGM approach, including method particulars and the targeted plant species, to check whether it is known for a potential to induce unintended changes. This should include but not be limited to off-target activity. In the case of genome editing applications consider if the particular biotechnological process has been optimized for precision, i.e., to result in a low level of off-target activity.
(2) Check, if information is available from previous use of comparable approaches which is indicating a certain potential for unintended changes/off-target activity.
(3) Assess the probability that genetically unlinked unintended modifications will be removed by crossbreeding used to develop a final product. This assessment should be based on the breeding history of the final product.
(4) Use robust bioinformatics tools to predict potential sites for off-target changes in the reference genome of the respective plant species, if available. In case no adequate (reference) genome sequence data is available, use a whole genome sequencing approach to check on actual off-target modifications (see point 6 below).
(5) Apply the available suite of *in vitro* test methods to identify a "superset of potential off-target cleavage sites" for a particular genome editing method (Akcakaya et al., 2018). This allows to check on the quality of the bioinformatics-based prediction of potential off-target sites. Consider if *in vitro* testing identifies potential activity at sites that are non-homologous to the genomic target sequence and thus not included in the prediction by bioinformatics tools.
(6) Based on the above and a wider set of potential off-target sites, use targeted sequencing to detect actual off-target changes at the predicted genomic loci. Use targeted sequencing also to assess the genomic region which is genetically linked to the desired modification(s), i.e., in the (wider) vicinity of the target sequence for the intended changes.
(7) Use whole genome sequencing to scan for unintended changes in a non-biased way in case unlinked modifications have not been removed and the used method protocols are not optimized for high specificity or the method is known to be associated with off-target activity and no robust prediction of off-target activity is possible. The appropriate comparator is the genome sequence of the parental plant line which was subjected to modification by nGM approaches.
(8) Assess whether any unintended changes might be of functional biological relevance. Consider if unintended changes might result in non-conservative nucleotide exchanges in coding sequences. Additionally consider whether unintended sequence changes might impact the regulatory function of the modified sequence.
(9) Check whether it is possible to assess the significance of unintended changes in terms of biological effects. The following information might be helpful for such considerations: reference sequence data, further sequencing information from different plant lines to assess the degree of natural variability of a particular genomic sequence and annotations of the functions of specific genomic sequences.
(10) Targeted or untargeted phenotyping should be used to assess the possibility of adverse effects resulting from unintended modifications/off-target effects. In particular such an assessment should be required in case fast-track approaches are used to develop the final product (e.g., modification of elite lines, few or no crossbreeding possible or applied following the modification step). Such assessments should also be required in the case potential phenotypic effects are associated with identified unintended sequence changes which cannot be readily removed from the final product by crossbreeding (cf. results of points 4, 8, and 9).

The recent ruling of the European Court of Justice confirms that in the EU plants developed by genome editing approaches are covered by existing biosafety legislation, in particular Directive 2001/18/EC, and are thus subject to the requirements for a premarket risk assessment according to the comprehensive general framework outlined in the directive (ECJ, 2018). If GM technology is involved in the development process of other nGM plants, similar risk assessment requirements apply.

EFSA has already conducted an initial evaluation for some nGM applications, i.e., plants developed through cisgenesis, intragenesis, and SDN-3 type applications of genome editing, as to whether and how specific risk issues should be considered for such nGM plants (EFSA–Panel on GMOs, 2012a,b). These studies should be revisited and used as input to develop robust risk assessment approaches for such applications. Similar evaluations need to be conducted for all nGM applications included in the ruling of the ECJ, particularly for emerging technologies like CRISPR-based genome editing which can be applied in many ways and with many variants. The experience available with risk assessments for nGM products according to the existing worldwide regulatory frameworks for biosafety should be taken into account during this exercise. However, at present the experience with such assessments is quite limited (Wolt, 2017), partly due to the decisions of a number of countries not to regulate some nGM plants (Waltz, 2018). Against this background of limited knowledge and experience we recommend that a case-specific risk assessment is conducted for nGM plants to address all relevant risk issues accordingly. Our technical analysis is thus in agreement with the outcome of the ECJ ruling.

CONCLUSION

A broad range of nGMs including genome editing is currently available and further methods allowing complex modification of plants are rapidly being developed. They are used to develop nGM plants with different traits and characteristics, which will be associated with different levels of risk. With respect to intended traits three categories of nGM plants can be distinguished (apart from further considerations regarding e.g., crop type, purpose of application, and use, etc., that have to be taken into account additionally):

(1) nGM plants with traits and usage known from conventional approaches and without adverse effects
(2) nGM plants with traits known from established GM plants, e.g., herbicide resistance or disease resistance, and associated with comparable risk issues
(3) nGM plants with traits which have not yet been established and thus need to be considered as novel.

Our study shows that nGM applications may be found for all three categories; the same applies for all sub-classes of genome

editing (SDN-1, SDN-2, and SDN-3). Therefore, regulation and risk assessment has to acknowledge that all nGM groups will be comprised of a mix of applications with lower as well as higher uncertainty regarding their level of risk/safety. In addition nGM applications are fairly new and only a few plants developed with these methods have been risk assessed for cultivation purposes so far. Against this background of insufficient knowledge and experience for a variety of applications, we argue that a general framework for biosafety oversight is further implemented for nGM plants, based on a case-specific risk assessment incorporating the following elements:

- Case-specific risk assessment requirements taking into account (i) the nature of the developed trait, (ii) unintended consequences of the modification introduced, (iii) the available experience with comparable products, and (iv) relevant protection goals specified by the respective countries.
- Appropriate molecular characterization to assess among other things (i) the unintentional presence of any transgenic inserts in the final product, and (ii) the presence of off-target modifications and other unintended genetic changes, which might result in adverse phenotypic effects.
- Phenotypic characterization to specifically test parameters related to plausible risk issues associated with a particular nGM plant.

This will require that the existing guidance for risk assessment of GMOs as established in the EU by EFSA be reviewed as to whether it is suitable, sufficient and appropriate for specific types of nGM applications. Specific guidance needs be developed which enables risk assessors to focus their attention and resources on issues of concern specific for the different applications and to use established and emerging tools for their assessment.

With a view to the development of ever faster and ever more complex and sophisticated breeding approaches this will not be an easy task. However, in our opinion the efforts will be worthwhile from a safety perspective and a better alternative to exempting nGM applications from biosafety assessments altogether.

AUTHOR CONTRIBUTIONS

ME conducted the study and drafted the manuscript. MD and MM contributed to data acquisition and analysis. AH, WR, RS, and FW contributed to the study design and implementation and edited the manuscript. All authors read and approved the manuscript for publication.

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Corrigendum: An EU Perspective on Biosafety Considerations for Plants Developed by Genome Editing and Other New Genetic Modification Techniques (nGMs)

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AUTHOR CONTRIBUTIONS

ME conducted the study and drafted the manuscript. MD and MM contributed to data acquisition and analysis. AH, WR, RS, and FW contributed to the study design and implementation and edited the manuscript. All authors read and approved the manuscript for publication.

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Detection and Identification of Genome Editing in Plants: Challenges and Opportunities

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Conventional genetic engineering techniques generate modifications in the genome *via* stable integration of DNA elements which do not occur naturally in this combination. Therefore, the resulting organisms and (most) products thereof can unambiguously be identified with event-specific PCR-based methods targeting the insertion site. New breeding techniques such as genome editing diversify the toolbox to generate genetic variability in plants. Several of these techniques can introduce single nucleotide changes without integrating foreign DNA and thereby generate organisms with intended phenotypes. Consequently, such organisms and products thereof might be indistinguishable from naturally occurring or conventionally bred counterparts with established analytical tools. The modifications can entirely resemble random mutations regardless of being spontaneous or induced chemically or *via* irradiation. Therefore, if an identification of these organisms or products thereof is demanded, a new challenge will arise for (official) seed, food, and feed testing laboratories and enforcement institutions. For detailed consideration, we distinguish between the *detection of sequence alterations* – regardless of their origin – the *identification of the process* that generated a specific modification and the *identification of a genotype*, i.e., an organism produced by genome editing carrying a specific genetic alteration in a known background. This article briefly reviews the existing and upcoming detection and identification strategies (including the use of bioinformatics and statistical approaches) in particular for plants developed with genome editing techniques.

Keywords: genome editing, new breeding techniques, GMO, detection, identification, SDN, ODM

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INTRODUCTION

For a genetically modified organism (GMO) and the derived food and feed products, the European genetic engineering legislation demands event-specific methods for detection, identification, and quantification before they may be authorized and placed on the market[1]. Market releases of organisms generated through random mutagenesis (resulting from, e.g., irradiation or mutagenic chemicals) do not require analytical methods for post-market identification and traceability, because

[1]Directive 2001/18/EC and Regulation (EC) No 1829/2003.

such organisms are exempt from the obligations of Directive 2001/18/EC on the deliberate release of GMOs. In contrast, organisms developed using genome editing (gene editing) are not exempt, as ruled by the European Court of Justice on July 25th 2018[2]. Consequently, the requirements according to the genetic engineering legislation for detection, identification, and quantification apply for these organisms and food and feed derived thereof. Market releases need to comply with the rigorous legal obligations for risk assessment, labeling, and traceability.

EU-authorized "classic GMOs" are detectable, identifiable, and quantifiable by polymerase chain reaction (PCR) methods, which target the stable integration site of "foreign" DNA elements in a genome, as this is a combination that does not occur naturally. Plants produced by the application of new breeding techniques (NBT) like genome editing, however, may lack integrations of any foreign DNA or corresponding genetic elements commonly used in "classic GMOs." The application of genome editing aims to minimize the amount of unintended off-target alterations, and subsequent backcrossing and selection steps help to limit the alteration exclusively to the target site without leaving other permanent changes in the genome (e.g., Wang et al., 2014). As a result, the genome sequence of a genome-edited plant may differ only minimally from its parental one (Zhang et al., 2014; Shin et al., 2016).

Genome editing techniques using nucleases can be categorized into site-directed nuclease systems (SDN) 1, 2, and 3 (EFSA, 2012; Podevin et al., 2013). SDN1 applications rely on the endogenous processes of non-homologous end-joining (NHEJ), which is the most common mechanism to repair double-strand DNA breaks in plants. Since NHEJ is an error-prone mechanism, random point mutations frequently occur at the repaired locus (Hsu et al., 2014; Bortesi and Fischer, 2015). Homology-directed repair (HDR) is an alternative repair mechanism, which the cell may apply if a template sequence is available (Sonoda et al., 2006). If this repair template differs by one or a few nucleotides and is otherwise homologous to the autochthonous sequence, the application will be categorized as SDN2 (EFSA, 2012). If longer DNA sequences, which might be of allelic, additional, or foreign origin, are site-specifically integrated into the target genome, this mechanism will be categorized as SDN3 (EFSA, 2012). Oligonucleotide-directed mutagenesis (ODM) does not require the introduction of a nuclease but uses a synthetic single-stranded oligonucleotide, which is complementary to the target sequence, to introduce precise, site-specific modifications of one or a few nucleotides by the cellular mismatch repair mechanism (Mohanta et al., 2017).

As compared to plants generated *via* conventional genetic engineering, the detection of plants obtained by the application of NBTs poses a couple of new challenges. These plants may not contain foreign DNA such as the commonly used cauliflower mosaic virus (CaMV) promoters and terminators (e.g., CaMV P-35S or T-35S). NBTs, including genome editing, offer the possibility to alter the nucleotide sequence specifically. The modifications are often as small as the substitution, insertion, or deletion (indel) of only a single nucleotide.

If genes coding for the genome editing components, e.g., the site-directed nucleases, are stably integrated into the genome of the recipient, the initially regenerated plant will contain foreign DNA. Through subsequent crossing and selection, at best, the locus harboring the integration will be segregated out completely. Then, the offspring used for further breeding will contain the intended genome-edited modification but will not harbor the foreign DNA (null-segregant). Alternatively, genome editing through vector based, transiently expressed nucleases and guide RNA may be applied (Zhang et al., 2016). If transgene-free genome editing is applied by introduction of transcription activator-like effector nuclease (TALEN) proteins or preassembled Cas9 protein-gRNA ribonucleoproteins into cells, no allochthonous DNA will be used and can be expected in the organism at any time (Woo et al., 2015; Metje-Sprink et al., 2018).

German governmental research and regulatory institutions published a scientific report on NBT in plant and animal breeding and their application in the area of nutrition and agriculture[3]. Here, we report the findings concerning detection and identification of genome-edited plants. We focus on whether or not

1. modifications of a plant genome can be detected analytically (detection of a specific sequence);
2. it is analytically possible to prove that a given sequence modification was induced by genome editing or any other specific technique (identification of the process); and
3. a plant generated through genome editing can unambiguously be identified (identification of the genotype).

Evaluating the different methods in this article needs to clarify a main characteristic of plant samples: A sample might be homogeneous, i.e., consisting only of a single genotype, or heterogeneous, i.e., a mixture of various genotypes. *A priori*, it cannot be decided whether a sample taken from a commodity is homogenous or heterogeneous. If it is essential to analyze a homogeneous sample in order to identify a distinct genotype, a single plant has to be tested.

ANALYTICAL METHODS FOR THE DETECTION OF SPECIFIC SEQUENCES

Various analytical tools are well established and routinely used for "classic" GMO detection. In the following sections, these tools are considered for the applicability for detection of genome-edited plants.

DNA Amplification-Based Methods

The most common method applied to analyze a locus of interest (e.g., a known genome-edited DNA sequence) is PCR. It requires the knowledge of the target DNA sequence of the modified

[2]https://curia.europa.eu/jcms/jcms/p1_1217550/en/

[3]https://www.bvl.bund.de/DE/06_Gentechnik/02_Verbraucher/Bericht_Neue_Zuechtungstechniken/gentechnik_Neue_Zuechtungstechniken_node.html;jsessionid=B1C3F310A72AB446ADAE015D0AC88497.1_cid350

locus and applies complementary oligonucleotides as primers and a polymerase for cyclic DNA amplification. A large number of standardized reference PCR methods for detection of transgenic constructs and of classical GMOs is available[4,5] and might be adapted to genome-edited plants.

If a known insertion is present, PCR-based methods will be state-of-the-art. PCR-based methods are highly specific and sensitive. Based on the experience from GMO testing, it should be feasible to establish event-specific PCR methods targeting larger nucleotide sequence changes induced by genome editing (for example SDN3). Short sequence changes (substitutions or indels of one or a few nucleotides) induced by SDN1, SDN2, or ODM should also be detectable using a specific probe, for example, TaqMan real-time PCR or digital PCR (Stevanato and Biscarini, 2016). Single nucleotide polymorphism (SNP) genotyping approaches can be used to detect very small sequence differences of one or a few nucleotides, provided an adequate reference sequence is available (Huggett et al., 2015; Broccanello et al., 2018). For heterogeneous samples, it was shown that an optimized SNP assay based on digital PCR can detect one mutant within up to 100,000 wild types (Jennings et al., 2014). However, it is questionable whether it will be feasible to develop a robust and specific PCR-based quantification assay for the presence of genome-edited material that is applicable for routine testing of, e.g., composite food samples at the EU-regulative decision levels of 0.9 or 0.1% of genetically modified material (Emons et al., 2018).

DNA Sequencing-Based Methods

Conventional chain termination (Sanger) sequencing will be suitable for the *targeted* detection of known sequences even if the modifications are small. Especially from homogeneous samples, the altered locus can be amplified and sequenced. It might be unsuitable for heterogeneous samples, but massive parallel sequencing of a specific locus using next generation sequencing (NGS), so-called targeted deep sequencing, is a feasible approach for food and GMO analytics and might be adapted for genome-edited plants (Fraiture et al., 2015; Staats et al., 2016). Efforts and costs for detecting (and quantifying) a known genetic sequence difference can be significantly reduced as compared to whole genome sequencing (WGS).

WGS is increasingly used as an analytical method, including for GMO detection (Wahler et al., 2013; Pauwels et al., 2015; Holst-Jensen et al., 2016). WGS requires no prior information on a specific genetic alteration and can be applied as an *untargeted* detection approach for unknown alterations. NGS platforms can produce millions of small DNA sequence reads in parallel, which need to be processed and compared to some reference using bioinformatics pipelines. Therefore, an adequate reference genome sequence for the respective plant is an indispensable prerequisite for the analysis. The reference genome should be derived from the parental plant, as substantial sequence differences are to be expected even between different lines of the same species, different ecotypes, and between the offspring of one parental plant (Ossowski et al., 2010; Zapata et al., 2016).

Furthermore, the application of WGS is increasingly challenging the larger the genome in question is and the more repetitive sequences are present in the genome. This applies for a variety of crop plants, e.g., the genome of the allohexaploid common wheat (*Triticum aestivum*) (Feldman and Levy, 2012). WGS might find its limitations if applied for the analysis of heterogeneous or contaminated plant samples.

If generated sequence data reveal foreign DNA sequences, it is likely that the genetic modification was introduced intentionally either by genome editing (SDN3) or conventional genetic engineering[6]. However, detected sequences derived from other species need to be carefully evaluated, and their integration into the genome needs to be verified. WGS may generate sequence information not only from the target organism but also from a wide array of contaminants, endophytes or pathogens.

DNA Hybridization Assays, Protein- and Metabolite-Based Methods

There are a number of alternative analytical approaches (e.g., Southern Blot, DNA Microarrays) that are used to characterize a GMO, but these are of minor relevance for the detection of genome-edited plants (Lusser et al., 2011). DNA hybridization assays generally require a large amount of genetic material and have a comparably low sensitivity. Their specificity also depends on the length of the modification. Therefore, they can only be considered for the (targeted) detection of longer altered nucleotide sequences and/or integrated foreign DNA. From our perspective, they are unsuitable for the detection of small or single nucleotide differences.

Protein-based methods such as immuno-based assays (e.g., ELISA) are applied for "classic" GMO detection (e.g., the transgenic gene product). In addition, mass spectrometry (MS) methods such as MALDI-TOF are available (Lusser et al., 2011). However, alterations detected *via* protein-based approaches need to be confirmed by subsequent DNA analyses.

Metabolite-based methods employing chromatography in combination with mass spectrometry (GC-MS, LC-MS) and nuclear magnetic resonance (NMR) are routinely used for the detection and identification of a broad range of substances. They may allow to detect qualitative differences in a (genome-edited) plant metabolite profile and to identify specific substances, if the analyzed sample is homogeneous, unprocessed, and assuming an appropriate reference is available (Lusser et al., 2011; Frank et al., 2012; Kumar et al., 2017). However, their potential as a detection method is considerably limited because the metabolite pattern is highly dynamic and fluctuating in response to developmental and environmental conditions (Verma and Shukla, 2015). Hence, a detected difference in the metabolite profile is no proof of a genetic modification but merely a hint. Therefore, metabolite-based methods might

[4]http://gmo-crl.jrc.ec.europa.eu/gmomethods/
[5]http://www.euginius.eu/euginius/pages/home.jsf

[6]The integration of nucleic acid sequences of foreign organisms can, albeit very rarely, also occur naturally, as seen in the sweet potato, which was shown to contain *Agrobacterium* genes (Kyndt et al., 2015).

serve as a tool for screening, e.g., for known metabolites specifically produced through the application of genome editing, but any findings need to be confirmed by subsequent DNA analyses.

CONSIDERATIONS FOR THE IDENTIFICATION OF THE PROCESS

After the detection of a specific sequence that is different to the reference, it needs to be clarified whether this sequence occurred naturally or whether it was likely introduced by a genome modification technique. To our knowledge, the application of conventional mutagenesis techniques, such as irradiation or mutagenic chemicals, as well as genome editing applications do not leave specific imprints in the genome. Even for the conventional genetic engineering techniques, it may be impossible to unequivocally identify the specifically applied technique for the integration of foreign DNA, e.g., *Agrobacterium*-mediated or biolistic transfer.

Current analytical strategies allow assessing the similarities between sequence data. They do not allow determining how a sequence alteration was introduced – by genome editing (targeted mutagenesis), classical (untargeted) mutagenesis, or whether it occurred spontaneously. This is in line with the report "New Techniques in Agricultural Biotechnology" of the European Commission's Scientific Advice Mechanism (SAM, 2017). If the developer describes how an alteration was induced, then it can obviously be linked to the applied technique.

In case the genes coding for the genome editing components are absent, it cannot be deduced from the altered sequence which specific process has been used. For this reason, it cannot be distinguished between conventional genetic engineering and genome editing. We will therefore use the term "genome modification" in the following. However, bioinformatics and statistical considerations might help to evaluate whether a detected sequence was potentially introduced by genome modification.

Bioinformatics

Generally, mutations in genomes of living cells are probably the result of repair mechanisms that are known to be error-prone (Manova and Gruszka, 2015). Many studies have been published to profile the changes that can arise from this natural phenomenon (Salomon and Puchta, 1998; Puchta, 1999; Kirik et al., 2000). Li et al. (2016) published that WGS data of 41 rice plants sequenced a few generations after damaging their DNA with ionizing radiation and their parental plant. An evaluation of these data showed that deletions were more frequent and (on average) larger than insertions (**Figure 1**). This observation is consistent with what is known about the mechanisms of DNA repair (Puchta, 2005). Insertions larger than 26 bp were not observed, but 15% of the detected deletions were larger than 25 bp. Further studies on rice and *Arabidopsis thaliana* report similar results after induced random mutagenesis (Hirano et al., 2015; Li et al., 2016; Du et al., 2017).

However, considerably longer deletions were observed as well (**Figure 1**)[7]. In addition, introgression lines harboring chromosomal or segmental substitutions or additions are further examples of long insertions and deletions (Rabinovich, 1998). For this reason, it is impossible to identify the applied technique purely based on the length of a detected indel.

Statistical Considerations

Lusser et al. (2011) used a simplifying calculation to estimate the minimum length of a unique random sequence in a genome by correlating the genome size with the possible number of combinations for this sequence length. The report of Lusser et al. "assumed that in the case of a plant genome, information on a DNA sequence of at least 20 nucleotides is needed to be in a position to consider a certain DNA sequence as unique and to identify it as the result of a deliberate genetic modification technique." This estimation exclusively applies to insertions of a sequence of the given length.

In a similar way, the genome sizes of several plant species for the estimation of the length of a sequence which can be statistically considered as unique has been compiled in this paper (**Table 1**). The probability calculations show that a sequence of 14–17 bp, depending on the genome size of the respective organism, is theoretically expected to be unique. These estimations are based on the simplifying assumption that the four bases are equally distributed and occur statistically independent. However, the complexity of the altered sequence, the amount of repetitive sequences, and the diversity of the genomes within a species are not taken into account.

Only an insertion of a larger sequence, for instance, of a transgene inserted by SDN3, might provide information that can be used for the analyses of its origin. In case a sequence from a different species is detected *via* WGS, it was most likely intentionally introduced into the analyzed genome[6]. If a construct of consecutive foreign genetic elements (e.g., a combination of promoter, coding sequence, and terminator from different species) is identified, it will indicate the application of a genetic modification technique. Search packages like BLAST (Altschul et al., 1990) or k-mer based tools like NIKS (Nordström et al., 2013) can be used to find such DNA sequences within WGS data. Modifications of the foreign DNA, for example, the codon optimization, may impede their identification.

Genome editing techniques can also be applied to introduce targeted mutations of single or a few nucleotides distributed over various loci within one genome (Svitashev et al., 2015; Braatz et al., 2017; Shen et al., 2017). These may be detectable using WGS, but detected alterations need to be evaluated in relation to randomly occurring mutations and considering breeding schemes, i.e., pedigree information and ancestor genotypes.

[7]It should be kept in mind that the publicly available data analyzed here were produced by bioinformatics tools that are not expected to report long structural variants (i.e., 50 bases or more as defined by the Structural Variation Analysis Group).

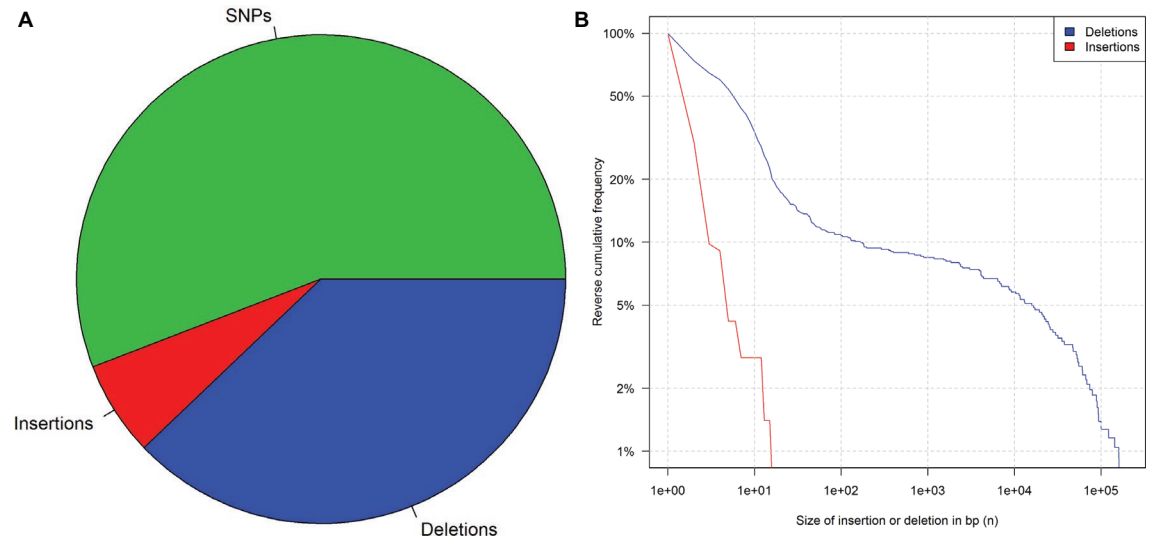

FIGURE 1 | Mutation frequency in 41 rice plants after irradiation and three generations of propagation. **(A)** Pie chart of SNPs, insertions, and deletions. **(B)** Reverse cumulative frequency distribution of indels that are at least n base pairs long. Data from Li et al. (2016), supplementary.

TABLE 1 | Genome sizes of selected (crop) plant species in megabases (1 Mb = 106 bases) (see NCBI, 2018, Sep 6) and the minimal length of a random sequence required to be theoretically unique in a genome of the respective size (simplified assumption purely based on combinatorial possibilities of the four bases within each genome, no other parameters considered).

(Crop) plant species	Haploid genome size (Mb)	Minimum sequence length for theoretical uniqueness in a genome of the respective size (nt)
Arabidopsis thaliana	119.67	14
Oryza sativa	374.42	15
Solanum tuberosum	705.93	15
Brassica napus	976.19	15
Glycine max	1017.57	15
Zea mays	2135.08	16
Triticum aestivum	13916.90	17

The expected increase of available genome sequence information in combination with developments and advances in bioinformatics analyses and experience with genome-edited plants will contribute to the improvement of the reliability of these approaches.

PROBLEMS FOR THE IDENTIFICATION OF GENOTYPES

In this section, the question will be discussed, whether the genotype of a genome-edited plant within a plant sample can unambiguously be identified. If a known sequence that is specific for a genetic modification, e.g., a foreign DNA fragment, can be detected in the sample, then the sample will contain a genetically modified genotype that can be identified.

However, most modifications produced by genome editing are very small, down to the substitution, deletion, or insertion of one single nucleotide, which might also occur naturally in non-genome-edited plants (Fauser et al., 2014; Wang et al., 2014; Jacobs et al., 2015). In such cases, the genotype of a modified plant is almost identical to that of the non-modified counterpart, and accurate experimental genotyping is needed to unambiguously identify the genotype. Here, WGS might be considered useful, but it faces a number of substantial problems, e.g.:

1. If the sample is heterogeneous, the identification of a specific genotype will be hampered by the amount and number of other genotypes in the sample. Furthermore, the amount of natural variation in the sample will blur the analysis. If the fragment length of the WGS approach is too short, the linkage between polymorphisms, either naturally occurring or introduced by genome editing, cannot be deduced and the genotypes cannot be determined. Hence, genotyping a heterogeneous sample does not allow identifying individual genotypes in most cases.
2. Avoiding such problems with heterogeneous plant samples, individual plants need to be investigated. For WGS, sufficient amount of DNA is needed. In case of seed samples, a single plant needs to be grown and probed instead of the seed. To avoid missing genotypes, DNA of several plants has to be isolated and sequenced separately, which increases the effort drastically.
3. A high-quality database of all genotypes of genome-edited plants is needed as a reference to unambiguously identify the unique genotype. However, to our knowledge, there is

no database providing high-quality genotypes of all genome-edited plants at the present. Furthermore, naturally occurring mutations need to be considered when comparing a sampled sequence to the database. Finally, as mutations and recombination occur naturally during each propagation, there will be low likelihood to trace genotypes that are the offspring of genome-edited plants if the offspring is not included in the database.
4. Sequencing bias of the sequencing technology might lead to an underrepresentation of the genomic region of interest.
5. Due to the small size of the modifications, sequencing errors and other bioinformatics problems increase the potential of false-positive predictions in comparison to conventional GMO analytics.

These problems will be further intensified if the genome of the species is large and/or contains redundant sequences, e.g., in wheat or maize. The amount of time needed and the costs incurred to precisely genotype wheat or other plants with larger genomes seems to render analysis of mixed samples or tests for contaminations infeasible.

CONCLUSION

In general, DNA-based procedures are most suitable for the detection of specific sequences in a genome. Without knowledge of the modification, the range of applicable DNA-based methods is limited. PCR requires at least the precise nucleotide sequence information of the locus; thus, PCR cannot be applied if this information is unavailable. Therefore, for the untargeted detection of sequence differences, WGS is currently considered the method of choice, provided an adequate reference genome sequence is available. Once a difference is revealed, this knowledge may be used to develop a targeted (PCR-based) detection method.

Hybridization methods are unsuited to detect very small differences, and the applicability of protein-based and metabolite-based methods for detection is limited. All of them are unsuitable for the routine analysis of commodities.

In contrast to classical genetic engineering, where common or broadly used transgenic elements like typical promoters or terminators combined with a target sequence are used, genome-edited (SDN1 and SDN2, SDN3-based allele exchanges) sites do not carry foreign DNA such as "screening targets," which makes to our knowledge an untargeted detection of unknown genome-edited loci impossible in most cases. This will challenge market surveillance testing of seeds or food and feed products.

In case a genome sequence difference between two plants was detected, it is challenging to decide whether this difference was introduced using genome editing techniques. Provided that several preconditions apply, bioinformatics and statistical approaches can help to estimate the probability whether genome editing was used. For these considerations, the size and the information encoded in this sequence are essential. For longer insertions, the similarity to DNA of foreign species might be an indicator but can be blurred due to codon optimization. In case of any other differences, additional information as for instance pedigree information in combination with genetic information of the ancestors might help. However, if such information is not available, it will be almost impossible to unambiguously decide on basis of purely statistical approaches, whether or not detected sequence variations were caused by genome editing techniques.

The emergence of further reference genomes or pan-genomes might help to handle some of these problems (Emons et al., 2018). However, using the concept of a pan-genome for the identification of specific genome modification techniques is questionable due to sexual reproduction, introgressions, induced mutagenesis, naturally occurring mutations, and other evolutionary processes. Even with pan-genome information available, to our knowledge, it is not possible to decide for a small difference, e.g., a SNP or a short indel, whether it occurred naturally, whether it was introduced by mutagenesis using chemicals or radiation, or whether it was introduced by genome editing.

The genotype of a plant from a homogeneous sample might be identified in specific cases, e.g., in the presence of specific sequences. However, it will be much harder for most practical cases. As mentioned above, the identification of specific genotypes in heterogeneous samples (commodities) demands a number of essential prerequisites which are commonly not given. However, if the prerequisites are met, the analyses will be very expensive and time consuming. All these considerations are based on an appropriate documentation, e.g., origin and pedigree, of the samples that have to be analyzed. Unambiguous detection of hidden admixtures will still be impossible.

DATA AVAILABILITY

Publicly available datasets were analyzed in this study. These data can be found here: https://www.cell.com/cms/10.1016/j.molp.2016.03.009/attachment/ecde2a05-a059-4556-90bd-e1dd7650f003/mmc2.xls.

AUTHOR CONTRIBUTIONS

LG and JK equally explored the core of the topic with regard to detection and bioinformatics methods and prepared the manuscript. All authors contributed equally in the discussion and conclusions, reviewed, read, and approved the manuscript.

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Dealing With Rejection: An Application of the Exit–Voice Framework to Genome-Edited Food

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Genome editing has been hailed as both a revolutionary technology and potential solution to many agriculture-related and sustainability problems. However, owing to the past challenges and controversy generated by widespread rejection of genetic engineering, especially once applied to agriculture and food production, such innovations have also prompted their fair share of concern. Generally speaking, much of the discussion centers on the inadequacy or uncertainty of current regulatory regimes, partly owing to the vastly different approaches in the European Union and United States. Insofar as this focus on regulatory regimes is stimulated by the desire to bridge the divide between proponents and critics of genome editing, it risks losing sight of an essential aim of regulatory action: effectively responding to and fostering trust in consumers and the public. In this article, we thus assign priority to understanding the contours of individual dissatisfaction and its related responses. Toward this end, we apply and extend Hirschman's exit–voice framework to bring together, synthesize, and give much-needed substance to the diverse expressions of dissatisfaction and discontent with novel genome-editing technologies. Through the resulting synthetic framework, we then identify and evaluate which governance approaches can prevent actions seen to be problematic and, moreover, open up the space for a more active public. In this context, we devote specific attention to (i) use of labeling as a means to enable "exit" of consumers from markets and (ii) public deliberation as a possible expression of "voice." Indeed, both options are proposed and utilized in the context of genome editing, e.g., as a way for skeptical consumers to express their viewpoints, seek change in prevailing food systems, and navigate the conflicts and tensions from applying unique sets of values to assess the balance of risks and benefits. So far missing, though, is an evaluation of how well such efforts offer effective means for public expression, which is why we also link this framework to the wider issue of consumer sovereignty. Having done so, we conclude with a brief commentary on the potential and limitations of both options in the existing institutional framework of the EU.

Keywords: CRISPR, exit and voice, food innovation, food labeling, genome editing, governance, public deliberation

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INTRODUCTION

Genome editing has been hailed as a revolutionary technology and the potential solution to many agriculture-related and sustainability problems (Baltes et al., 2017; Zilberman et al., 2018a). The new possibilities offered by genome editing, particularly via novel methods like CRISPR-based systems, however, also entail that existing governance solutions for genetically modified (GM) food are rendered (at least partly) obsolete. It thus becomes unclear how applications of genome editing in the food sector *should* be governed and regulated, or whether any special regulation is in fact necessary at all. Multiple opinions on this subject have already been voiced (e.g., Araki and Ishii, 2015; Huang et al., 2016; Kuzma, 2016; Malyska et al., 2016; Pollock, 2016), and even more in the wake of the recent judgment by the European Court of Justice that "all organisms obtained by mutagenesis," even those resulting from genome editing, are identical in terms of the associated risks to health and environment (ECJ, 2018). Regardless of underlying differences in the process involved, and in contrast to the approach set forth by the relevant authorities in the United States (Waltz, 2016; USDA, 2018), use of any mutagenic[1] technique to alter genetic material "in a way that does not occur naturally by mating and/or natural recombination" is now likely to require the same level of regulatory scrutiny across the European Union (ECJ, 2018).

Herein we find vivid expression of perhaps the central divide in the literature on the regulation of genome-editing products: between so-called product- and process-based approaches. In specific, the former often assigns priority to scientific risk assessments, thereby resulting in an emphasis on the "substantial equivalence" of products derived from genome editing with those engendered by "natural" processes. According to this approach, the prevailing criterion for deeming a product to be safe, whether developed by conventional breeding, genetic engineering, or genome editing, is whether it has a substantially different effect on a range of outcomes, e.g., human health and environmental impact, as compared with products available on the market (OECD, 1993; USDA, 2018). However, if there is no evidence for any such differences, then the products should be viewed identically from a regulatory point of view, irrespective of the breeding approach applied. Recently, in fact, the USDA (2018) (implicitly) upheld the determination of "substantial equivalence," pointing to the fact that no foreign DNA had been inserted as a reason that CRISPR-based systems did not require "special" regulatory oversight.

On the other hand, process-based approaches assign more specific attention to whether there are fundamental differences with explicit respect to the underlying processes themselves. In the case of genome-editing techniques, with CRISPR-based systems currently occupying the cutting-edge here (Brinegar et al., 2017), it is both the greater precision to make changes at a specified location in the target DNA—thus the use of "editing"—and the combinatorial capacity to simultaneously enable many such changes (multiplexing; Barakate and Stephens, 2016) that render them distinct from "conventional" genetic engineering. At the same time, there is quite recent evidence that the CRISPR-based systems might also result in unwanted deletions and complex rearrangements of DNA (Kosicki et al., 2018) and that cells edited using such systems could be more susceptible to cancer (Haapaniemi et al., 2018). Given the growing (scientific) evidence of a connection between such problems and the underlying processes, this provides one argument supporting a more process-based approach (i.e., given that the nature of the effects extends beyond those for changes to a single product or product characteristic). Moreover, it has been argued that such an approach could provide greater scope to better consider issues such as the potential for consumer acceptance, perceived "naturalness" of biotechnology (Hartley et al., 2016; van Hove and Gillund, 2017; Pirscher et al., 2018), or the rate at which modifications occur (ECJ, 2018) when assessing the possible risks. Taking a step beyond the assessment of risks in (controlled) real-world settings, such an approach would highlight the wider relationship between technologies and the social, scientific, and technological contexts in which they would be applied (see Sjöberg, 2002). Currently, such a broad understanding of what constitutes "risk" is rarely taken into consideration within most prevailing regulatory approaches for GM food.

Given the nature of recent developments in the domain of genome editing, and the resulting rise in regulatory uncertainty, a novel analytical framework is necessary to synthesize and reconcile these disparate perspectives. In this regard, this article seeks to venture beyond the extant debate about, e.g., if the regulatory approach in the EU is justified and whether genome-editing should not also be entitled to a "mutagenesis exemption" (Purnhagen et al., 2018). Instead, we highlight that, for better or worse, whenever a country has decided against giving free rein to the products of genome editing and/or genetic engineering, this is prompted by the expressed discomfort and anxiety of large swaths of the general public (e.g., Gaskell et al., 2010; Hess et al., 2016; Cui and Shoemaker, 2018). What is required, as a result, is a deeper engagement with the public, which is itself predicated upon a greater understanding of the contours of individual dissatisfaction and its related responses. To facilitate this, we first bring together, synthesize, and give much-needed substance to the ways in which people express discontent with new genome-editing technologies. And second, through the resulting synthetic framework, we can then identify and evaluate which governance approaches can prevent actions seen to be problematic and, moreover, open up the space for a more active public to express criticism or support. In other words, looking past trade-offs between the potential benefits of genome editing and widespread opposition to genetic engineering, we wish to explore whether facilitating a more active role for the general public in regulatory decision-making may not only improve acceptance, but also partly compensate for the (perceived) inadequacy of current regulatory regimes. Accordingly, we aim to shed light on the ability and opportunities afforded to consumer-citizens to express their discontent with genome-edited food—as well

[1] i.e., the targeted modification of single base pairs in the DNA. In EU law, conventional, non-targeted mutagenesis (via exposure to radiation or acids) is however exempted from GM regulation, notably, owing to the fact that it has been "traditionally used without proven harm for public health or the environment" (ECJ, 2018, p. 3).

as options available to proponents of genome editing and/or regulatory officials wishing to better take consumers' opinions and concerns into account. To achieve this, we apply and extend Hirschman's (1970) exit–voice framework to explore the contours of dissatisfaction and its related responses and then offer insights into a suitable governance approach for genome-edited food products. Hirschman's framework is unique in its potential to illuminate foundations of consumer and citizen engagement with products, producers as well as regulators. Applying the framework to the case of genome-edited food, we give specific attention to the use of labeling as a governance solution facilitating "exit" of consumers from markets, and to public deliberation as an expression of "voice." As such, our analysis is grounded in the actual responses of consumer-citizens toward the potential market introduction of genome-edited food, whether this entails the controversy of GM food or more novel positions related to CRISPR-based systems. Instead of focusing on how acceptance of GM food can be improved (Araki and Ishii, 2015; Kolodinsky and Lusk, 2018), this framework thus institutes a "two-sided" understanding of governance. In specific, we contend that new regulatory approaches, whether in the form of labeling schemes enabling "exit" or deliberative mini-publics promoting a diverse, participatory type of "voice," are crucial for ensuring that public viewpoints and concerns are taken into account in the political and social discussions of genome-edited food.

The article is structured as follows: in section Hirschman's Exit–Voice Framework and Its Application, we provide an overview of Hirschman's exit–voice framework and discuss some applications relevant to food consumption. In section GM Opposition and Genome Editing, we introduce genome editing in the context of opposition toward GM food. In section Current Debate on Governance of Genome-Edited Food, we briefly summarize the current state of the debate on governance of genome-edited food. In section Exit and Voice in the Context of Genome-Edited Food, we apply Hirschman's framework to the case of genome-edited food: after a general discussion of the role of exit and voice in this context, we analyse the manifestations of and preconditions for both. In section Conclusions, we offer conclusions.

HIRSCHMAN'S EXIT–VOICE FRAMEWORK AND ITS APPLICATION

The Exit–Voice Framework

In his seminal book *Exit, Voice, and Loyalty: Responses to Decline in Firms, Organizations, and States* (Hirschman, 1970), the economist and social scientist Albert O. Hirschman enumerated a flexible framework for analyzing the diverse responses of consumers or users to dissatisfaction and discontent with the perceived quality of goods or services provided by private or public institutions. In this framework, a consumer/user has two main options to express dissatisfaction: exit or voice. Exit consists in refraining from consuming the good or service in question by, e.g., switching to a substitute good offered by another supplier. It represents, so to speak, a fundamentally market-based response as a result, and is thus broadly in line with conventional economic theory (Franzini, 2016; John, 2017). Voice, conversely, consists in various forms of expressing one's discontent in a way that directly reaches the producer/supplier, and specifically the management of the firm—whether through petitioning, protesting, lobbying, becoming more generally politically engaged, etc. It is thus a response that is more inherently political and participatory and which can be engaged in collectively or, more generally, "any attempt at all to change, rather than escape from, an objectionable state of affairs" (Hirschman, 1970, p. 30). In addition, this endows voice with a greater degree of flexibility and the ability to modulate how much dissatisfaction is expressed. Depending on the level of discontent, one can alternatively sign on to an petition; canvas directly to elected representatives; knock on doors in one's community to gather support; or sue the government or relevant firm if a problem is deemed sufficiently egregious. By going beyond just "voting with one's wallet," voice thus provides one with the ability to convey more information than would be possible through exit alone.

With regard to the firms involved, or more generally those to whom dissatisfaction is addressed, Hirschman's framework also offers insights for how best to get their attention. In fact, one of the chief advantages of the framework lies in its ability to highlight mechanisms and opportunities available to individuals (as consumers and citizens) to push for changes in products or practices with which they are dissatisfied. First, it is crucial how the focus here lies on exploring the sub-par performance of firms, why this occurs, and how "temporary and remediable lapses" may be resolved. And, indeed, we highlight this phrase for how it signals these problems, as perceived by the individual consumers, to be (implicitly) understood as more or less correctable, assuming one uses the suitable mechanism or leverages sufficient pressure. Decisions about which strategy is most suitable for a given situation—i.e., gauging level of discontent, potential responsiveness of the institution, the number of viable substitutes available, how likely is a restoration of quality, etc.—are therefore crucial. Accordingly, at the center of the relationship between exit and voice we highlight a key trade-off: voice is more preferable when exit is not practical; exit more likely when transaction costs of voice are prohibitively high, or after prior efforts at voice have failed to bring about results. Moreover, though both represent responses to the declining quality of a good or service, the nature of the relationship between them—and thus which one is thus preferred—is likely to vary across contexts.[2] Regarding public services, for instance, the availability of an exit option has actually been shown to foster further deterioration in service quality, even if this is the opposite of what customers intended. This may occur because e.g., the finances of bureaucracies are "insulated" from

[2]Conversely, Hirschman underscores that the dynamics of what he calls the "management reaction function" could conspire to put the firm beyond saving, no matter the level of pressure exerted. Notably, a mismatch between the timing of consumer action and ability of the firm to respond, perhaps due to competition in the relevant sector, could result in the needed feedback coming too late. For instance, if customers are slow to respond to a change in quality, before then doing so *en masse*, by the time management receives this information, it might be too late to engage in the necessary remediation that would stem the tide of customers leaving.

market pressure and not overly responsive to "market signals" or since those customers who opt to withdraw are often the ones with the necessary resources and expertise to ultimately enable change. Consequently, Hirschman (1970) concludes that state monopolies can, surprisingly, often be welfare-enhancing, e.g., if lack of exit options forces one to utilize another strategy to which bureaucracies are relatively more responsive: i.e., voice. Indeed, it is exactly in cases where customers are "locked in" that voice is most likely to be effective, namely as a way to compensate for the diminished reliability of exit. This example also makes clear how the level of competition in a given sector might represent a key determinant of the relative effectiveness of voice vis-a-vis exit: notably, if a customer only has a narrow set of alternatives for expressing her preferences, or the profitability of firms does not depend only on the quality of their offerings, then recourse to exit will likely be hamstrung.[3] In this way, one can also observe the vital role of consumers/customers qua "agents of competition," that is, assuming the necessary conditions are in place for them to perform it. On the other hand, exit and voice can and do complement each other in some circumstances: the threat of exit (instead of its silent realization) can lend powerful support to voice. For Hirschman, this makes exit "a last resort option that individuals do not want to take" (John, 2017, p. 515) in most settings, especially as this may prevent the departing members from reaping the benefits of any of those subsequent improvements in quality that their exit has made possible. Of course, this turns out to be less of an issue in market settings, e.g., the choice to switch from one product to another, where exit is most often "temporary" in nature.

Exit and Voice in Subsequent Literature

In the ensuing decades, a large and diverse literature building upon Hirschman's framework has emerged. The framework has recently been applied to topics as diverse as behavior of farmers' associations in agricultural conflicts (Alpmann and Bitsch, 2015), the responses of communities of football fans to commercialization (Kiernan, 2017), vaccination policy (Geelen et al., 2016), maternal risk anxiety (Smyth, 2017), Euroscepticism in the European Parliament (Brack, 2012), and the persistence of Cuban socialism (Hoffmann, 2005). While Hirschman's own applications (e.g., Hirschman, 1978, 1993) and much of the literature have focused on the decline in "[political] organizations and states," along with a sizeable body of research on exit and voice in the context of public services, relatively little attention has been given to exit and voice as strategies available to consumers (or consumer-citizens) in the marketplace. However, amidst the increasing contestation over food in the public discourse and a diminishing trust in food systems (Murdoch and Miele, 1999; Mazzocchi et al., 2008; Meyer et al., 2012), Hirschman's framework seems highly relevant. In the following, we briefly review a collection of the publications that have applied the exit–voice framework to the context of food consumption.

In spite of the relatively limited number of studies in this setting, the available literature turns out to not only be quite diverse but also to advance various improvements to the original framework. Light et al. (2003) deviate from Hirschman's original focus on *individual* responses to declines in quality and focus instead on "collective manifestations of voice." They distinguish between two types of voice: *vertical*, directed at those responsible for the (perceived) deteriorations in product or service quality, and *horizontal*, directed at others who are "in the same boat" (Light et al., 2003, p. 477). They further note an example for each, namely organized protests and citizen/consumer associations, respectively. In this way, we grasp how the audiences for the two activities differ, with the latter seeking to build consensus among fellow consumers/citizens and the former speaking directly to power. This approach is then applied to early anti-GM protests in the US to underscore, *inter alia*, how voice was essentially the only option then available to consumers. According to the authors, this was explicitly tied to the absence of GM food labeling and, as a result, the absence of the necessary preconditions to render exit effective.

Meanwhile, focusing in particular on the rise in fair-trade certified products and vegetarianism, Newholm (2000) outlines the potential for and limitations of exit and voice as signaling devices available to ethically motivated consumers. Specifically underlining the insufficiency of exit as a "standalone" option for improving food systems, he notes that "peoples" [sic!] preferences cannot simply be read off their purchase behavior in the market' (p. 159). In fact, empirical studies have not been able to establish any direct link between motivations and attitudes, on the one hand, and consumer choices on the other (e.g., Bamberg and Möser, 2007; Grunert et al., 2014) and also that consumer behavior is not a good predictor of political attitudes and behavior (Hamilton et al., 2003). Newholm (2000, p. 161) instead argues that: "Consumer voice on the other hand, far from being unreliable, is the major source of business information," while at the same time stressing that, at least in some cases, it is ethical concerns that are behind changes in buying patterns (that is, exit). In effect, the overall message is that, in the context of consumer ethics, not only are exit and voice both important but, in fact, given their varying strengths and weaknesses, they can be seen to be complementary to a large extent.

Representing a further step in this direction, Keeley and Graham (1991) have further argued that, actually, exit, and voice can each be disentangled into two distinct "values," such that there end up being four possible constellations for responding to decline: *passive acceptance*; *internal change effort*; *quiet exit* and *vociferous exit*. In this way, they lay out not only how exit and voice might work together but also how this functions to diminish some of their respective shortcomings. For instance, they stress that the "trouble with exit, [. . .] is that it permits firms to unfairly externalize system maintenance (feedback) costs by shifting these to exiting individuals—who may prefer (and, in fairness, deserve) a voice" (Keeley and Graham, 1991, p. 353). Informed by an empirical case analysis in the context of environmental risks, Zuindeau (2009)

[3]Indeed, the relative effectiveness of a given option also depends on the type of decision involved, i.e., not only the availability of substitutes but also potential costs of making a wrong choice. On this point, Hirschman (1970, p. 41–42) underscores that "the sheer number of available goods and varieties in an advanced economy favors exit over voice, but the increasing importance in such an economy of standardized durable consumer goods requiring large outlays works in the opposite direction."

similarly proposes an extension of Hirschman's framework that disentangles active from passive responses as well as the implications of differing levels of dissent across groups (some dissatisfied, others satisfied). In Zuindeau's (2009) interpretation, exit can be counterproductive if the "exiteers" are those who had previously offered strong voice; conversely, voice can also be legitimizing and thus similarly undermining criticism. For this reason, he identifies four "key variables" able to influence the viability of exit and voice: the spatial extent of the problem in question; uncertainty around the problem; potential damages; and conflicts of interest involved. Rather interestingly, the last three variables are very similar to some points often stressed in conceptualizations of the precautionary principle, including in the context of GM food (e.g., Stirling, 2017). In fact, one can observe strong parallels between Zuindeau's characterization of "global risk," i.e., its having unlimited spatial area, strong uncertainty, and very high potential damages, and the long-standing conception of DNA technologies in the literature on so-called technological hazards (Fischhoff et al., 1978; Slovic et al., 1985; Slovic, 1987). Particularly noteworthy in this context is Zuindeau's (2009) concept of informational voice, which takes the form of a request for information or a demand for further study and research that would lead to understanding the issue at stake better. In other words, voice ends up being modulated to the point that it is neither expressing a well-defined viewpoint nor seeking an outcome that is particularly clear-cut; rather, it wants to assert, if anything, that there still may be a degree of uncertainty around the underlying science, and perhaps that other "values" may also be required to come to an ultimate determination about its acceptability. In this way, we observe a type of voice that "defers" to the expertise of others, while nonetheless entreating them to take into account a broader perspective than might previously have been the case.

Before we apply those insights to genome-edited food and its governance, we first briefly review the history of GM food opposition and how genome editing differs from older GM techniques. It is crucial to underscore at this point, however, that the case of GM food serves as a probe or lens for exploring the broad category of genome-editing technologies applied in this domain. Instead of assigning undue importance to any one type of technology, we argue in the ensuing sections that there is a "systemic" component to much of the dissatisfaction that is expressed, and thereby rendering such criticism relevant though not necessarily specific to any given technology.

GM Opposition and Genome Editing

There is a long history of public opposition toward genetically engineered food, particularly in Europe, where currently almost no GM food is being produced, or consumed (although it is fairly common to use GM feed in animal husbandry; Zilberman et al., 2018a). Before the advent of genome editing, skeptics focused mainly on environmental and health risks (Pirscher et al., 2018). There are multiple reasons for this. First, early GM techniques were rather imprecise, as it was not possible to determine exactly where a DNA snippet would be integrated into the DNA of the target organism. This thus gave rise to fear of unintended modifications and side-effects. Second, the focus was almost entirely on transgenesis, i.e., transmission of genes across the boundaries between species (or even kingdoms). Consumers have, however, been repeatedly found to be much more skeptical of transgenic than cisgenic food (Delwaide et al., 2015; Edenbrandt et al., 2018). Third, the vast majority of GM varieties was developed and sold by multinational companies such as Monsanto, DuPont, and Syngenta, which have long been viewed skeptically by consumers and civil society.[4] Relatedly, GM food has been viewed, fairly or unfairly, as compatible mainly with highly intensive, industrialized, and environmentally harmful variants of agriculture (e.g., Gomiero, 2018). This is often linked to the fact that most commercial GM crops were bred for herbicide-resistance or *bt* (pest resistance) traits (Bennett et al., 2013) and that their use has led to pest resistances (Perry et al., 2016).

Genome editing, especially since the advent of CRISPR/Cas (Jinek et al., 2012), has significantly changed the picture across all three dimensions. First, genome editing is much more precise than earlier GM techniques, allowing for modifications of the genome at precisely specified locations and with few unintended, off-target mutations—though recently, a number of publications have questioned this claim (Schaefer et al., 2017; Haapaniemi et al., 2018; Kosicki et al., 2018). Second, the emphasis is more on non-transgenic modifications, including cisgenesis, targeted mutagenesis, gene silencing, and gene knockout (Bartkowski et al., 2018). Third, at least in the case of CRISPR/Cas, due to the low-cost of the technology's application and its higher flexibility, the heavy involvement of large multinational companies is no longer as essential as hitherto (Bartkowski et al., 2018). Accordingly, a shift in the public debate can be observed—today, environmental and health risks play less of a role; rather, the focus is shifting toward issues of naturalness, problem framing and, still, patents and property rights (van Hove and Gillund, 2017; Pirscher et al., 2018). Meanwhile, the general skepticism to GM food increasingly entails questions of the purpose of and need for "technical solutions" (van Hove and Gillund, 2017), a shift that also offers striking parallels to the older Golden Rice debate (Kettenburg et al., 2018). Drawing on the wide-ranging research of Sjöberg (2002), we could see all this as evidence of the increasing attention to the wider context in which technologies are introduced, implemented, and adopted. Instead of focusing only on perceptions of a technology like genome editing (and its associated hazards), this then draws into focus the relationship between the technology and its societal, scientific, and technological context in order to explore and understand attitudes toward risk. In fact, Sjöberg (2002) highlights three factors that are characteristic of the wider context of technology: whether a technology is readily replaceable, beliefs in the uncertainty of scientific knowledge, and the sense that its use represents "tampering with nature." Not only is each shown

[4] A significant exception is Golden Rice, which was developed in a non-commercial context (though Syngenta has been involved), but has been still targeted particularly by environmental NGOs such as Greenpeace. For an in-depth study of the Golden Rice debate, see Kettenburg et al. (2018). For a critical discussion of why Golden Rice has not been widely adopted, see Stone and Glover (2017).

to be among the most significant determinants for attitudes toward technologies, but they are especially impactful for gene technologies (Sjöberg, 2002). Moreover, the fact that all three are increasingly prominent in the shifting discussion of new genome-editing technologies also connotes that, as some of the more technical shortcomings of older-generation approaches are overcome, we can expect there to be more scope to consider the wider context to which technologies will have to relate—rather than their immediate and unequivocal acceptance. Thus, while the improvements offered by genome editing have the potential to change the public perception of GM food, the more short-term development is likely to be the greater engagement with novel types of arguments, and as a result, a continuation in the status quo where, at least in the EU, the majority of citizens remain skeptical of GM food (Twardowski and Małyska, 2015).

Current Debate on Governance of Genome-Edited Food

The novel possibilities offered by genome editing, particularly CRISPR-based systems, have also brought to attention the shortcomings of existing regulatory regimes. For instance, as observed by Wolt et al. (2016), novel genome-editing techniques "do not readily fit current definitions of genetically engineered or genetically modified used within most regulatory regimes." This has given rise to an (ongoing) debate about the proper governance regime for genome-edited food, as well as substantial differences in opinion, even among regulatory officials in various developed economies. For instance, in the US, the Department of Agriculture has decided that a gene-edited non-browning mushroom (*Agaricus bisporus*) can be cultivated and sold without any oversight, as it was created by "knocking out" the gene responsible for browning and without the introduction of foreign DNA (Waltz, 2016). More recently, the USDA has expounded upon this through its assessment that new genome-editing techniques, notably CRISPR, do not require any "special" regulation, specifically because these methods neither make use of nor rely on anything that may qualify as a "plant pest" (USDA, 2018). Conversely, albeit for somewhat distinct reasons, the European Court of Justice recently came to the conflicting determination that "all organisms obtained by mutagenesis" are identical in terms of their associated potential risks, and irrespective of any differences in the underlying technical process (ECJ, 2018). Not only does this raise the question of the appropriate level of regulatory scrutiny for the products of genome editing, as many commentators rushed to point out, but now there is the further issue of whether and how any "transatlantic" disparities in regulatory approach can be reconciled.

The broad debate on genome editing governance, which is likely to continue after the ECJ ruling, has been largely framed as the choice between product-based and process-based regulation (e.g., Hartung and Schiemann, 2014; Araki and Ishii, 2015; Huang et al., 2016; Sprink et al., 2016; Wolt et al., 2016). In the EU, what is currently employed best represents a process-based approach, implying that greater oversight is needed for any plant created using a technology classified as GM. However, arguing that non-transgenic genome editing "is by nature similar to the use of spontaneous variants or induced mutations in conventional breeding, with the advantage that only the desired change is introduced," Huang et al. (2016) for instance "strongly advocate product-based rather than technology-based regulation" (p. 110). This would imply that most genome-edited crops would not be treated as GM products, and therefore should not be subject to the same regulations. Indeed, the advocate general of the European Court of Justice expressed a very similar viewpoint in his opinion to the court back in January (Purnhagen et al., 2018).

Such "evidence-based" or "science-based" approaches have been criticized as being founded upon the fallacious assumption that it is possible to make far-reaching societal decisions on an objective basis: "Empirical evidence matters, but human interpretation brings meaning to that evidence, and multiple perspectives can strengthen understanding" (Kuzma, 2016, p. 167). It has been further pointed out that "it is wishful thinking to believe that, by simply classifying products of NBTs [new breeding techniques] as non-GMOs, their commercial potential will be realized" (Malyska et al., 2016, p. 532). In fact, adoption and acceptance of novel products and technologies depends on both a range of stakeholders across the supply chain and a multitude of factors, some of which might not necessarily be deemed "objectively" relevant (Scheufele et al., 2007; Sarewitz, 2015; Baum, 2018). Malyska et al. (2016, p. 532) therefore contend that "the key issue is not whether new crop varieties are as safe as those developed by conventional plant breeding and thus fall outside the scope of current GMO legislation, but whether society perceives them as such." In other words, the crucial issue is not whether there is definite evidence of a proof of an issue for human health or the environment, especially if there are widespread beliefs in the uncertainty of scientific knowledge (Sjöberg, 2002). Nor is the crucial issue even the pursuit of regulatory certainty, at least not for those actively engaged in developing and commercializing the new technologies. Instead, it is primarily a matter of public acceptance and legitimacy. Hence, what is most urgently required is a far-reaching societal dialogue on the (perceived) benefits and risks of genome editing, rather than one that only seeks to find technocratic "evidence-based" solutions (Jasanoff et al., 2015; Bartkowski et al., 2018) that draw upon and make use of only some types of evidence, perhaps to the detriment or ignorance of others.

Exit and Voice in the Context of Genome-Edited Food

Adopting the perspective of a consumer-citizen, the distinction between exit, and voice as means to express discontent (and thereby offer feedback to producers/suppliers) only becomes relevant once genome-edited food products are already on the market. Before this time, there is nothing to exit from and, as such, any discontent about the *possibility* of market introduction can only be expressed by means of voice, as has been done for example in the debate spurred by the advent of

CRISPR/Cas (section GM Opposition and Genome Editing).[5] Given the various calls from those advocating for a product-based regulation that may facilitate the quicker introduction of (at least) non-transgenic genome-edited food products to the market (section Current Debate on Governance of Genome-Edited Food), the subsequent analysis thus orients itself around the counterfactual that genome-edited food is already available on the market. Whether this occurs because one country—e.g., the United States—has taken more immediate steps to "deregulate" such products or a few firms have shouldered the greater regulatory burden to bring the products to market is not so important—only that some products do exist on the market. For what follows, we will chiefly focus on two issues: the role(s) of exit and voice in the present context; and their manifestations and preconditions.

The Role of Exit and Voice

The main role of both exit and voice is to express dissatisfaction and discontent with the existing state of affairs. As detailed in section GM Opposition and Genome Editing, many consumer-citizens have expressed their opposition toward GM foods in the past, by means of political protests and in choice-elicitation surveys.[6] While genome editing, with its focus on non-transgenic modifications, may alleviate some of the public's concerns (Delwaide et al., 2015; Edenbrandt et al., 2018), there are still other concerns that go beyond health-related and environmental risks (van Hove and Gillund, 2017; Pirscher et al., 2018; see section GM Opposition and Genome Editing). Thus, it is fair to assume that, in the event of allowing genome-edited food products on the market, a significant and widespread level of concern and skepticism is likely to surface.

In this context, it is helpful to distinguish between two levels of dissatisfaction regarding GM food. First, there is product-related dissatisfaction, based particularly on perceived environmental and health risks. Here, we have to further distinguish between risks that are more private (health) or public in nature (environment), given that they have different consequences for the selection of response strategy. Notably, whereas exit is likely to represent a desirable strategy for risks that are perceived to be private, public risks cannot be sufficiently tackled in this fashion, because of the strong potential for externalities. Accordingly, I may be able to narrowly protect the health of myself and my family by means of exit (i.e., by not buying GM food), but if my main concerns center on the risks posed by GM crop cultivation for the environment, exit can neither solve the problem as long as production of GM food continues, nor indeed if the effects of this cultivation exert an indirect impact even on those not directly involved in their consumption. Here, voice is thus the potentially more effective strategy, as protection against these more public risks can only be achieved collectively—in the extreme, for instance, via a ban on activities like the cultivation of GM crops. In fact, it has been revealed that, once we enter in the context of public goods and externalities, market behavior only turns out to be loosely correlated with political behavior (Hamilton et al., 2003). Hence, the more voice-inflected forms of political activism prove to be a more appropriate strategy with respect to public-good concerns in the food context.

Second, there is the dissatisfaction more generally related to the food systems, of which GM and recently genome-edited crops end up being only one (perceived) manifestation of a broad, more symbolic bundle of (unwanted) characteristics (Gomiero, 2018). Bundled together, an observer of the current debate can thus find an assortment of issues such as market power, the shift toward industrialized, monoculture-based cultivation, distributions of property rights perceived to be unfair and, more generally, an unequal distribution of risks and benefits across groups within a society. This would suggest that, for at least some segments of the public, development and commercialization of GM food is understood to be entangled with the wider economic and societal circumstances into which these products would be introduced (see Sjöberg, 2002). Of course, it might be, and indeed frequently has been, objected that such perceptions are inherently biased, and thus in need of correction (cf. Stirling, 2008; Torgersen, 2009). Nonetheless, there are a variety of reasons to not simply dismiss such concerns out of hand, not least of which is the fact that the evaluations of experts have been revealed to severely underweight the importance to the public of socio-economic issues (Scheufele et al., 2007; Sarewitz, 2015). Attributing such concerns simply to "bias" would therefore run the risk of misunderstanding the reasons for dissatisfaction, not to mention the degree to which it exists.

More crucially for the role of exit and voice in the context of genome-edited food, it is necessary to recognize how broad societal, technological, and scientific conditions can incite not only an increase in the level of dissatisfaction but also prompt it to take one form over another. For instance, Schütz and Wiedemann (2008) have demonstrated how the risk perceptions of novel technologies are influenced by the identity of the beneficiaries. When a small- or medium-sized enterprise is most likely to benefit from their development, and not a multinational corporation, it is notable that people tend to assign lower risk probabilities to the likelihood for toxic damages, negative environmental impacts, and even those unknown risks yet to be considered. This speaks to the significance assigned to not just technology but rather the nature, scale, and identity of its introduction and implementation into (existing) economic systems. Similarly, Betten et al. (2018) find, somewhat contrary to expectations, that most people are neither inherently for nor against synthetic biology; instead much of the criticism stems from core values about the relationship of society with science and technology as well as general feelings of discontent with the prevailing context. As such, if there is anxiety about wider trends in technology development, for instance because of the potential impacts for employment or the greater prospect of firm consolidation, such anxiety might then manifest itself as an ostensibly "irrational concern" about one specific technology, that is, because it is not only not viewed as a potential

[5]In this regard, the facility and relevance of comparisons/contrasts to other technologies, with genetic engineering serving as perhaps the most notably, represent a crucial basis for being able to produce the type of counterfactual "analysis" that will allow decisions and criticisms in line with the values which one espouses.

[6]In absence of marketed GM food in the EU and of GM labeling in the US (except for Vermont), no opposition could be expressed via market behavior.

solution but rather as something that could make things worse. Broadly speaking, the crucial point is that *it is not necessarily the technology itself that arouses societal unease but rather its (perceived) engagement with existing socio-techno-economic systems* (Jasanoff et al., 2015; Baum, 2018).

With regard to expressions of voice and exit, moreover, this also opens up the specific possibility that what I may wish to exit from, or raise my voice against, is not a particular product but rather the whole food (production) system. Thus, while it is clear that exit and voice are supposed to communicate discontent and dissatisfaction, in the domain of food, the reasons for discontent and dissatisfaction are potentially greater and more complex than in many other areas. Of course, this need not be unique to the food sector and yet, whereas genetic engineering has been deemed acceptable if used for other purposes, notably pharmaceuticals, and plant protection (Frewer et al., 1995; Knight, 2006; Christoph et al., 2008), applications to food have frequently "amplified" the controversy of novel genome-editing technologies (Frewer et al., 2002; Pidgeon et al., 2003). As a result, there are many reasons to believe that the evolution of discussions in the food sector could follow their own unique logic. On the one hand, we see burgeoning interest in many developed countries regarding the quality and provenance of the food one eats and growing appreciation of environmental, health-related, and socio-economic impacts of conventional food systems. Given that this is coupled to the advent of innovative arrangements such as organic food, farmers' markets, community supported agriculture and fair trade, there is a change in both the quantity and quality of consumer involvement within the food sector. Reflecting the increasingly diverse, multi-dimensional responses available, for instance, a person who is "fed up" with established food systems can express their dissatisfaction by buying less from a given firm; protesting the particular activities with which they take issue; supporting related policies by contacting their representative; or, at a more systemic level, "voting with their wallets" by frequenting farmers' markets or becoming a member of a box scheme, rather just switching brands or choosing to buy organics. Which of these available strategies would best be able to not only express dissatisfaction but also inspire a desirable reaction by those in charge, however, depends on whether a variety of preconditions are in place to ensure their effectiveness.

Manifestations of and Preconditions for Exit and Voice

As already indicated in the previous sub-section, exit and voice can communicate discontent and dissatisfaction in a variety of fashions when it comes to food in general and genome-edited food in specific. Given the range of manifestations that may emerge as a result, it is crucial to explore the extent to which, depending on the specific context and purpose of the activity, the conditions and requirements of success could vary. For instance, if the existence of alternatives is necessary to render exit effective, the increasing manifestation of such activities is unlikely to take place in the absence of broader changes. Rather, we would expect reliance on exit to occur in response to the availability and diversity of alternatives on offer. And, if the scope of dissatisfaction is linked with established food systems at large, then the alternatives would have to be of a similar type as well—that is, alternative food systems.

Exit

As suggested above, dissatisfaction in the food sector can occur at two distinct levels: the level of products and the level of systems. In this respect, we see one of the crucial ways in which this sector represents a clear departure from others that have previously engaged the attention of exit–voice researchers (section Hirschman's Exit–Voice Framework and Its Application). Indeed, exit has typically been understood as a strategy that is more relevant at the level of products, for instance, because of the way that we individually bear any related risks (and collect the benefits) of our food choices. Further undermining our capacity to express dissatisfaction with entire food systems, there is the added impracticality of "exiting" the food system of a country, by opting for instance to purchase all food from Canada instead of the United States if the former were to adopt GM labels or support family farmers. Aside from leaving the country, this would leave critical consumer-citizens with "nowhere else to go."

However, as alternative food systems have become available, the scope of choice that is afforded to consumer-citizens even at the level of systems has increasingly grown. Organic agriculture is often perceived as one such system (Reganold and Wachter, 2016; Gomiero, 2018), specifically as it positions itself as a solution to the perceived deficiencies of the industrialized, environmentally harmful, and excessive concentration of conventional food systems. Of course, it should be noted that the degree to which it is in fact a clear alternative, at least in terms of environmental impact, has been called into question (e.g., Meemken and Qaim, 2018; Tal, 2018). Even if the large scale forms of organic agriculture may not drastically differ from existing approaches, there are others adopting a more regional character, e.g., through community-supported agriculture or other "independent" arrangements, so alternatives do exist, thus offering a greater degree of "exit potential" for consumers who wish to extricate themselves.

In any case, exit generally requires the capacity to distinguish between alternatives. In the more extreme case of "complete" exit from conventional systems, buying only (regional) organic food could signify a viable option as "[o]rganic management systems do not use genetically modified organisms (GMO) or their derivatives, except vaccines, in all stages of organic production and processing" (IFOAM, 2017). However, taking such recourse would not only require the complete detachment from conventional food systems but is also likely to be quite costly, both financially (Seufert and Ramankutty, 2017) and in terms of the effort needed to identify and purchase food of a suitable quality. Moreover, given the increasing specialization and "industrialization" of organic farming, the potential of this strategy to serve as a way to escape the conventional food system is somewhat diminished. As it becomes more and more difficult to differentiate "authentic" organic producers, i.e., those who inhabit the original ideals of the system, from those who do the bare minimum to attain the desired premiums, ever

more effort and attention is required to make an informed decision. This represents, in fact, a long-standing issue in the literature on consumer welfare, notably, the requirements for choice that is actually "free." On this point, and proposing his deeper understanding of what constitutes consumer sovereignty, Scitovsky (1962) has drawn a strong distinction between the ability of markets to cater to so-called "minority preferences" and "majority preferences." Pointing to the often-unattended downsides of pursuit of economies of scale, he observes that what tends to pass for "variety" in satisfying the desires of most people turns out to signify an illusory choice offered among products that are distinct only superficially, and mostly identical in their core characteristics. As a result, this offers any consumer with somewhat atypical tastes not only an increasingly narrow set of alternatives on which to express their preferences but also more limited prospects to pursue genuine "exit" if this is deemed to be desirable. Limitations on the range of alternatives that are genuinely distinct, that is, not just in relation to a few peripheral features but also for domains of more fundamental importance to society, environment, etc., are thus a radical constraint on the effectiveness of exit.

While Scitovsky and others (e.g., Sirgy and Su, 2000) are able to sketch the wide context in which consumers become less sovereign, this literature focuses less on how this impacts the actions and decisions of the consumers themselves. For this, we must look at Hirschman (1970), specifically in relation to the archetype of "quality connoisseurs." In specific, these individuals are both most accustomed to a high level of quality and, accordingly, more likely to be disappointed with declines in product quality. One potential explanation for this would pertain to the growing complexity and uncertainty involved with ascertaining the quality of products within modern economies, not least because of the growing technological sophistication even in the area of food production. As such, if one had a background in microbiology, they might then be (given suitable levels of time and interest) more able to research the competing claims about the safety and efficacy of genome-editing technologies than someone who is less expert. In fact, according to Hirschman (1970), such actors play an essential role for the broad operation of exit and voice, e.g., from their greater willingness to engage in "opinion leadership" by assembling their fellow citizens or directly reaching out to management. At the same time, if there is a higher-quality but more expensive substitute, these people are just as likely to abandon the firm and exit in favor of this alternative.[7] We therefore observe that such individuals are more likely to engage in exit and voice: the desire for this quality occurring even and in spite of the costs of doing so.

However, even if these "connoisseurs" are more likely to be motivated and willing to retain their desired level of quality, this alone is no guarantee that they will actually be able to do so. On the one hand, this is a result of the tendency of there to be a relative paucity of alternatives at the higher-quality end of markets. Contrasting with the more typical clustering of products at the low-quality, low-price end of the spectrum, it turns out that the choosiest may necessarily have less to choose from. Even if they have a greater opportunity to leave, this lack of choice can thus serve as a check on the speed with which connoisseurs will exit in favor of the greener pastures elsewhere. In other words, possessing a greater amount of resources offers no guarantee that this is matched by an increasing quantity of alternatives, nor even a larger product assortment than those with fewer "opportunities." As a general point, likelihood of engaging in exit thus reflects the trade-off between the number of suitable alternatives that exist and the quality preferences of individual consumers.

How then are consumers who might favor "exit" likely to respond? On the one hand, it may be argued that, even if complete exit is not feasible, a more "partial" exit, that is, one that balances personal costs against social benefits, can still have an impact. For instance, dissatisfaction with the quality of the offerings of a firm (or the entire system) could lead a consumer to reduce their amount of consumption, e.g., by purchasing from another firm or, in the case of the entire food system, frequenting more farmers' markets or even starting a home garden. In the latter case, we therefore find one of the few "true" alternatives for exiting from the current food system, notably, substituting one's own production and/or just consuming less overall. Nonetheless, to the degree that the concerns of an individual are public in nature, it turns out that any kind of "individual" exit only represents an imperfect solution, for reasons similar to those discussed above. That is, whenever the impacts of food production affect the quality of "public goods," of which the environment is perhaps the clearest example, these are necessarily diffuse and non-exclusive in nature. For this reason, even if one is able to "escape" having conventionally produced food on one's table, it is not possible to escape the negative externalities of conventional food production in a more general sense. In the words of Hirschman (1970, p. 104), this results in a situation where "[i]n spite of exit one remains a consumer of the output or at least of its external effects from which there is no escape."

Instead of cause for cynicism, this leads Hirschman to explore alternative ways in which exit can effect change, notably, by ensuring that one's exit directly contributes to desired improvements. Recognizing that there in fact limitations on individual exit, greater emphasis is therefore placed on the *timing* of one's exit, i.e., to ensure that it not only expresses dissatisfaction but is effective in doing so. For someone to best avoid hypothetical damages, it could then turn out to be useful to forestall exit as long as possible, thereby guaranteeing one retains a modicum of influence to be exercised from within. In the words of Hirschman (1970), this however results in a shift in the reading of the situation to where "the alternative is now not so much between voice and exit as between voice from within and voice from without (after exit)" (p. 105). In such a scenario, we might conceive of "exit" within the prevailing system as basically recurring over time, by taking the form, e.g.,

[7]Here it is useful to note that the reasons for doing so are not explainable in terms of the (looser) budget constraint alone. Rather, according to the exit–voice framework, this is a matter of retaining the level of quality to which they are accustomed and, what is more, the difficulty of achieving the same outcome by means of voice alone.

of a consistent choice of the brand that attempts to minimize its harmful impacts or of the exact offering of a given brand that best satisfies consumer concerns.

Besides signaling the rather limited scope for "exit" in this context, the foregoing highlights how the effectiveness of exit depends, first and foremost, upon the ability to distinguish between, e.g., genome-edited and non-genome-edited products. Having labels on GM food is thus one of the preconditions for choice to be effective, including for products that are "only" genome-edited. Contrary to calls for the product-based regulation of genome-edited, non-transgenic crops, we thus note that there are also more informational and expressive reasons for adopting a process-based approach. In other words, if consumers perceive such labels as useful for making choices, regardless of whether they see the underlying technologies as problematic, their mere absence could raise "red flags" where none were present before. At first glance, this might seem counterintuitive; however, there is growing evidence that, not only are attitudes toward GM food not affected by the existence of labels (Kolodinsky and Lusk, 2018), but that their absence could spark concern, even for those who might be more likely to accept such products. Instead of labels possibly prejudicing the public against genome-editing technologies, it could be their absence which proves to be more of an issue if individuals are indeed to be asked to make informed decisions.

While useful, it still remains that labels ought not be taken to be "sufficient" for the effectiveness of exit, especially given the range of other factors involved. Some of these have been addressed in the rather extensive literature on the economics of labeling (McCluskey et al., 2018; Zilberman et al., 2018b). A particularly important question is "What should be labeled?" There has been a proliferation of labeling schemes in recent years, all of which, by claiming to provide different kinds of information, both relevant and irrelevant, induce a constant risk of information overload (Verbeke, 2005). In this way, consumers are confronted with the "paradox of choice," whereby the overwhelming amount of alternatives and products, while generally assumed to be beneficial by standard economic theory, ends up reducing welfare (Schwartz, 2004). According to Scitovsky (1962), for choice to actually be "sovereign" and free, it is necessary for consumers to actually be able to evaluate the alternatives available—also without this requiring them to invest an unreasonable amount of time or energy to do so. Accordingly, if labels fail to clearly distinguish products in ways that the public can understand, e.g., by allowing too many exemptions or creating multiple levels of "non-GMO," or make it difficult for certain groups to track down relevant information, e.g., by solely employing QR (or quick response) codes, this would undercut their ability to support decision-making. Partly owing to such shortcomings, the quality perceptions of labels have been shown to vary across contexts. Indeed, perceptions of healthiness and sustainability have been tied to the type of retail format where products are sold (van Rompay et al., 2016; Baum and Weigelt, 2019). If, however, the value of an organic label in a supermarket exceeds the value of one in a discounter format, labels are then no longer able to convey the same information in all situations, or to serve as an unbiased basis for information provision. Indeed, it has been illustrated that many consumers therefore question the reliability of organic and fair-trade labels (Jahn et al., 2005; Janssen and Hamm, 2012), even going so far as to dismiss them as "marketing tools" that fail to provide what is promised (Rousseau, 2015).

From the perspective of the exit–voice framework applied here, there is one general problem with exit that cannot, however, be solved by labeling, no matter the accommodations that are made: if the aim is to signal dissatisfaction with specific characteristics of a product or production system, exit turns out to be of very limited relevance since it is an imprecise signaling device. Producers usually do not know why exactly it is that a consumer decides to exit, given that exit is carried out in relation to the product (or system) in full (Hirschman, 1970; Newholm, 2000). In addition, use of exit as a standalone strategy suffers from the same fatal flaw of collective-action problems: that is, change in and of the system (here: food system) cannot be triggered by the actions of one individual. Not only is there the potential for firms/institutions to ignore the activities of any one individual (or handful of individuals), there is the further problem that such activities, instead of giving rise to a "virtuous circle" where other consumers opt to take part, might just as well trigger a higher incidence of free-riding behavior. As exit is ex definitione an individual-level strategy, it thus requires the complement of other strategies to be a contribution to collective action. Enter voice.

Voice

Having outlined the manifold limitations to the effectiveness of exit, the foregoing might provide the impression that consumers are increasingly "captive" to commercial interests. Almost 20 years ago, Sirgy and Su (2000) thus asserted that the capacity of "sovereign" consumers to exercise an unconstrained freedom of choice has now become "more of a fiction than a fact." In specific, the authors note, *inter alia*, the diminishing expertise, motivation, and opportunity of individuals to make decisions broadly in the interests of societal welfare to explain why they are unable to hold firms to account. Given the strictures of "an increasingly high tech world," they then propose that consumers are replaced by the wider set of stakeholders as ultimate arbiters of business performance—thereby absolving the former of any specific, deeper responsibility. Consumers are thus no longer treated as sovereign, but simply another actor group whose interests must be considered when making decisions of broadly societal relevance.

Holding to our stated aim of facilitating a more active role for the public, we however call into question the validity of their conclusions. Firstly, the tendency to neglect the average citizen and her interests, or to suppose that engaging in "self-regulation" on their behalf is sufficient, is often one of the broad complaints lobbed against the established system of food production, and as a result against the commercialization of genome-edited products (Stirling, 2008; Torgersen, 2009; e.g., Jasanoff et al., 2015). Furthermore, the foregoing seems to suppose that, should individuals be limited in their efficacy as consumers, they would then have no other recourse for making their dissatisfaction known. Conversely, the dialectic of the exit–voice framework elucidates that, if use of exit is

forestalled, this opens up a greater likelihood to focus attention on opportunities to engage in voice (Hirschman, 1970, pp. 70–72). Throughout his examination, Hirschman (1970) focused mainly on those voice options available to individuals that are not institutionalized: be it protests, boycotts, petitions, letter-writing campaigns, etc. In the context of genome-edited food, we can therefore see manifestations of voice through, for instance, the widespread "March(es) Against Monsanto"—which first emerged, in fact, in response to the failure of a ballot initiative in California that would have required GM labels on food products—and omnipresent petitions, whether from consumers, scientists, and non-governmental organizations (NGOs), urging firms and regulatory agencies to label or outright ban these products. Of course, given the strong differences in opinion here, it is unsurprising that "counter-petitions" pronouncing the safety and desirability of these products are also widespread—best exemplified by the widely-publicized letter "supporting precision agriculture (GMOs)," signed by 135 Nobel laureates, and identifying efforts of Greenpeace against GM crops as a potential "crime against humanity."

While the fraught nature of the debate should come with little surprise, these examples are useful for a few reasons. First, they illustrate both the prevalence and variety of manifestations of voice, not to mention the diverse actors who could engage in such activities. For instance, in addition to the "voices" of consumers, there are many recent cases of leading experts also speaking out for, e.g., a moratorium on human germline editing by means of CRISPR-Cas (Baltimore et al., 2015) and a ban on the field-testing and development of gene drives until "open and international discussions" have an opportunity to occur (Esvelt and Gemmell, 2017; Noble et al., 2017). In fact, owing to the way that public knowledge about new technologies tends to substantially lag behind that of experts, the initial expressions of voice are most likely to emanate from those with the most experience working with and developing them. Second, the examples also point to a crucial limitation on the exercise of voice, which is of importance given that it extends to Hirschman's framework more generally. Notably, all the various manifestations mentioned here, while clearly able to express the dissatisfaction of the consuming public, fall short of supporting a more direct engagement with the relevant decision-making processes. From the perspective of the *governance* of genome-edited food, the more relevant issue is thus how the voice option can be institutionalized in order to be more accessible for critical individuals and groups wishing to have recourse to it. Institutionalization of voice in this manner is crucial, in that it secures the deeper embeddedness and integration of voice within political decision-making processes and mechanisms, thus allowing expressions of voice to be more effective (as its addressees are likely not only, or not even mainly, the producers of genome-edited food but rather regulators). On the one hand, we observed in section The Role of Exit and Voice that many of the concerns surrounding GM food (including genome-edited food) are public in nature; therefore, their (re)solution requires collective action. What is more, given the (perceived) deficit of legitimacy in this domain, it is principally crucial to engage with and integrate the public more deeply—especially with the limited effectiveness of exit here (section Exit). With these facts in mind, we would like to step beyond Hirschman's original framework and introduce some insights from the theory of deliberative democracy that may be instructive in the present context.

Starting off broadly, Jasanoff et al. (2015) have already emphasized the importance of public deliberation among stakeholders for reaching a legitimate solution to the controversies enfolding genome-edited food. Especially given the extent of the stakes involved, going beyond particular breeding techniques to also encompass more fundamental questions regarding the future of the food system, a broad debate on applications of genome editing to the food domain, embedded in a more general debate about the food system as a whole, appears warranted. As already indicated above, the parties to the current GM food debate currently confront each other in a quite antagonistic fashion, which may be interpreted as an instance of "deep moral disagreement," i.e. a situation in which "parties to a dispute do not recognize the legitimacy of each others" [sic!] values' (Dryzek, 2013, p. 337). In such a case, public deliberation can serve as a useful tool to uncover a "normative meta-consensus" that would allow the parties to at least recognize the legitimacy of each other's positions (without necessarily agreeing on a specific course of action) (Dryzek, 2013). The existence of this kind of mutual respect on both sides represents a fundamental precondition for the more effective use of voice in this context, even if this ideal is rarely fulfilled in reality. When looking for instance at the nature of the discourse from Greenpeace and other non-governmental, civil-society organizations on the one hand and notable advocates of GM food on the other, it is readily clear that any kind of meta-consensus is presently lacking. We have already mentioned the charge levied against the activities of Greenpeace by the assembled Nobel signatories above; in addition, note the title of an influential paper from the "father" of the Green Revolution, Norman Borlaug (2000): "Ending World Hunger: The Promise of Biotechnology and the Threat of Antiscience Zealotry." On the other side of the divide, activists tend to attack scientists voicing pro-GM opinions as being allegedly paid by Monsanto.

This shows that while the importance of civil society as a herald of collective voice cannot be overemphasized (Habermas, 1996), in situations of deep moral disagreement additional institutionalized voice options and a deliberate broadening of the debate are needed. Examples of successful deliberative processes in deeply divided societies such as Northern Ireland (Luskin et al., 2014) demonstrate the broader potential for institutionalized deliberation to help bridge even strong differences of opinion. In the context of genome-edited food, Bartkowski (2019) discusses using deliberative mini-publics, i.e., moderated small-group discussions including testimonies by expert witnesses, to facilitate a societal process aiming at shared understanding. It has been argued that such mini-publics can be a helpful complement to conventional, representative-democratic political processes by contrasting "majority opinions" (e.g., the widespread skepticism toward GM food, including genome-edited products) with such shared understandings reached by small, in-depth group discussions of representative samples of the population (Lafont, 2017). In fact, experimental results suggest that mini-publics can

influence public opinion (Ingham and Levin, 2018). However, careful design is necessary for such deliberative institutions to work properly (Aasen and Vatn, 2013): for instance, the case of UK's 2003 "GM Nation?" public debate has been invoked as a negative example because participation was based on self-selection (Goodin and Dryzek, 2006), thus potentially leading to biased results.

As showed by the recent decision of the ECJ, there is urgent need for new GM legislation that is up to the task of dealing with genome editing. At the same time, there is a need for a rational debate among the parties, including not only biotechnology companies, scientists and anti-GM NGOs, but also the broader public. Innovations such as mini-publics might help institutionalize voice and thus offer consumer-citizens an opportunity to participate more actively in the debates currently characterized by deep moral disagreement. Moreover, such institutionalized voice opportunities have the potential to generate more understanding of the underlying motives of the participants, including general dissatisfaction with the modern food system. Last but not least, if properly institutionalized, mini-publics may help legislative bodies navigate the complex, morally charged field of GM regulation so as to identify legitimate solutions to the currently inadequate (in face of genome editing) GM law. In fact, public consultations have been applied to inform the EU agricultural policy, and stakeholder consultations are already part of EU's GM food chain governance (Bengtsson and Klintman, 2010).[8] Strengthening and extending these institutions by the inclusion of more deliberative elements, possibly also in cooperation with the European Parliament as the democratically legitimized legislative body of the EU, would be a viable step toward resolving the controversies of genome editing.

CONCLUSIONS

In this paper, we have applied and extended Albert Hirschman's exit–voice framework in order to shed light the proper governance of genome-edited food. Starting from the premise that it is not the type of governance approach that matters most but whether governance proves suitable to not only enable consumer-citizens to express and react to sources of dissatisfaction but also open up space for the public to assume a more active role, we analyzed the dominant expressions of voice and exit in relation to genome-edited food. We specifically argue, first, that opposition in many cases signals the existence of a deeper dissatisfaction with conventional food (production) systems and their negative externalities: for environment, society, human health, and animal welfare. Criticisms about GM food, for instance, are not therefore specific to any one technology or product application, but rather share aspects that are consistent across all others that highlight and draw out similar concerns. Second, we posit that much dissatisfaction with and skepticism toward the biotech industry could thus reflect the lack of effective recuperation mechanisms, whether exit or voice. As a result, what is perceived as unfair or misplaced criticism—from the point of view of proponents and actors in the food industry—could represent a delayed response on the part of consumer-citizens to previous grievances, specifically because of their previously limited outlets available to them for expressing their dissatisfaction. Also, calls from both science and industry to reduce options of exit (via product-based regulation) might well contribute to the dissatisfaction. If this is the case, improvements in the availability of exit and voice could go a long way to also reducing the levels of "unfair" criticism. Based on these points, we considered possible manifestations of exit in this context as well as the conditions that are required for these strategies to be effective. Ultimately, we conclude that, in situations where dissatisfaction extends to the food system as a whole (channeled as a result into the opposition toward GM food, among other things), exit turns out to be of limited relevance. In part, this is a reflection of the nature of the problems themselves, most notably, that the "goods" (or "bads") in question do not just affect discrete individuals but are instead more public in nature. As a result, the ability to find solutions not only eludes the grasp of a single individual, instead requiring that collective action take place, but it will also be difficult for any individual to completely "isolate" themselves from the wider consequences of the system in place. In other words, consumers can select the types of food they serve for dinner but not whether or not the environmental or societal consequences of the food system (if any) have an impact on their daily lives.

Nonetheless, although exit is only an imperfect strategy, it is still likely to be relevant in some contexts, most notably for influencing the decisions of certain firms. For exit to serve as a viable option in this regard, it must be possible for consumers to distinguish between the alternatives on offer—thus making labeling a necessary condition. This does not mean, however, that labeling is *per se* sufficient for effectively expressing dissatisfaction across all contexts, not least because of the risks of information overload and often-circumscribed variety of alternatives from which individuals are able to choose. Given the limitations on the exit option, we therefore turned to voice and, in line with our aim of studying options to foster more institutionalized forms of action, we extended Hirschman's original framework by introducing some insights from the theory and practices of deliberative democracy. We emphasized the deep moral disagreement that characterizes the current state of the debate on GM food (including genome-edited food) and stressed the potential of institutionalized voice (e.g., deliberative mini-publics) to diversely inform and orient a more wide-ranging societal debate into genome-edited food and, more broadly, the future of the food system. We see potential to extend existing institutional structures in the EU to enable institutionalized voice and contribute to crafting new GM food regulations, adequate for genome editing technologies.

The foregoing conceptual analysis, however, leaves many questions open, partly given its reliance on a few requisite simplifications. For instance, we have ignored the distinct variants of labeling approaches (mandatory vs. voluntary, governmental vs. self-declared vs. third-party), as these are both

[8]However, Bengtsson and Klintman (2010) note that a major problem of the stakeholder consultations by the European Food Safety Agency (EFSA) and the Directorate General for Health and Food Safety (DG SANTE) is that they do not include the general public in their concept of "stakeholders."

less important for the present analysis and, moreover, covered in much greater detail in the relevant literature (e.g., Zilberman et al., 2018b). Nevertheless, further analysis of the comparative strengths and weaknesses of the varied approaches against the background of our findings would be interesting and informative. With regard to voice, we have implicitly assumed a rather idealized account of deliberative democratic institutions. There is, in fact, a large literature that highlights the limitations and weaknesses of such practices, such as the constraints of power dynamics and the unclear role of emotions (Mendelberg, 2002; Chilvers, 2009). Nonetheless, what specific consequences these limitations have in the context of genome-edited food must be left for future research. Perhaps most fundamentally, there is a deeper need for information about the types of conclusions that institutionalized voice—whether by mini-publics or some other format—can reach in the context of genome-edited food, as well as how these may best be used to inform and orient public policy. Further research in this vein is urgently needed.

Last but not least, assuming that a product-based regulatory approach is not ultimately deemed to be democratically legitimate, there are many questions about which kind of governance regime could best balance the benefits and costs of genome-editing products in the food domain. Indeed, the recent judgment by the ECJ (2018), by lending support toward further risk assessments and value-based discussions, is much more likely to represent the beginning of a wider debate into this topic than offering the last word. In this regard, we contend that further progress in application of the exit–voice framework here can prove useful by, *inter alia*, helping to establish the preconditions and institutional forms necessary for such strategies to be able to effectively express (and resolve) the sources of popular dissatisfaction with the food sector.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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Indigenous Perspectives and Gene Editing in Aotearoa New Zealand

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Gene editing is arguably the most significant recent addition to the modern biotechnology toolbox, bringing both profoundly challenging and enabling opportunities. From a technical point of view the specificity and relative simplicity of these new tools has broadened the potential applications. However, from an ethical point of view it has re-ignited the debates generated by earlier forms of genetic modification. In New Zealand gene editing is currently considered genetic modification and is subject to approval processes under the Environmental Protection Authority (EPA). This process requires decision makers to take into account Māori perspectives. This article outlines previously articulated Māori perspectives on genetic modification and considers the continuing influence of those cultural and ethical arguments within the new context of gene editing. It also explores the range of ways cultural values might be used to analyse the risks and benefits of gene editing in the Aotearoa New Zealand context. Methods used to obtain these perspectives consisted of (a) review of relevant literature regarding lessons learned from the responses of Maori to genetic modification, (b) interviews of selected 'key Maori informants' and (c) surveys of self-selected individuals from groups with interests in either genetics or environmental management. The outcomes of this pilot study identified that while Māori informants were not categorically opposed to new and emerging gene editing technologies *a priori*, they suggest a dynamic approach to regulation is required where specific uses or types of uses are approved on a case by case basis. This study demonstrates how the cultural cues that Māori referenced in the genetic modification debate continue to be relevant in the context of gene editing but that further work is required to characterize the strength of various positions across the broader community.

Keywords: indigenous, gene editing, Māori, ethics, regulation, New Zealand

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BACKGROUND

Gene Editing

All living organisms contain long molecules of DNA which are inherited between generations. The total sum of DNA from an organism is referred to as its "genome," which itself includes all of its "genes." An organism's DNA affects how it looks and how it behaves. DNA can change spontaneously, generating new "mutations" (or "variants") that can have a visible effect.

For example several mutations of the eye color character have occurred during human history. Similarly, key mutations in traits such as grain yield and milk production selected by farmers have contributed to crop domestication and agriculture. In the last century and since the discovery of the structure and importance of DNA as the molecule encoding life, several techniques have been developed to artificially alter genes and genomes. The latest of such technique is "gene editing."

Gene editing is a technology that enables scientists to alter the DNA of an organism in a very precise way. The technique relies on the CRISPR-Cas9 (clustered, regularly interspaced, short palindromic repeats—associated protein Cas9) system that is capable of recognizing a specific DNA motif in the genome. The Cas9 protein then cuts it the DNA sequence to produce double stranded breaks, which can be fixed by the repair system in a non-homologous end joining manner with variable sizes of insertions or deletions and therefore generates DNA mutations (Jinek et al., 2012; Chang et al., 2016). Gene editing has some key differences with other techniques used to generate DNA mutations. Radiation-based mutation using gamma-ray irradiation generates many DNA mutations across the genome in a random fashion. Radiation is used extensively in plant breeding to generate new traits such as seedless table grapes. Unlike radiation, gene editing only targets a precise location in the genome. Another method that has been used in the last 40 years is genetic modification using transgenics (often referred to as GM or GMOs). The principle of transgenics is to insert an entire gene into the host genome. The inserted gene often comes from a different organism. For example, insect-resistance Bt maize, eggplant, and cotton result from inserting a gene from the bacteria *Bacillus thuringiensis*. Gene editing is being promoted as a more precise technology that could be used to amend an existing gene rather than inserting foreign genes into an organism.

Potential Applications

Gene editing is bringing both profoundly challenging and enabling opportunities for applications in human health, natural resource stewardship and primary production. In medicine, gene editing has already been approved for use in patients to make immune cells attack cancer cells or to mutate HIV virus DNA to stop it from replicating (Tebas et al., 2014; Reardon, 2015). In agriculture, gene editing is being used to create more hardy, nutritious and productive plants and animals (Shan et al., 2013; Wang et al., 2015). In conservation, researchers may be able to use gene editing to introduce a sterility gene into a pest as part of a pest-eradication programme, or spread a malaria resistance gene in mosquitoes (Hammond et al., 2016).

The broad range of potential applications of gene editing has the potential to re-ignite ethical debates generated by earlier forms of genetic modification. In Aotearoa New Zealand, genetically modified organisms (GMO's) have been regulated since the establishment of the Environmental Risk Management Authority (ERMA) through the Hazardous Substances and New Organisms Act (1996), responsibilities which transitioned to the Environmental Protection Authority (EPA) through the Environmental Protection Authority Act (2011). Early applications of gene technologies to create transgenic organisms were met with public outrage, leading to the establishment of a Royal Commission of Inquiry into Genetic Modification. Over 10,000 public submissions were considered by the Commission in the development of its report, which has led to no genetically modified crops being grown in New Zealand (Royal Commission on Genetic Modification, 2001). Māori were significant contributors to the debates on genetic modification and regulatory processes provide specific recognition of Māori values within decision-making processes for new organisms including GMOs (Cram et al., 2000; Environmental Protection Authority, 2016).

The variability surrounding the regulation of gene editing in the international context has led to the recent establishment of a Royal Society of New Zealand (RSNZ) Gene Editing Panel to engage the public in discussions and provide advice to the New Zealand Government on potential options for regulation (Royal Society of New Zealand, 2016, Royal Society of New Zealand, 2017a,b). Gene editing is currently considered genetic modification and therefore non-human gene-edited organisms are classified as "new organisms" and therefore are subject to approval processes under the EPA, a process which includes the incorporation of Māori perspectives.

Literature Review

The literature suggests there are more Māori positioned on the anti-GM end of the spectrum (Gardiner, 1997; Cram et al., 2000), however a distinction is apparent between GMOs for commercial production with no clear cultural or environmental benefits and those that might provide direct community benefit (Roberts and Fairweather, 2004; Smith et al., 2013). GM projects that had a clear benefit or genuine contribution to communities and the environment were received more positively. The literature provided some consistent messages about the key Māori cultural concepts and values relevant to biotechnology and genetic research. There is a general consensus that *whakapapa* (genealogy) sits as the key concept for Māori communities. The second most commonly acknowledged cultural value is *mauri* (life essence), followed by *mana* (power/authority) and *kaitiakitanga* (guardianship). A number of other Māori terms are also used in the course of writing about Māori and biotechnology issues such as *mātauranga* (indigenous knowledge), *tikanga* (protocols), *Papatūānuku* (earth mother), and *tangata whenua* (indigenous people, literally people of the land). Culturally based concepts have also been associated with specific functions. For example, concepts related to "Consultation and Relationships" include *kawa* (customary principles), *tika* (right, correct), and *manaakitanga* (to care for, look after), while *tapu* (sacred, restricted), *taonga* (precious), *wairua* (spirit) are associated with the status of DNA and *tākoha* (gift) to the sharing of DNA (Beaton et al., 2016; Hudson et al., 2016a). **Table 1** highlights the most commonly discussed Māori concepts and values.

This article outlines previously articulated Māori perspectives on genetic modification, then considers the continuing influence of those cultural and ethical arguments within the context of recent developments in gene editing, and finally explore with key Māori informants how cultural values might be used to

TABLE 1 | Reference to key Māori concepts.

Concept:	Whakapapa	Mauri	Kaitiakitanga	Mana
Te Momo, 2007	•	•	•	
Te Mata Ira (Hudson et al., 2016a)	•	•	•	•
Hutchings and Reynolds, 2005	•	•	•	
Mead, 2017	•	•		
Roberts, 2005	•	•		•
Tipene-Matua, 2006				•
Wilcox et al., 2008	•	•		•
Environmental Protection Authority, 2016		•	•	•
He Tangata Kei Tua (Hudson et al., 2016b)	•	•	•	•
Waitangi, 2011	•	•	•	

analyse the risks and benefits of gene editing in the Aotearoa New Zealand context.

METHODS

This project is a part of a New Zealand government funded research programme led by Plant & Food Research Ltd on "Turbo-breeding for New Zealand's plant industries" primarily focusing on the adoption of new breeding technologies in the horticulture sector. A component of the project explores the co-innovation interface, the interface of cultural and commercial interests and concerns, with a view to identifying processes that support Māori organizations to participate in research and commercialization activities involving gene editing technologies. The aim of this part of the project was to assess the on-going relevance of Māori concepts in the context of gene-editing. Data was collected through three key activities; a literature review; key informant interviews; and an electronic survey.

A review of 38 key publications between 2005 and 2017 provided the foundation for this project. The review covered literature relating to Māori and biotechnology, genetic modification, and genetic research with a particular focus on the Māori values, concepts and perspectives that have previously been articulated. The review became the basis for a discussion document, Māori Perspectives & Gene Editing: A Discussion Paper (Mead et al., 2017), which informed preliminary discussions with a number of agencies and Māori networks, such as the EPA's Ngā Kaihautu Tikanga Taiao (Māori Advisory Body), Te Herenga Network (National Māori Network of Iwi Environmental Practitioners), Te Tira Whakamataki (the Māori Biosecurity Network), the Biological Heritage National Science Challenge, and public consultation exercises on gene editing led by the Royal Society of New Zealand.

Ethics Approval was gained from the University of Waikato's delegated research ethics committee within the Faculty of Māori and Indigenous Studies for the collection of data from key informant interviews and an electronic survey which used the same set of open questions. The questions emerged from the key concepts identified in the literature review and focused on the potential applications and opportunities associated with gene editing, the key issues and concerns associated with gene editing, whether gene editing should be considered the same as genetic modification, and the relevance of key Māori values and concepts (**Table 1**) to understanding gene editing.

Eight key informants (2 × males, 6 × females) were purposefully selected from the researchers networks with interests in plant health, environmental health, human health, business, research, public understanding, and public policy to provide a range of informed Māori perspectives. Four of the key informants are active researchers albeit not in genomic sciences, and the others have strong relationships with researchers and communities. They were chosen because of the roles they play as translators between science teams and Māori communities and general familiarity with both scientific research and Māori perspectives. Key informants were interviewed separately and an electronic survey was shared with members of the Te Herenga Network (National Māori Network associated with the Environmental Protection Authority) and the SING Alumni Network (Summer Internship for Indigenous Genomics programme). The survey, which resulted in nine additional responses, was used to broaden the range of perspectives and reduce the potential bias associated with the key informant interviews. All participants were Māori and the responses to each question in the interviews (KI) and the survey (SR) were grouped and analyzed manually by the researchers using guided thematic analysis (Coffey and Atkinson, 1996). The process of coding empirical material to the research questions and emerging themes, was conducted across key domains, including potential benefits, concerns, and the relevance of Māori values and concepts.

RESULTS

Interview and Survey Responses

What Do You See Are the Potential Applications/Opportunities Associated WITH GENE Editing?

Participants saw different opportunities for gene editing to support their communities aspirations in horticulture, conservation, maintaining the health and biodiversity of the environment, or to address human health issues. A range of potential applications were identified including preservation of endangered species of plants and animals, new health related therapies, protecting biodiversity, creating health and food security, sequencing of rare threatened and endangered endemic species (and their medical chemotypes), human health, environmental restoration, sustainable enterprise, pest control, and pest eradication. One participant noted a primary interest as ensuring that gene editing was stopped.

What Do You See Are the Key Issues/Concerns That Arise From Use of Gene Editing?

Participants concerns about the use of gene editing centered on the risks of adopting this technology. The risk of unintended consequences, whether it is possible to do rigorous

assessments of the potential downstream effects, the reversibility of any genetic modifications, ethical considerations, and effect on kaitiaki (guardians) responsibilities were highlighted. Participants identified an innate risk from a cultural perspective that needs to be managed to limit unethical or unauthorized modifications. A number of the participants expressed a view that mixing genetic material from different species is unnatural and there was also a degree of anxiety about editing an organisms genome especially for economic gain.

An issue was raised about the benefits to society and concerns that technology favors the wealthy and tends to increase inequities through the commodification of resources. Some participants had concerns about the use of gene editing in the environment and others were more concerned about its use in humans. Specific issues were raised in relation to editing genes in the human germline because it is passed down to future generations and there is no information about the long-term effects. Risks to the environment were associated with the release of modified organisms into the environment where commercial interests exclude wider community benefits. Concerns were also expressed about the level of experimentation required before benefit emerges and how the community are kept up to date with what types of activities are underway. Fostering public conversations about genetic modification are necessary as public understanding lags well behind the current state of expert knowledge and technical capability.

> In NZ law, gene editing is considered the same as genetic modification. In other countries gene editing is treated differently from GM. Do you think we have the right legal approach in NZ or do you think the law should be amended in light of the developing technology?

Strictly speaking gene-editing is a form of genetic modification and while some participants considered gene editing and genetic modification to be the same thing, others saw a spectrum of gene technologies.

> "This highlights an issue regarding terminology and general understanding of genetic technologies. Genetic modification encompasses a spectrum of technologies with transgenics on one end and modern gene-editing on the other." (Survey Response, SR7)

It was generally accepted that a regulatory regime should cover both Genetic Modification and Gene editing as a precautionary approach was necessary. The level of regulation ranged from "*No GMO in New Zealand*"*(SR5)* to "*Only in the laboratory and total containment*" *(SR6)* to "*after strict substantive and procedural decision-making*" *(SR4)*. Participants recognized the inconsistencies arising from the treatment of all genetically modified organisms in the same way and while there was some sympathy for the differences between technologies, the status quo allows all gene technologies to be monitored appropriately. Some of the participants felt that inter- and intra-specific genetic modification should be treated differently and possibly on a case by case basis. However, any change would require more consultation with Māori and the wider public to assess the effect on Māori rights and interests.

Do You Think Gene Editing Can Support Kaitiaki Responsibilities and Under What Circumstances?

Gene editing technologies are potentially one tool in the toolbox to protect and save species or be used to enhance health. Considerations will include the intent of the use, how kaitiaki understand the science, and whether its use disrupts or enhances the relationships they have with taonga species.

> "Values-based organisations can use technologies to support their aspirations and in that respect they have a duty to explore all avenues that support kaitiaki responsibilities for taonga species." (Key Informant; KI1)

The participants generally felt that gene editing could support kaitiaki to exercise their responsibilities and in some situations will be forced to consider extreme options like gene-editing to deal with intractable "wicked problems," where all the choices appear on a spectrum of ethically challenging options. This might arise in terms of pest control as a tool to protect and enhance wildlife or as a way of correcting a variant of a gene known to be responsible for a disease. Where species extinctions are occurring, kaitiaki might explore gene-editing as an option. These decisions would be by hapū (sub-tribes) or iwi (tribes) as to whether this is an appropriate technology to support their responsibilities.

Do You Think Whakapapa Is Affected if You Introduce DNA Into One Species From Another Species? Is This the Same Case if You Edit DNA Within the Same Species?

All the participants thought that whakapapa was affected by introducing DNA from one species into another through genetic modification. Some kaumatua (elders) are against interspecies transfer while others are less concerned, and the participants expressed mixed opinions in relation to the effect of gene editing on whakapapa. Some participants felt it was dependent on the extent of the edit as some variation within the same species or sub-species is expected. The effect on whakapapa was also thought to be connected to the relationality between the species sharing or exchanging DNA. Where DNA associated with genes that are shared in different species is exchanged, this will have less impact on whakapapa. However, if a transferred gene does not have a naturally occurring sequence, then this could be seen to be cutting across whakapapa links. Some felt that gene editing definitely affects whakapapa but that could also be in a positive direction.

> "Altering genes changes the genetic make-up of an individual but can be viewed similarly to an organ transplant. Whether it is ethical to change or introduce DNA into a species is another thing. For me whakapapa is lineage and your ties to whanau and your ancestors and that doesn't change with the introduction of foreign DNA. It could be viewed as enhancing your lineage to some or diluting it to others." (SR8)

Is There a Difference Between Applying Gene-Editing for "Taonga Species" and Introduced or Commercially Produced Species?

There were mixed views on whether there was a difference when applying gene editing to a taonga (precious) species or an introduced or commercially produced species. It was clear that Māori should have a say in relation to both but the difference arose from the nature of the relationship Māori have with taonga species through Treaty of Waitangi obligations and indigenous rights.

> "Māori should have the final say for approving editing in taonga species. For introduced or commercially produced species, all groups in NZ should be consulted (including Māori)." (SR3)

> "The only difference between gene editing in taonga species and introduced or commercial species is that our responsibility to taonga species means that there is a much greater impetus to ensure minimal disruption to the whakapapa, mana, and mauri of these species."(SR7)

Some felt that all species are interdependent and therefore taonga but would need to consider their different degrees of importance and how to deal with hybridity. Exotic species have been incorporated within rongoa Māori (traditional medicine) formulations since colonization and as such Māori walk in two worlds. Regardless of whether gene editing was being used for taonga species or other species the risks involved and ethical considerations are the same.

Is Mauri of a Species/Person Affected if the Gene-Edit Mimics a Natural Mutation/Variant?

The effect on mauri (life essence) represents one of the key moral dilemmas associated with genetic modification. Most people believe the life force is changed by gene editing but there were variations on this theme. Some felt that mauri was not affected if the change occurs naturally, and others mentioned that the effect on mauri can be both positive and negative. The effect on the mauri is related to the nature and size of the change including the heritability of the new characteristics. There was a concern expressed around the unintended effects of genetic variation and whether that changes the long term resilience of a species.

> "Naturally occurring mutations/variants often occur due to environmental changes which enable adaptation to occur in the species. I feel a gene edit can either enhance or reduce Mauri depending on the phenotypic outcome of that species." (SR8)

> If mana is recognised through Māori leading the project and the research objectives are to benefit Māori whanau (families), hapū (sub-tribe), or iwi (tribe) are the same concerns still relevant?

There was a general belief amongst the participants that enhancing mana through Māori leadership would increase the level of engagement and acceptance to a project involving gene editing however the same concerns about gene editing still apply. There was a feeling that all gene editing, whether it be for conservation, commercial, or indigenous interests, should be subject to standardized processes even though cultural protocols are specific to each tribal authority.

> "I don't believe it is necessary for Māori to lead a project, but it is critical that Māori are at least partners, to ensure that Mana is recognised." (SR7)

Participants thought it was important to define the space and reasoning for using gene editing technology including how the project and any data/intellectual property would be managed. Māori input into this process is necessary especially for taonga species and Māori should also consider the impact of our decisions on other indigenous peoples, especially if the intended use is to eradicate a species endemic to another country.

DISCUSSION

A small but significant portion of research exists concerning indigenous peoples' responses to bio/nano-technology, generally establishing the 'indigenous position' as one strongly against these developments and their commercialization (Gardiner, 1997; Harry et al., 2000; Leier, 2002; Reynolds and Smith, 2002; Hutchings and Reynolds, 2005; Mead and Ratuva, 2007). The prevailing critique has been that most 'bio/nanotechnology projects are inconsistent with Māori values, impinge on Māori rights and sovereignty, and continue a process where indigenous cultures, values, knowledge systems and even lives are marginalized and undervalued (Cram et al., 2000; Roberts and Fairweather, 2004; Cram, 2005; Hutchings and Reynolds, 2005; Te Momo, 2005; Hutchings, 2009).

Despite inclusion in existing regulatory processes and more positive interactions over the past decade (Hemara, 2006; Cheung et al., 2007; Te Momo, 2007; Hudson et al., 2012, 2016c) and the responses of participants in this project, a widespread social license for the use of gene-based technologies amongst the Māori community is unlikely in the short term. Generally, Māori do not oppose new and emerging gene editing technologies *a priori*, but instead raise concerns as to how the technologies should be used and the rationale, objectives and consequences of choosing them. Individual subjectivities inform the process as personal preferences for particular technologies are grounded in their own values, experiences and knowledge (Te Momo, 2007; Smith et al., 2016). The experience of the participants played an interesting part in the identification and management of potential risk. Sometimes those with backgrounds in particular fields, for example the environment, were more comfortable with its potential application in that domain and highlighted risks associated with other areas like health. In other cases, the reverse was true where experience in a field highlighted the specific concerns for application in that domain. The general discomfort all the participants expressed was reflected in the desire for appropriate regulation and a sense that there will always be justifiable use-cases and unpalatable use-cases. This anticipates a more dynamic approach to regulation where specific uses or types of uses are approved on a case by case basis.

Māori participation in discussions on gene technologies is as much cultural and political as scientific [(Cram, 2005),

TABLE 2 | Impact of gene editing on Māori concepts/values.

Value/Concept	Value enhancement	Value diminishment
Whakapapa	Gene Editing does not involve the transfer of genes between species—**whakapapa** can be maintained and enhanced through the continued well-being of the species	Gene Editing introduces foreign DNA or involves changing the genome inter-generationally with negative consequences—**whakapapa** is diminished
Mauri	Gene Editing is being used to support human or environmental health—**mauri** is enhanced	Gene Editing is used for inappropriate purposes—**mauri** is diminished
Kaitiakitanga	Gene Editing may support or enhance resilience of ecosystems—**kaitiakitanga** is enhanced	Gene editing has unknown effects on the well-being of organisms and the ecosystem—**kaitiakitanga** is diminished
Mana	Māori are able to choose how gene editing is applied—**mana** is enhanced	Māori have no say in discussions about how gene-editing is used—**mana** is diminished
Taonga	Gene-editing supports commercial and cultural interests as identified by Māori—**Taonga** status is enhanced	Gene-Editing is used in ways that negatively affect taonga species—**Taonga** is diminished
Tapu	The use of gene editing is restricted and subject to a precautionary principle—**Tapu** is enhanced	The use of gene editing is widely approved for any purpose—**Tapu** is diminished
Wairua	Māori are involved in decision-making and are comfortable with the uses of the biotechnology—**Wairua** is enhanced	Māori are not involved in decision-making and don't know what's going on—**Wairua** is diminished
Kawa	Robust consultation and decision-making processes are developed, and Māori values inform the use of gene editing—**Kawa** is enhanced	Māori values are excluded from policy development and decision making processes—**Kawa** are diminished
Tika	Benefits of the research are shared equitably across the community—**Tika** is enhanced	Benefits are captured by commercial or special interest groups—**Tika** is diminished
Manaakitanga	Cultural protocols are developed to support the use of gene-editing—**Manaakitanga** is enhanced	No cultural support for Māori participation in gene editing activities—**Manaakitanga** is diminished
Tākoha	Recognition of Māori rights and interests to genome sequences and responsibilities associated with this—**Tākoha** is enhanced	No recognition of Māori rights, interests or responsibilities—**Tākoha** is diminished
Whanaungatanga	The use of gene editing supports a strengthening of whanau by addressing a key issue or concern—**Whanaungatanga** is enhanced	The use of gene editing does not contribute to addressing whanau issues or creates disruption in the whanau—**Whanaungatanga** is diminished

p. 62; (Hudson et al., 2010; Smith et al., 2013)]. Discussions on gene-based technologies cannot be divorced from discourse on land ownership and control over natural resources and debates traverse the spectrum of philosophical, social, ethical, and technical dimensions (Smith et al., 2013). Māori perspectives on biotechnology/genetic technologies frequently reference core cultural concepts as conceptual markers, derived from mātauranga Māori (indigenous knowledge) and tikanga Māori (Māori values), which are intrinsic to an indigenous way of viewing and living in the world. These cultural cues provide the basis for describing the cultural logic that underpins engagement in a culturally acceptable manner (Cram et al., 2000; Hudson et al., 2010, 2016c; Smith et al., 2013). This research demonstrates the cultural cues that Māori referenced in the genetic modification debate, and subsequent conversations about biotechnologies, continue to be relevant in the context of gene editing. The Māori concepts of *whakapapa* (genealogy), *mauri* (life essence), *mana* (authority), and *kaitiakitanga* (guardianship) feature prominently. *Whakapapa* and *mauri* relate to the organism itself and *mana* and *kaitiakitanga* refer to the relationship that people have with that organism. *Whakapapa* is a key reference point when talking about genetics or genomics (Hudson et al., 2016a) because it provides the foundation for how Māori construct their identities and their relationships with other species (Roberts, 2005, 2013; Hudson et al., 2007). *Mauri* relates to the distinctive and special nature of an organism including its right to life (Hudson et al., 2010; Mead, 2017). *Mana* relates to authority and provides a responsibility to act in the interests of the broader community (Mead, 2017). The expression of *kaitiakitanga* enhanced through the recognition of mana whenua status presupposes that Māori have authority over their lands and resources and that the use of gene technologies is done in ways that supports these outcomes (Thompson, 2018).

Participants in this study suggested that the effect of gene editing on Māori values is not always in a negative direction and it was suggested that whakapapa, mana, mauri, and kaitiakitanga might be enhanced through the use of gene editing technologies. This suggests that values based frameworks developed for other gene based technologies (Wilcox et al., 2008; Hudson et al., 2016c) will remain relevant in for gene editing applications. What the enhancement or diminishment of these Māori values might look like is summarized in **Table 2**.

According to New Zealand law, gene editing is not deemed distinct, rather it is seen as one of many processes, tools, methods, or products of genetic modification and as such is subject to the same regulations as any other GMO. A key issue here is whether it makes sense to regulate a technology rather than regulating the outcome or product of the technology. Gene editing will allow the generation of outcomes/products similar, or identical to those generated by technologies not covered by legislation. Gene editing does not necessarily insert

foreign DNA into the genome of the host organism and the DNA mutations resulting from gene editing involve small DNA sequence changes that cannot be differentiated from natural ones, even using modern sequencing technologies. The RSNZ is currently engaging the New Zealand public in debates about gene editing through a series of discussion documents (Royal Society of New Zealand, 2016; Royal Society of New Zealand, 2017a,b) and a public speaker series. The topic has recently been brought to back into the spotlight by comments from the outgoing NZ Chief Government Science Advisor who suggested that while the use of gene technologies continued to be heavily debated, from a scientific point of view "*There are no significant ecological or health concerns associated with the use of advanced genetic technologies,*" and that we need to engage society in debate that is "*more constructive and less polarized than in the past*." (Science Media Centre, 2018) (https://www.sciencemediacentre.co.nz/2018/07/02/changing-of-the-chief-scientist-guard-in-the-news/). The participants in this study wanted to engage in a constructive discussion to create a robust regulatory framework that addresses gene editing on a case by case basis and utilizes Māori values within the decision-making process.

SUMMARY

Gene editing is the most recent gene based technology promising benefits across health, environmental, and commercial domains. It emerges in the wake of decades old, ethically charged, debates about GMOs and transgenic applications which seared controversy about gene technologies into the public consciousness. As the New Zealand government considers whether to change the regulations around gene editing technologies it is supporting a new round of public consultation exercises. While Māori have expressed strong views and opposition to genetically modified organisms in the past, it is important to assess the continuing influence of those perspectives.

The participants demonstrated that Māori values and cultural concepts continue to inform Māori perspectives on biotechnology and their regulation. Whakapapa, mauri, mana, and kaitiakitanga provide a cultural scaffold for considering the philosophical, moral, ethical and technical dimensions relevant to the use of gene editing technologies. It is apparent that a range of views exist across the Māori community and that participants are prepared to consider the use of gene editing on a case dependent basis, especially where it aligns with Māori worldviews. Incorporating Māori values into decision-making processes could provide a balancing factor to ensure broader community interests remain a key consideration in the future use of gene editing technologies. The application of gene editing technologies heightens societal sensitivities about inequities as their use tends to prioritize commercial interests over community benefit (Smith, 2016). However, further research is required to characterize the strength of the various positions identified in this pilot study and to explore its relevance to other indigenous communities.

AUTHOR CONTRIBUTIONS

MH: contribution to framing and writing, analysis of surveys and interviews, primary editor. AM: primary data collector, contribution to framing and writing, analysis of literature, and development of tables. DC: contribution to framing and writing of scientific context, review of analysis and manuscript. NR, SM, and PW: data collection, review of analysis and manuscript. AA: contribution to writing of scientific context, review of analysis and manuscript, principal investigator.

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GLOSSARY

Aotearoa	New Zealand
Hapū	Sub-tribal grouping
Hui	Tribal meetings
Iwi	Tribe
Kaitiakitanga	Guardianship
Kawa	Customary principles
Mana	Power/authority
Manaakitanga	To care for/to look after
Mātauranga	Indigenous knowledge
Mauri	Life essence
Ngā Kaihautu Tikanga Taiao	Māori advisory body for Environmental Protection Authority
Papatūānuku	Earth mother
Rongoa Maori	Traditional medicine
Tākoha	Gift
Tangata whenua	People of the land
Taonga	Precious
Tapu	Sacred/restricted
Te Ao Māori	Māori worldview
Te Herenga Network	National Māori Network of Environmental Practitioners
Te Tira Whakamātaki	Māori Biosecurity Network
Tika	Right/correct
Tikanga	Protocols
Wānanga	Traditional learning spaces and activities
Whakapapa	Genealogy
Whānau	Family, includes extended family
Wairua	Spirit

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Roads Forward for European GMO Policy—Uncertainties in Wake of ECJ Judgment Have to be Mitigated by Regulatory Reform

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This article gives an overview of legal and procedural uncertainties regarding genome edited organisms and possible ways forward for European GMO policy. After a recent judgment by the European Court of Justice (ECJ judgment of 25 July 2018, C-528/16), organisms obtained by techniques of genome editing are GMOs and subject to the same obligations as transgenic organisms. Uncertainties emerge if genome edited organisms cannot be distinguished from organisms bred by conventional techniques, such as crossing or random mutagenesis. In this case, identical organisms can be subject to either GMO law or exempt from regulation because of the use of a technique that cannot be identified. Regulatory agencies might not be able to enforce GMO law for such cases in the long term. As other jurisdictions do not regulate such organisms as GMOs, accidental imports might occur and undermine European GMO regulation. In the near future, the EU Commission as well as European and national regulatory agencies will decide on how to apply the updated interpretation of the law. In order to mitigate current legal and procedural uncertainties, a first step forward lies in updating all guidance documents to specifically address genome editing specifically address genome editing, including a solution for providing a unique identifier. In part, the authorization procedure for GMO release can be tailored to different types of organisms by making use of existing flexibilities in GMO law. However, only an amendment to the regulations that govern the process of authorization for GMO release can substantially lower the burden for innovators. In a second step, any way forward has to aim at amending, supplementing or replacing the European GMO Directive (2001/18/EC). The policy options presented in this article presuppose political readiness for reform. This may not be realistic in the current political situation. However, if the problems of current GMO law are just ignored, European competitiveness and research in green biotechnology will suffer.

Keywords: GMO regulation, future policy, CJEU C-528/16, directive 2001/18/EC, genome editing, new genetic modification techniques (nGM), CRISPR/Cas, directed mutagenesis

INTRODUCTION

This article gives a brief overview (section The ECJ Judgment and Its Ramifications) of what kinds of problems the European Union's (EU) regulatory framework for genetically modified organisms (GMOs) faces in the wake of the ECJ judgment *Confédération paysanne a.o.* on directed mutagenesis (ECJ, 2018). In a second step (section Roads Forward), policy options are discussed that could avert a crisis for European agricultural innovators and a crisis of enforcement for regulatory agencies. This crisis results from the inadequacy of the current regulatory framework to proportionately, predictably, and enforceably regulate organisms that have been bred by genome editing.

The term genome editing as used in this article refers to a variety of new techniques, specifically techniques of directed mutagenesis using CRISPR/Cas9 (or similar site-directed enzymatic DNA cleavage or base-editing in the sense of SDN1/2; see Podevin et al., 2013), or other techniques such as oligonucleotide directed mutagenesis. These techniques allow breeding organisms in which the genetic material has been altered to an outcome that is genetically indistinguishable from the possible outcomes of conventional breeding, i.e., traditional breeding by crossing and natural variation as well as conventionally used techniques of chemically or radiation-induced random mutagenesis. Throughout this article, the term "genome edited organism" (GEO) refers to an organism that has been altered by such techniques to an outcome that cannot be distinguished from a conventionally bred variety or a naturally occurring variant thereof. Note that this use of the term does not include every alteration that is possible with these same techniques; especially it excludes transgenic modifications (e.g., SDN3 with CRISPR/Cas9 and donor DNA with a sequence from another species as repair template; see Podevin et al., 2013). That is to say, the definition of GEO used throughout this article is outcome based, not process based. The same techniques can also be employed to alter an organism in a way that is easily distinguishable from conventionally bred varieties or a naturally occurring variant. The focus of this article, however, is on organisms that cannot be distinguished.

A reference scenario for the cases discussed in the following is a GEO that contains a point mutation, which provokes a frameshift in the DNA code or changes the code to form a stop-codon, both of which may knock-out a certain gene (loss of function mutation). For example, resistance to powdery mildew in barley occurs naturally and is caused by a loss of function mutation in the *Mlo* gene. Genome editing can place a point mutation in the equivalent of that gene in barley varieties that are not mildew resistant yet or in other plants that are susceptible to mildew such as wheat or tomato (e.g., Acevedo-Garcia et al., 2017; Nekrasov et al., 2017). A point mutation, such as a frameshift mutation in the *Mlo* gene in a barley variety, can be detected by sequencing if the sequence of the parent organisms is known. But it is often not possible to identify the cause of the mutation, i.e., establish whether the mutation occurred naturally or by conventional random mutagenesis and subsequent backcrossing or through directed mutagenesis (see e.g., Lusser et al., 2012; Bartsch et al., 2018; Grohmann et al., 2019). Indeed, if the only alteration introduced is nothing else than one single base mutation, then it is not at all possible to identify the technique used. The reason for this is that a wide variety of mutations in the genome occurs constantly in nature, most often during reproduction. In particular, double strand breaks of the DNA occur naturally and are subsequently repaired by the natural cellular repair mechanisms of non-homologous end joining and homology-directed repair. Those are the same kind of breaks in the DNA that can be introduced by the CRISPR associated enzyme CAS9 (and others). And the subsequent cellular repair mechanisms (non-homologous end joining and homology directed repair) are also the same as used in directed mutagenesis used in directed mutagenesis. In addition, a fabricated nucleic acids template (donor DNA) may be introduced in the lab to control the outcome of the mutation event and, e.g., accurately reproduce an alteration that is known to have emerged naturally in that variety. In consequence, for some small alterations that blend well into the genetic background (such as a single nucleotide frameshift mutation) it is impossible (without additional knowledge such as e.g., lab reports) to identify whether they occurred naturally or whether they are human made. Such identification based on sequence data alone is indeed even impossible on theoretical grounds, unless a technique were to preferentially incorporate certain isotopes or leave an epigenetic pattern, which however is not currently known and most certainly might only apply to single techniques under very specific conditions. It is true that if more genes or more copies of a gene (e.g., in polyploid organisms) have been altered in the same fashion, probabilistic considerations could provide evidence for the use of genome editing or similar techniques. However, data on intraspecies variation (for specific varieties and even for specific loci) is quite sparse for most plant species and even for many agriculturally relevant crops (e.g., see Jiao et al., 2012 for an assessment of genetic changes in conventional maize breeding and note how sparse the data seems to be for this major crop). The lack of knowledge on natural occurrences of mutations makes it difficult to establish whether a mutation is reasonably possible to occur naturally or not (a brief overview on comparison with naturally occurring mutations is given by Custers et al., 2019). Thus, on sequence data alone, a small alteration as discussed in this scenario is only detectable if a comparator sequence is given. If no relevant information is given in addition to the sequence—e.g., when controlling imports of agricultural commodities–small alterations made by genome editing can often neither be detected (because of the reasons listed above) nor is the technique identifiable that led to the alterations. In particular, this would be the case in our reference scenario of *Mlo* locus altered barley.

For further discussion on existing and upcoming detection and identification strategies, see Grohmann et al. (2019).

THE ECJ JUDGMENT AND ITS RAMIFICATIONS

On July 25th 2018, the European Court of Justice delivered its judgment in a case concerning among others the scope of the

mutagenesis exemption in the European GMO Directive (ECJ, 2018). For a detailed analysis of the judgment see e.g., Seitz (2018) or Faltus (2018), a brief analysis in English is given by Purnhagen et al. (2018b) and Garnett and Beck (2018). The ruling has the following implications:

(1) The GMO definition in Art. 2(2) i.c.w. Annex I A of the GMO Directive 2001/18/EC[1], taking into account the reference to techniques of genetic modification in Art. 3 i.c.w. Annex I B, applies to all organisms in which the genetic material has been altered by techniques of mutagenesis (ECJ, 2018, 32-38). This means that it does not matter what the alterations to the genetic material of an organism are and whether they are extensive or insignificantly small, any organism bred with a technique of (random or directed) mutagenesis is in legal terms a GMO.

(2) The exemption for organisms produced by mutagenesis from the obligations of the GMO Directive (mutagenesis exemption in Dir. 2001/18/EC, Art. 3 i.c.w. Annex I B) applies only to techniques of mutagenesis that "have conventionally been used in a number of applications and have a long safety record" (the Court here refers to Rec. 17: ECJ, 2018, para 44-46, 48-53). Referring to the precautionary principle (para 47-50), the Court further interprets the aforementioned wording of Rec. 17 as not applying to techniques "which have appeared or have been mostly developed since the Directive was adopted" (ECJ, 2018, para 51). Thus, techniques of genome editing—which have been mostly developed after adoption of the Directive in the year 2001—are not exempted from the obligations of GMO law.

In consequence, the ECJ does not differentiate between GEOs and transgenic organisms in all respects. Both are regulated as GMOs and subject to the same obligations, i.e., risk assessment, expiring market approval, post-release monitoring, liability, labeling. Indeed, the Court's reading of the Directive's GMO definition and mutagenesis exemption also applies to "downstream" directives and regulations that interact with the GMO definition i.c.w. Annex IB of the GMO Directive (ECJ, 2018, para 60-68; Purnhagen et al., 2018b).

This verdict has left scientists, breeders as well as officials from regulatory agencies perplex (the competent authorities of several countries, among them Sweden and Germany, assumed a differential treatment of GEOs before the ECJ judgment, e.g., see BVL, 2017; Eriksson, 2018a). The ruling ultimately reflects the fundamental problem of European GMO law: Long before the request for a preliminary ruling had been addressed to the ECJ, the legislator failed to acknowledge and incorporate decades of technologic development, especially the capacity of new techniques to alter the genetic material of organisms to a result that is indistinguishable from conventional breeding or natural variation.

Three major uncertainties result from the failure to account for technological change:

First and foremost, regulatory agencies will have a hard time implementing the verdict because they do not have the means to enforce compliance with GMO legislation in the case of fraudulent or unintentional non-declaration (cf. Faltus, 2018). Several large exporting nations of agricultural products outside of the EU have chosen to regulate (at least some) GEOs no different from conventionally bred varieties, thus not requiring any tracing or labeling of those GEOs (cf. Sprink et al., 2016; BMEL, 2018; Duensing et al., 2018; Wolt and Wolf, 2018). Non-declaration is then particularly likely to occur in international trade with agricultural commodities and it is precisely where regulatory agencies will fail for a variety of reasons. GEOs are in practice not distinguishable from conventionally bred varieties (discussion above). Since the technique is regulated as conventional breeding in several non-EU jurisdictions, soon a large number of varieties will be brought to market outside of the EU, without any notification procedures in most countries. European regulatory agencies would now have to somehow keep track of all of them, in order to identify them. This affects the enforcement of the regulations on deliberate release of GMOs (as generally laid down in the Directive 2001/18/EC), the enforcement of the regulations on (unintentional) transboundary movements of GMOs (as laid down in Regulation EC No 1946/2003[2]), the ability of authorities to enforce the compliance with traceability regulations (as laid down in Regulation EC No 1829/2003[3] and No 1830/2003), the ability of authorities to enforce the 0.9% tolerance threshold for conventional products contaminated by GMOs (as laid down in Regulation EC No 1830/2003[4]) and finally the ability of authorities to enforce the EU's zero tolerance policy for unauthorized GMOs, particularly in the case of agricultural commodities. For such regulatory enforcement, there are hardly enough inspectors and technical means (such as next-generation whole genome sequencing machines) as well as not adequate means for investigation with which to retrace complex malpractices if only isolated accidental mis-declaration of agricultural goods is given as probable cause. In fact, cases of unauthorized release that have only been noticed after years of malpractice exist even with conventional transgenic GMOs such as in the petunia case (Bashandy and Teeri, 2017), despite the fact that transgenic organisms should be comparably easy to identify. In addition, sooner or later a few rouge breeders that release GEOs in their gardens or fields might come to public attention, similarly to the case of "CRISPR cabbage," where the involved plant geneticist mocked authorities by stating "if I don't tell you, [which alterations I made to the cabbage planted in

[1] *Directive 2001/18/EC of the European Parliament and of the Council of 12 March 2001 on the Deliberate Release into the Environment of Genetically Modified Organisms and Repealing Council Directive 90/220/EEC*. 106, 1–39. Available online at: http://eur-lex.europa.eu/eli/dir/2001/18/oj (accessed March 1, 2018).

[2] *Regulation (EC) No 1946/2003 of the European Parliament and of the Council of 15 July 2003 on Transboundary Movements of Genetically Modified Organisms (Text with EEA relevance)*. 287, 1–10. Available online at: http://data.europa.eu/eli/reg/2003/1946/oj (accessed March 1, 2018).

[3] *Regulation (EC) No 1829/2003 of the European Parliament and of the Council of 22 September 2003 on Genetically Modified Food And Feed (Text with EEA relevance)*. 268, 1–23. Available online at: http://data.europa.eu/eli/reg/2003/1829/oj (accessed March 1, 2018).

[4] *Regulation (EC) No 1830/2003 of the European Parliament and of the Council of 22 September 2003 Concerning the Traceability and Labeling of Genetically Modified Organisms and the Traceability of Food and Feed Products Produced from Genetically Modified Organisms and Amending Directive 2001/18/EC*. 268, 24–28. Available online at: http://data.europa.eu/eli/reg/2003/1830/oj (accessed March 1, 2018).

my garden] you will not find out" (Kupferschmidt, 2018). Public interest in such cases will not cease while regulatory agencies remain unable to enforce the ECJ judgment outside of medium to large-sized breeding companies that apply for commercial permits and patents for their new breeds. Such a situation can erode confidence in Union legislation and in the capability of authorities to keep food and feed products on the European market safe.

Second, uncertainties arise regarding the specifics in the procedure of approval of GMOs (for a brief overview of the assessment procedure and its challenges, see Halford, 2019; Schiemann et al., 2019). Currently passing an application for placing on the market of a GMO (especially for cultivation and in some member states even for field trials) represents a significant regulatory barrier for innovators. The big driver of cost of such an application is its long and unpredictable duration, as a notifier might be asked to provide additional evidence midway through the process of application for authorization of GMO release. Compared to conventional GMOs however, the uncertainties for innovators that want to bring GEOs to market are far superior, because is yet unknown how applications of all kinds will be handled in the case of organisms that resemble conventionally bred varieties (i.e., for field trials, for market-release of non-food&feed and for food&feed). For example, when applying for market-release, notifiers must provide detection and identification techniques (unique identifier) in order to reliably distinguish the GMO in question from any other organisms (Directive 2001/18/EC, Annex III A, sec. II, C, 2(f) and Annex III B, sec. I & II, B, 5, as amended by Commission Directive 2018/350)[5]. Will the regulatory agencies just accept a reference to the specific DNA-sequence of the alteration? Or will they refuse some GEOs on the account that the alteration is too small to allow for reliable identification? Or will they even resort to asking breeders to incorporate a transgenic "marker sequence" to facilitate tracing of GEOs? Similarly, it is not clear yet which methods of identification will be accepted with regard to the environmental risk assessment that is intended to ascertain among others whether the alteration of a GMO is not transferred to the environment (especially if the GEO in question is not necessarily distinguishable from organisms present at the site of release). How regulatory agencies will implement the judgment regarding such issues is yet unknown and most companies will stop product development for the European market under these uncertain conditions.

Third, wide-ranging uncertainties remain concerning the interpretation and the legal effects of the ECJ judgment.

- How safe is safe enough? The ECJ, following the referring court, qualified techniques of directed mutagenesis as techniques of which "the risks for the environment or for human health have not thus far been established with certainty" (ECJ, 2018, para 47). Based mainly on precautionary considerations and on the wording of Recital 17 (which requires a "long safety record"), the Court judged that these techniques are not excluded from GMO law by the mutagenesis exemption. However, knowledge on the safety of these technologies will change over time, as does the length of their safety record. This raises legal uncertainties as to what happens when—at some point in the future—the technology of genome editing can be considered safe with certainty (e.g., if in 20 years genome editing is routinely used for medical applications). Will the judgment have to be interpreted differently then?
- Which technologies exactly are excluded from the obligations of the Directive? The court held that the mutagenesis exemption does not apply to "techniques/methods of mutagenesis which have appeared or have been mostly developed since adoption of the Directive," that is to say since the year 2001 (ECJ, 2018, para 51). Finding out which technologies are meant requires a thorough historical study of breeding techniques in that time. Note that the history is quite intricate. Some techniques of directed mutagenesis have appeared well before the year 2001 and had even some history of development and use in plants, albeit not widespread commercial use (e.g., oligonucleotide-directed mutagenesis in maize or tobacco, see Beetham et al., 1999; Zhu et al., 1999). On the other hand, some techniques of chemically or radiation induced random mutagenesis in use today can be considered to have been mostly developed after the year 2001, even more so regarding commercial applications in plant variety breeding (e.g., ion-beam mutagenesis, see Matsumura et al., 2010). In fact, the methods and techniques used in "conventional" mutation breeding do also progress rapidly and markers, dosages as well as mutagens used today significantly differ from the ones used at the time of adoption of the Directive–technological progress did not stop (a brief overview is given by Oladosu et al., 2016). In short, which technologies exactly are excluded by the judgment (ECJ, 2018, para 51) is not evident. But did the Court really make the interpretation of the mutagenesis exemption dependent on the details of a historical study of mutagenesis breeding techniques? If not, then the judgment must somehow explain a categorical distinction that underlies the historical argument. Indeed, in the buildup of the interpretation of Rec. 17 (ECJ, 2018, para 45-51) the ECJ assigns different categories of risk to all random mutagenesis techniques on the one hand and all techniques of directed mutagenesis on the other. Making reference to the findings of the referring court, the ECJ regards the techniques of directed mutagenesis (in bulk) as a risk on account of their ability "to produce [. . .] varieties at a rate and in quantities quite unlike those resulting from the application of conventional methods of random mutagenesis" (ECJ, 2018, para 48). Thereby it clearly does not hold a historical argument applying to all newer mutagenesis techniques equally, but it differentiates between random techniques of mutagenesis on one side and directed mutagenesis on the other. The Court however did not further clarify wherein the

[5]Commission Directive EU No 2018/350 of 8 March 2018 amending Directive 2001/18/EC of the European Parliament and of the Council as Regards the Environmental Risk Assessment of Genetically Modified Organisms. 67, 30-45. Available online at: http://data.europa.eu/eli/dir/2018/350/oj (accessed September 12, 2018).

risk consists in such a case. Does the Court deem the speed of the breeding process as a risk in itself or the ease of application of new techniques of directed mutagenesis? At least one of the two seems to be the case and this argument seems to be more important to the court than the historical details of the technological development. However, all new breeding technologies could be a risk in itself in this sense. How then will different kinds of future technologies be valuated, if they should allow an improved efficiency in breeding? Agreement on which techniques of mutagenesis are excluded from the obligations of GMO law after the ECJ judgment and which not is hardly possible unless the ECJs criteria are clarified. This is not least the case for new random mutagenesis techniques (i.e., not directed mutagenesis) that have been mostly developed after adoption of the Directive (and concomitantly do/did not have a long safety record).

- How far-reaching are the implications of the judgment? It is clear that the judgment also impacts directives and regulations "downstream" of the GMO Directive (i.e., regulations that depend directly or indirectly on and/or are affected by the mutagenesis exemption of the Directive). For instance, the ECJ was clear that the judgment is also relevant to the common catalog of varieties of Directive 2002/53 (ECJ, 2018, para 58-60). But do some of these considerations also apply to the Directive 2009/41/EC[6] on the contained use of GM micro-organisms (cf. Kahrmann and Leggewie, 2018)? Either way, some member states might have to revise their own national GMO laws and regulations as a consequence of the judgment, as their wording does not conform to the new interpretation of the mutagenesis exemption. Furthermore, the judgment raised a methodological question regarding ECJ case law. Did the Court mean to set a precedent and (generally or under specific circumstances) revert to a historic reading instead of a dynamic interpretation of undefined legal terms? If that were the case, the judgment would have a huge and lasting effect on biotechnology law (Seitz, 2018).

Only further clarifications by the ECJ can finally settle these legal uncertainties. Given current uncertainties, it is possible that within the next years another national court will refer similar or entirely different questions regarding Directive 2001/18/EC in a preliminary ruling procedure, that have an effect on the interpretation of the judgment. In fact, concerned individuals or organizations who have the time, risk-readiness and funds might take the initiative and initiate a law suit to probe the ECJs judgment (see corresponding remarks in **Table 1**). In addition, a national court would have to be willing to draft appropriate questions. On the other hand, there is the option of legal change.

Any of these three categories of uncertainties weighs heavily on research and development decisions, not only in large multinational companies but also all the way down to basic research in plant science and agricultural systems (Smyth and Lassoued, 2019; Zimny et al., 2019). It is not surprising that calls to urgent action are soaring, such as a recent call signed by scientists from 118 life sciences research institutions (VIB, 2018). If the situation remains unchanged, this will soon result in loss of competitiveness for Europe's green-biotech industry, for breeding and seed industries and for European agriculture. In addition, trade disruptions and concomitant economic consequences might follow if the zero-tolerance policy is indeed enforced and imports are halted by authorities based on suspected low-level presence of unapproved GEOs (Ryan and Smyth, 2012; cf. Kalaitzandonakes et al., 2014). Even a WTO trade dispute might ensue, similar to the *USA, Canada et al. vs. EU* cases DS291-293 on the import of GMOs to the European market, that ended in disadvantage for the EU (cf. WTO Reports of the Panel, 2006). Indeed, ten countries have already taken issue with the disruptive consequences of the ECJ verdict for international trade, demanding to "avoid arbitrary and unjustifiable distinctions between end products derived from precision biotechnology and similar end products obtained through other production methods" (WTO, 2018).

It is now the task of the Union legislator to update regulation in order to adequately reflect recent developments in breeding techniques and to prevent a crisis of enforcement.

ROADS FORWARD

Any way forward from the status-quo has to address present uncertainties and deliver solutions tailored to new breeding techniques. A number of possible courses of action are feasible and allow for a sustainable development of European GMO policy that has the potential to bring Europe back on track in agricultural biotechnology (see **Table 1**).

Make Use of Flexibility Within Current Legal Framework

GEOs do not fit squarely into the current regulatory framework (discussion above), mainly because legally they are GMOs but biologically they often are indistinguishable from organisms bred by conventional techniques that are not regulated as GMOs. Regulatory agencies therefore need to use the flexibility they have legally in their disposition to tailor regulatory processes to GEOs.

There are two main ways of authorization of GMOs in the EU, depending on the goal of the applicant (Voss, 2006; Roïz, 2014): Authorization for the deliberate release into the environment of a GMO (according to Directive 2001/18/EC) is the "default" way of authorization. It is possible to apply for an authorization for placing on the market of a GMO for any commercial purpose, i.e., cultivation, importation or transformation of GMOs into industrial products (according to Directive 2001/18/EC, part C). Typical examples are the importation of GMO flowers or the placing on the market of MON810 seeds for cultivation. It is also possible to apply for deliberate release for non-commercial purposes, i.e., an experimental release such as a field trial (according to Directive 2001/18/EC, part B). Authorization for placing on

[6]*Directive 2009/41/EC of the European Parliament and of the Council of 6 May 2009 on the Contained Use of Genetically Modified Micro-organisms.* 125, 75–97. Available online at: http://data.europa.eu/eli/dir/2009/41/oj (accessed March 1, 2018).

TABLE 1 | Table of genome editing directed policy options for different actors. Sorted by timing.

Action	Actors	Start of effect[a]	Effect
Make use of flexibility within current legal framework: – Transparency: revise guidance documents explicitly addressing GEOs – Predictability: if not scientifically warranted, do not ask for additional information or further assessments – Lower barriers for GEO authorization, especially for field trials and non-food&feed release	Regulatory agencies in member states and on European level	1 year	(Slightly) lowering legal uncertainty
Amend Commission Implementing Regulation (EU) No 503/2013: – Scale process of approval to allow differential treatment for different types of gmos, i.e., explicitly addressing geos – Relax ERA duration and number of locations, adapt to hypothesis of risk – 90 days feed studies only where hypothesis of risk – Relax monitoring modalities to hypothesis of risk	EU commission	2-5 years	Lowering cost for innovators
Update Annexes II, III, VI, VII of GMO Directive 2001/18/EC: – Scale process of approval to allow differential treatment for different types of GMOs, i.e., explicitly addressing GEOs	EU commission	2-5 years	Lowering cost for innovators
Probe ECJ judgment by putting new cases to trial: – Clarify what is a long safety record for mutagenesis – Clarify if every smallest base edit really leads to GMO in the sense of Dir. 2001/18/EC Art. 2(2) – Clarify how judgment is enforced regarding non-detectability	Various stakeholders	3-10+ years	Lowering legal uncertainty
Amend, supplement or replace GMO law (primarily Dir. 2001/18/EC): – Amend Dir. 2001/18/EC Art. 2(2) or Art. 3 i.c.w. Annex IB to exclude geos – Amend Dir. 2001/18/EC Rec. 17: What is long safety record? – Supplement by specific act for geos – Replace by outcome-based regulation (e.g., novel trait) – Replace by sectorial law	EU parliament & commission	5-10+ years	Solves all issues, including enforcement issue
Develop new technologies (speculative): – Alternatives to altering germline DNA for breeding e.g., epigenetic modifications, mRNA interference or proteome modifications are not currently regulated – Alternative employments of techniques of conventional (by chemicals or radiation, *in vivo*) mutagenesis	Basic research	Decades	Circumventing regulation

[a] *All timespans given in table are only rough estimates. The values for "start of effect" are estimated based on the following evidence: The duration of release or amendment of regulations by the parliament and/or by the commission can be estimated based on the history of e.g, Regulation (EC) No 1829/2003 and Commission Regulation (EC) No 641/2004. Regulation (EC) No 1829/2003 was adopted by the commission on 25.7.2001 and the date of its official publication was 22.9.2003, i.e., 2 years (without timespan for implementation). In the case of Commission Regulation (EC) No 641/2004, which is based on Regulation (EC) No 1829/2003 the date of effect is 7.4.2004, which is presumably even less than one year after drafting. Other regulations have been released within similar timeframes. The duration of the committee procedure of article 27 i.c.w. Art. 30(2) of the GMO Directive 2001/18/EC can be estimated on prior instances of its application. On 8.3.2018 changes to various Annexes of the GMO Directive were implemented (Commission Directive EU No 2018/350) involving a committee procedure, whereby the first draft was published on 10.11.2016, giving the procedure a total timespan from drafting to publication of c. 1.5 years. The estimate was heightened to 2–5 years since changes to the annexes of Directive 2001/18/EC are only effective in combination with amendments in the corresponding regulations. The estimates of 2–5 years given above are slightly more generous, to account for the politically delicate nature of the amendments and a longer timespan required by authorities to apply new legislation in the regulatory process. Amendments to the Directive have been brought into force on four dates (07.11.2003; 21.03.2008; 02.04.2015; 29.03.2018) between publication of Directive 2001/18/EC and Nov 2018 (see history of amendment available on http://data.europa.eu/eli/dir/2001/18/oj), which allows to deduce a span of 3-8 years. The duration of a major redrafting and replacement of the entire Directive was estimated as 5-10+ years based on the timeframe it took for Directive 2001/18/EC of 12 March 2001 to be drafted and replace its antecessor Directive 90/220/EEC of 23 April 1990. The process of redrafting did of course not start immediately after publication of 90/220/EEC but only a few years later. The first proposal by the commission was published on 26 Nov 1997, hence the estimate of a minimum of 5 years. After implementation into the directive of the above legislative procedures on European level, transposition by member states might take about 1.5 years (e.g., this is the timeframe for Commission Directive EU 2018/350). The duration of legal actions that involve a clarification of GMO law is very unpredictable and the vague span of 3-10+ years reflect this fact. Finally, research and development of new breeding techniques is ongoing (e.g., CRISPR mediated epigenetic modification) but technologies developed in basic research usually take decades to be transferred to market readiness.*

the market of GMOs as food and feed is the way to go for all products that are intended for food or feed use and contain GMOs, are produced from GMOs or contain GMO ingredients (according to Regulation 1829/2003 and Commission Implementing Regulation EU No 503/2013). An authorization for cultivation of a GMO for food and feed production can also be obtained in this way. Obtaining authorization for the import of GMO commodities is easier than for cultivation. While MON810 is the only GMO currently authorized for cultivation in the EU (but opted-out by 19 EU member states), numerous GMOs are authorized for various uses other than cultivation (see the GMO registers at https://webgate.ec.europa.eu/dyna/gm_register/index_en.cfm and http://gmoinfo.jrc.ec.europa.eu/gmc_browse.aspx; pers. comm. by a reviewer).

To carry out these processes of authorization, multiple regulatory agencies are involved on all levels from EU to the member states regions in some cases. Member states institutions alone are responsible for authorization of field

trials (national risk assessment and national authorization) but member states and EU institutions are jointly responsible for market authorization of GMOs (for import of commodities, for cultivation and as food and feed), in which case an EU-wide authorization process including risk assessment is carried out by EFSA and supported by national authorities (Directive 2001/18/EC[1] part B and part C respectively, and Regulation 1829/2003). In addition, in the case of cultivation in a certain member state, obtaining a national risk assessment is required.

In practice, regulatory agencies are always subject to member states' or the union's political interests and–legal obligations set aside—their attitude regarding smooth and efficient administrative procedures goes a long way toward providing an innovation friendly regulatory landscape. In the next few months, all regulatory agencies (partly independently on EU level and in member states) will decide on how to implement the ECJs judgment. They have considerable freedom in doing so and their decisions will certainly at least slightly reshape the regulatory landscape—for better or for worse.

To address the aforementioned uncertainties and avoid a disproportionate regulatory burden for GEOs, the following considerations should guide regulatory agencies in implementing the ECJs judgment:

First and foremost, given the complex situation in the aftermath of the judgment, transparency is key. To reduce uncertainty regarding GEOs, it is important that regulatory agencies communicate transparently how GEOs will be treated by publishing new guidelines (e.g., EFSA guidance documents) that explicitly cover organisms gained by all relevant new techniques and different kinds of alterations. All possible obstacles in the process of application that could arise with GEOs should be addressed in guidance documents in order to (at least slightly) lowering legal uncertainty. It is the duty of regulatory agencies to ensure a high predictability of regulatory decisions and transparent and simple guidelines are a first step.

Second, the process of authorization should take account of the indistinguishability of GEOs from conventionally bred organisms. Thereby, it is the regulatory agencies duty to evaluate organisms gained by new techniques on scientific basis alone. While the ECJ has invoked the precautionary principle based on the mere possibility of risks (ECJ, 2018, para 48) as a reason to place new techniques under GMO regulation, the risk assessments in applications for authorization of GMOs within the bounds of those laws can only be based on scientific method and scientific evidence. GEOs that cannot be distinguished from conventionally bred varieties cannot involve more or less risks than these conventionally bred varieties themselves, unless the risks do not stem from the alterations made to the genetic material of the organism but instead from the methods used (for instance by introducing unintended alterations that amount to a relevant new trait but somehow escape our notice; it is a bit ironic then that the ECJ judgment, para 48, designates haphazard alterations as not presenting a risk in organisms altered by chemical and radiation-induced mutagenesis whereas it is presumably regarded as the main source of risk in GEOs). Anyhow, there is currently scientific consensus that the new techniques are in principle safe (Leopoldina, 2015; Gao et al., 2018; VIB, 2018; cf. Diekämper et al., 2018) and this consensus is successfully paving the field for medical applications of the same techniques (e.g., Baylis and McLeod, 2017; Ginn et al., 2018). Pre-release monitoring is a legal requirement and therefore at least a standard evaluation has to be conducted also on GEOs, even if no particular risks are expected to be associated with the specific alterations introduced into the organism. But it is not possible to empirically assess risks that are not known and cannot be foreseen, i.e., for which there is no scientific hypothesis to test for. When confronted with such risks, scientists have two options. A disproportionate option is to test various kinds of evidence at random and hope for a serendipitous discovery of a hazard. In such a case however, there is no scientific measure by which testing can be completed and no reason for preferring one kind of evidence over another (in simple words: how is someone to decide at which point to end testing, if nothing is found?). The only judicious option is to perform a few standard tests and focus on facilitating early detection in post-release monitoring (in simple words: if nothing relevant came up in standard testing, assume all is good. But if ever a hazard comes up, be ready and react quickly). Therefore, as long as not even a hypothesis is given as to how an organism, e.g., barley with an *Mlo* point mutation, that has been bred by genome editing has higher risks than another organism bred by "conventional" techniques, e.g., barley with the same *Mlo* point mutation, then testing should stop after a few empirically meaningful standard tests. This second option is the one that regulatory agencies should apply to GEOs, as long as no hypothesis of risk is given, neither from the technology nor from the organism's traits.

Indeed, the GMO Directive (2001/18/EC) leaves some freedom to tailor the regulatory process to cases of low risk, such as most GEOs:

First, it was one of the goals of the 2001 amendment to fixate (and thereby shorten) the duration of single steps and the overall duration of the process of application for authorization of GMO release (Voss, 2006). The timespan from application to decision could be reduced in principle to a swift 6–9 months for market-release in the case of cultivation (for an in-depth discussion of timing in the process of authorization see Voss, 2006). To keep up with the timeframe envisaged in the Directive it is however crucial that authorities do not ask for additional information after notification, as this allows prolonging the timespan beyond the norm. Consequently, the Directive stresses that authorities requesting additional information require a solid reason for so doing (cf. Directive 2001/18/EC, Art. 6(7), Art. 14(4), Art 15(1), Art 18(1)). As GEOs in most cases do not present a scientifically grounded risk (discussion above), there can be no warranted reason for an authority to prolong the process by asking for further tests or additional information, unless in response to new scientific evidence.

Second, the Directive (2001/18/EC) allows for adapting the modalities of assessment to different types of GMOs and their different concomitant risks. Its Art 7(1) allows for the application of differentiated (simplified) procedures if sufficient experience has been obtained by releases of certain GMOs in certain ecosystems and sufficient evidence of safety is

available (Directive 2001/18/EC[1] Art. 7(1) i.c.w. Annex V). For placing on the market of certain types of GMOs, a competent authority or the Commission may propose to derogate from the general requirements for the notification procedure Directive 2001/18/EC[1] Art. 16(1). And on scientific grounds such as low risk, an application might dispense with part of the information for post-release handling Directive 2001/18/EC, Art. 13(2). In addition, for the environmental risk assessment, the information required "may vary depending on the type of the GMOs concerned" [Directive 2001/18/EC, Annex II (B)]. In fact, individual applications for release shall not be required to present information "where it is not relevant or necessary for the purposes of risk assessment in the context of a specific notification" (Directive 2001/18/EC, Annex III, as amended by Commission Directive 2018/350). In short, risk assessment can and should be tailored to the type of GMO in question and GEOs are definitely a special type of GMOs (cf. similar arguments by Bratlie et al., 2019 and Eriksson, 2018b).

Third, the Directive does not prescribe specific scientific methods of risk assessment (as is normal for a legal act of this category), i.e., specific qualitative vs. quantitative methods, which varieties to compare with, in the lab or in the field, null hypotheses, sample sizes, specific values for statistical significance etc. It simply lists different risks that have to be assessed and maintains that studies have to conform to usual standards (Directive 2001/18/EC, Annex II (C), as amended by Commission Directive 2018/350). Consequently, authorities could draw more strongly than they do now on different kinds of readily available evidence including theoretical considerations and evidence from published scientific literature on similar types of GMOs. In particular, it should in most cases suffice to "refer to data or results from notifications previously submitted by other notifiers" cf. Directive 2001/18/EC, Art. 6(3) and of course to evidence from "releases of the same GMOs [...] outside the Community" cf. Directive 2001/18/EC, Art. 13(3); note that in the case of GEOs we will soon have abundant information. The type of scientific evidence (e.g., from a study conducted on the whole organisms vs. from theoretical considerations that estimate risks) and the level of detail required in response to each subset of considerations should be allowed "to vary according to the nature and the scale of the proposed release" (cf. Directive 2001/18/EC, Annex III). Note that this is not meant as a cut on rigor of risk assessment but only as a methodological change within the bounds of scientific method choice. Why should from a scientific standpoint a single locally confined field trial be more representative for the effects of a specific trait than knowledge from its agricultural use across decades? In the case of GEOs the traits are often already known from decades of cultivation in conventionally bred varieties. In the most simple terms: Systematic reviews and evidence maps based on known risks have to suffice when no scientific hypothesis is available against which empirical testing can be done, which is the case for many GEOs (as discussed above).

Of course, the flexibility extant in the GMO Directive is a breadless argument in some respects. On the one hand, even if a soft administrative change along these lines was accomplished, applications for placing on the market of GMOs could still get stuck in the committee voting procedures. Consequently, the timespan from notification until committee voting might decrease, but after voting there wouldn't necessarily be more successful authorizations than today—unless member states bring into better alignment their committee votes or other incentives are given (as e.g., the instatement of a "GMO opt-in mechanism" proposed by Eriksson et al., 2018). On the other hand, regulatory agencies are not free to act on the Directive directly but they have to take into account how the Directive has been transposed in the member states. In addition, while the GMO Directive (2001/18/EC) is the centerpiece of GMO legislation, other regulations are in place that narrow the flexibilities discussed above. Especially the regulations for GMO food and feed (discussion of Commission Implementing Regulation EU No 503/2013[7] below) do lay out the process in much more detail and in a more restrictive manner that leaves less freedom to treat GEOs any differently than transgenic GMOs. In practice, most GMOs apply for market approval as food and feed. Still, there are field trials and releases for non-food&feed purposes (e.g., industrial enzymes or raw materials for the production of biopolymers, biofuels, paper, starch etc.; EFSA, 2009) that do not in principle fall under that regulatory regime and could therefore profit from more flexibility, as regulated according to part B and part C of Directive 2001/18/EC respectively. And the Commission Implementing Regulation (EU) No 503/2013 is not itself completely devoid of flexibility, e.g., its Art. 5(2) concedes that an application may have to fulfill less requirements if this can be justified for the GMO in question.

The take home message is that if member states and their regulatory agencies are willing, slightly defusing legal uncertainties for GEOs is already possible by adjusting procedures and communicating transparently, even before tackling legislation. While the aspect of flexible implementation should not be underestimated, especially since it is the first thing that will happen, the fundamental problems of GMO law cannot be solved by such means.

Amend Commission Implementing Regulation (EU) 503/2013

Applications for authorization for placing on the market of GMOs as food and feed are regulated among others by the Regulation (EC) No 1829/2003 on Genetically Modified Food and Feed which is implemented by the Commission Implementing Regulation (EU) No 503/2013 on Applications for Authorisation of Genetically Modified Food and Feed. While the Regulation 1829/2003 and other regulations leave a lot of flexibility for regulatory agencies to shape the process of assessment, the Commission Implementing Regulation (EU) No 503/2013 lays down concrete criteria and prescribes scientific methods to be applied by regulatory agencies. The two most time

[7] *Commission Implementing Regulation EU No 503/2013 of 3 April 2013 on Applications for Authorisation Of Genetically Modified Food and Feed in Accordance With Regulation (EC) No 1829/2003 of the European Parliament and of the Council and Amending Commission Regulations (EC) No 641/2004 and (EC) No 1981/2006 (Text with EEA relevance)*. 157, 1–48. Available online at: http://data.europa.eu/eli/reg_impl/2013/503/oj (accessed March 1, 2018).

consuming and costly scientific requirements of the assessment process are:

(1) The environmental risk assessment, which has to be carried out with field trials on a minimum of 8 sites that have to be representative for the GMOs receiving environment and often cover multiple years [cf. Commission Implementing Regulation EU No 503/2013, Annex II, II. Sci. Req. 1.3.2.1(b)].
(2) The mandatory toxicology assessment by 90-day feeding studies on rodents (cf. Commission Implementing Regulation EU No 503/2013, Annex II, II. Sci. Req. 1.4.4.1).

Both these assessment procedures have been scientifically supervised and evaluated. The procedure of environmental risk assessment has been criticized repeatedly for leading to results that are not comparable, as no common test protocol is implemented (e.g., Hilbeck and Otto, 2015; Priesnitz et al., 2016; Fernández Ríos et al., 2018). Moreover, it has recently been suggested to adapt the methods of risk assessment for new types of GMOs (Duensing et al., 2018). The current regime of 90-days feeding studies for toxicology assessment has been criticized, as "the performance of rat feeding trials with whole food/feed for the risk assessment of a GM plant would not result in additional information pointing at possible health risks" as compared to less expensive studies and biochemical characterization (G-TwYST, 2018; cf. similar results in GRACE, 2018). In other words, both these tests are lengthy and expensive but do not seem suited to reveal new risks that cannot also be investigated by other means. These results call for a change in assessment procedures for all GMOs.

Regarding GEOs in particular, the addition of a caveat explicitly exempting them from such studies (90-day feeding studies and extended field trials), provided that the alterations introduced are deemed non-hazardous based on theoretical considerations and currently available scientific evidence, would constitute a decisive improvement. With any such update to Commission Implementing Regulation (EU) No 503/2013, the European Commission can significantly lower regulatory hurdles for agricultural innovators. On the other hand, GEOs would still be subject to all other obligations of GMO law and many applications that are geared toward more agricultural sustainability, with small alterations that improve existing varieties, will not become economically attractive unless exempted from all obligations of GMO law.

Update Annexes II, III, VI, VII of GMO Directive 2001/18/EC

An additional option for lowering the regulatory burden for innovators bringing GEOs to market is an update to technical progress to Annexes II, III, VI, and VII of the GMO Directive, which is provided for by statute with recourse to a Committee Procedure that has to be initiated by the European Commission (Directive 2001/18/EC, Art. 27 i.c.w. Art. 30(2), referring to Committee Procedure of Decision 1999/468/EC). An explicit exemption for GEOs for some of the obligations laid down in these Annexes might have an effect if followed by a diligent implementation in the concomitant regulations. However, as a single measure, upgrading the annexes is not adequate to mitigating the uncertainties for GEOs, as the Directive already leaves relative freedom to deal with different types of GMOs and individual cases (discussion above). By contrast, the fundamental problems lie in the main body of the GMO Directive (2001/18/EC) and its Annex IB, as these define the scope of the law. This part, however, cannot be tackled by a Committee Procedure but requires an ordinary legislative procedure.

Amend, Supplement or Replace GMO Law (Primarily Directive 2001/18/EC)

Being more transparent and flexible regarding regulatory procedures (section Make Use of Flexibility Within Current Legal Framework) and lowering the costs of applications for market approval of GEOs (section Amend Commission Implementing Regulation) is not enough. The most significant uncertainties stem from foreseeable problems with enforcement, particularly when taking into account global trade (discussion in section The ECJ Judgment and its Ramifications). Regarding GEOs specifically, it has to be somehow ensured that organisms that cannot be distinguished from organisms bred with conventional techniques, which currently do not fall under the obligations of the GMO Directive, are regulated equally–or at least in a pragmatic manner similarly, also taking into account economic feasibility. Solving this issue is the centerpiece to finding a solution to the new uncertainties in European GMO regulation.

The following options could accomplish this task, for example (in any combination):

- Amend the GMO Directive (2001/18/EC), for example by (A) revising the wording of the GMO definition in Art. 2(2) in order to exclude organisms that could also have been obtained by conventional breeding techniques or/and (B) updating the mutagenesis exemption in Art. 3 i.c.w. Annex IB to additionally exclude GEOs (cf. Dutch Proposal, 2017) or/and (C) clarifying what constitutes a "long safety record" in the sense of Rec. 17.
- Supplement the GMO law by a new act that specifically applies to GEOs, at least among others. This is maybe politically more feasible and efficient than opening up the entrenched debates on adequate regulation for GMOs in general. It has also been suggested to transfer competencies back to member states for specific cases (cf. the idea of an "opt-in Directive" by Eriksson et al., 2018; slightly outdated scenarios for regulation of NBTs in Purnhagen et al., 2018a). The drawback of additional acts is that the core of GMO law will still have to be revised at a later stage, as new technological developments will again put pressure on old GMO law, such as epigenetic modifications or gene drives (gene drives are not GEOs in the sense of this article but transgenic organisms and the scientific community is very much aware that they present high risks unless very specific conditions are met, cf. Akbari et al., 2015; Brown, 2017).
- In terms of complete replacement of GMO law, there are plenty of options. An obvious choice would be to develop an outcome-based regulation (e.g., novel trait), as this is a

means to ensure that the law automatically keeps up with technological progress (cf. Huang et al., 2016). Opponents of the current horizontal legislation of GMO release might propose replacement by sectorial regulation, as this allows a more customized treatment of e.g., plant varieties with higher starch content vs. e.g., future medical applications that may require a permit for release. A new tiered approach has recently been proposed by the Norwegian Biotechnology Advisory Board (Bratlie et al., 2019).

This article does not provide further discussion and evaluation of such options. There is no shortage of ideas for renewed legislation and it is far too early to hypothesize on which options might find political majorities more easily. In order to solve the issue of GEOs, the broad goals of amending, supplementing or replacing legislation are clear: (1) Updating the regulatory framework in a way that specifically addresses GEOs, including issues of regulatory enforcement. (2) Making the regulatory framework more flexible to future developments in green biotechnology and allowing fast and predicable incorporation of recent scientific evidence. (3) Finding a balance between precautionary regulation and allowing sustainable innovation. European legislation is a lengthy process and that is why the involved actors have to start acting soon.

Although this is speculative, in several decades new technologies will be developed that might be shaped by the current regulatory hurdles for GMOs. For example, techniques of conventional mutagenesis, which are currently exempted from all obligations for GMOs, might be developed further and maybe employed differently, in order to allow much faster and efficient breeding, e.g., in combination with future enhanced molecular markers. And in the long run, it might become possible to circumvent all aspects of the current regulatory regime by developing breeding techniques that do not change the "genetic material" of an organism (see remarks in **Table 1**). Such future breeding techniques could draw on e.g., epigenetic modifications (cf. Thakore et al., 2016), CRISPR-interference (cf. Dominguez et al., 2016), mRNA interference or proteome modifications. Either way, as new breeding methods develop over the next decades, the gap between the scope of the current regulatory framework and technical possibilities will widen and put additional pressure on a complete overhaul of European GMO law.

CONCLUSION

Companies and research institutions that employ new breeding techniques are confronted with considerable legal uncertainties after the recent ECJ judgment. While in principle uncertainties tied to the process of application for authorization of GMO release can be addressed by procedural changes on a lower level, problems of enforcement with organisms that are indistinguishable from the result of conventional breeding techniques cannot be solved without an amendment of European GMO legislation. There are various options for legal change that all share the common necessity of treating organisms that are indistinguishable from non-GMOs equally if they are devoid of known additional risks—that is, to exclude them from most or all obligations of GMO regulation as well. This is by no means a statement for less rigor, as organisms with novel traits that are associated with risks should still be assessed and regulated thoroughly.

Any such solution however requires the constructive involvement of European institutions and member states. The roads forward presented in this article are thus mere possibilities in an optimistic scenario that presupposes political willingness to act. This may not be realistic in the current political situation. However, if the problems in GMO law are just ignored a state of crisis will ensue: Regulatory agencies will struggle to enforce GMO-regulation, international trade relations will be affected, European agriculture loses an opportunity for sustainable innovation and jobs in research and development will be relocated elsewhere.

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The author confirms being the sole contributor of this work and has approved it for publication.

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OPINION
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Politicizing the Precautionary Principle: Why Disregarding Facts Should Not Pass for Farsightedness

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In his seminal book "The Death of Expertise" Thomas Nichols (2017) explores how "ignorance became a virtue" (Kakutani, 2017) in public debates on controversial issues in the United States, revealing a growing hostility toward scientific expertise. A similar trend can be observed in Europe, especially when it comes to the regulation of agricultural biotechnology.

In response to widespread public concerns about the potential risks of genetically modified organisms (GMOs) in food and agriculture, the EU legislative bodies passed Directive 2001/18/EC on the deliberate release of GMO into the environment in the year 2001.[1] As for the history of safe use, this so-called GMO Directive implies that there is a fundamental difference between crops improved by means of genetic engineering and crops improved by any other established types of breeding technology, including classic mutagenesis, which is widely considered to be a more uncertain manipulation of the plant DNA than genetic engineering (SAM and High-level Group of Scientific Advisors, 2017). The GMO Directive is meant to follow the Precautionary Principle (PP), which has been defined in detail by the European Commission (EC) in its "Communication on the Precautionary Principle," published in the year 2000 (EC (European Commission), 2000). It states that the PP should adhere to the general principles of risk management, which include (a) the principle of proportionality between the measures taken and the chosen level of protection; (b) the principle of nondiscrimination in the application of the measures; (c) consistency of the measures with similar measures already taken in similar situations; (d) the examination of the benefits and costs of action or lack of action; and (e) review of the measures in the light of scientific developments. This interpretation of the PP is scientifically sound and has a long track record in national and international environmental policy. Yet, by treating genetic engineering as an environmental risk in a broad sense, the GMO Directive has more in common with toxic waste regulation than with the registration of a new plant variety (Sprankling and Salcido, 2018). As such, the new regulation did not help address the EU's de-facto moratorium on biotech products in place since 1998 and thus induced major exporters of GM crops to submit a first request for consultation with the World Trade Organization (WTO) on May 13, 2003, on the consistency of the GMO regulation in Europe with WTO rules.

In 2006, the dispute settlement panel of the WTO took a decision on the case "European Communities Measuring and Affecting the Approval and Marketing of Biotech Products" (DS291, 292, 293). The panel faulted the European Union for causing undue delay in the approval of biotech products and pointed at the fact that the additional safeguard measures applied by EU member states were not based on proper risk assessment as required by the WTO Agreement on the Application of Sanitary and Phytosanitary Measures (SPS Agreement). The SPS Agreement endorses the use of the PP as long as it is combined with an effort to gain more science-based information on the potential

[1] Directive 2001/18/EC was an amendment of Council Directive 90/220/EEC on the deliberate release into the environment of GMOs, passed in 1990 (see http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CELEX:31990L0220:EN:NOT).

risks and eventually adjust regulation correspondingly.[2] The EU and its member states made such an effort by spending hundreds of millions of Euros on risk research on GMOs (EC (European Commission), 2010); yet, they failed to take any action based on the insights gained from this research. The main finding, which was also reaffirmed by all national academies worldwide, was that there are indeed risks, but that these risks are also well known in conventional agriculture. As a result, the EU should have taken appropriate action to better align its regulatory approach to GMOs with the risk management principles that underpin the PP. But for that purpose, risk management would have to shift from a process-based to a product-based approach of risk assessment. This has not happened because the PP ceased to be a tool of responsible risk management, but instead became a convenient excuse to postpone approval decisions by pointing out that off-target effects in the breeding process and indirect adverse effects resulting from the commercial use of GMO cannot be excluded entirely; however, the likelihood of such effects to occur often turns out to be lower in the case of GMO than with unregulated classical mutagenesis or conventional breeding due to the higher degree of precision and efficiency of advanced biotechnology, the more accurate identification of off-target effects, and the more strict monitoring requirements (Lazebnik et al., 2017; SAM and High-level Group of Scientific Advisors, 2017:58).

Despite the highly preventive EU regulatory framework, a few GM crops, being considered "safe," eventually won temporary approval for cultivation in the EU; but many EU member states continued to prohibit them in their territories claiming "safety concerns." In response to this disregard of EU regulation, the EC proposed in 2015 to amend the legislation so that Member States are free to restrict or prohibit the cultivation of EU-authorized GM crops on their territory on the basis of grounds that divert from those assessed by the harmonized set of Union rules as outlined in Directive 2001/18/EC and Regulation (EC) No. 1829/2003 on GM food and feed. By including broadly defined "social concerns," the resulting Directive (EU) 2015/412[3] has the effect that the application of the PP ceases to be limited to potential danger for which there is credible scientific evidence (Hansson, 2016).

Lawmakers and judges in the EU nevertheless continue to invoke the PP as justification for banning GMOs (Alemanno, 2007; Lamping, 2012; Heubuch, 2016). These preventive measures may not be unpopular since numerous advocacy groups concerned with the environment, sustainable agriculture, and consumer interests will praise them as being farsighted.

The disregard of the principles that underpin the PP is not just a phenomenon among politicians with a clear antibiotech agenda, but also prevalent in the field of ethics. In the Report on the Precautionary Principle, published by the Swiss Federal Ethics Committee on Non-Human Biotechnology (EKAH (Swiss Federal Ethics Committee on Non-Human Biotechnology), 2018) in spring 2018, the committee members point at the ethical foundations behind the principle and describe it as a tool to protect society from potentially harmful consequences of scientific and technological advances. The cover page of the report features Pandora's Box as a symbol for all the evils that may result from the advances in modern plant breeding. Suggestively, it visually links the risks of genetic engineering with the risks of nuclear plants, toxic waste, and oil spills and contrasts it with pictures of healthy Swiss agricultural landscapes and happy farmers. Unsurprisingly, the committee reaches the conclusion that the new breeding techniques (NBTs) that involve gene editing should be regulated like genetic engineering in food and agriculture in order to protect society and the environment. The committee does not refer to the safe track record of existing GMOs in the market, nor does it cite the recent detailed expert assessment by the Science Advisory Group of the EC (SAM and High-level Group of Scientific Advisors, 2017) of the different gene-editing techniques. Moreover, it does not address the ethical issues related to the instrumental use of the PP for political ends, especially by lobbying groups that benefit from the status quo (Aerni, 2018). In this sense, the EKAH report once again treats the PP as a tool to make disregard look like far-sightedness and, as such, anticipates the decision of the European Court of Justice (ECJ) on July 25, 2018.

The ECJ issued an explanation toward the French High Court (Conseil d'Etat) as to what extent NBT falls under the category of GMO as defined by Directive 2001/18/EC.[4] It stated that organisms obtained by NBT are to be considered GMOs. This would also apply to point mutations generated by NBT as they would not fall under the express "GMO"[5] exemption of mutagenesis in Annex 1B of the Directive comprising conventional techniques of mutagenesis that would have a long safety record. Unlike the report of the Swiss ethics committee, the ruling of the ECJ may have serious consequences for the future of science in Europe. By interpreting Directive 2001/18/EC in a way that would subject NPT to GMO regulation, the ruling may render the cultivation of crops that have been bred with even the least invasive forms of gene-editing in a limbo of legal uncertainty in Europe. As for the imports of such crops into the European Union from countries that have already decided to not subject NBT to the same burdensome regulations of GMOs, such as the United States, Argentina, or Chile (Eriksson et al., 2019), there will be great technical and political difficulties to ensure the same costly separation and corresponding labeling of bulk agricultural commodities. The EC's Joint Research Centre confirms that it will be impossible to understand if a point mutation derives from a spontaneous event or a human intervention (Emons et al., 2018). As a consequence, the European rapid alert system[6] might collapse.

The European retailers, which campaigned in advance of the ECJ decision to subject gene-editing techniques to the

[2] https://www.wto.org/english/tratop_e/dispu_e/cases_e/ds291_e.htm (visited on February 12, 2019).

[3] https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=celex%3A32015L0412 (visited on February 12, 2019).

[4] InfoCuria—Case-law of the Court of Justice. Case Number C-528/16.

[5] The ECJ follows Directive 2001/18/EC by regarding organisms obtained by means of techniques/methods of mutagenesis as genetically modified organisms, yet they are explicitly excluded from the scope of the Directive and therefore not subject to GMO regulation. But if any Member State will ask for the repeal of this rather contrived exclusion, even classical mutagenized plants may have to go through the same regulation like GMOs. Such a decision may be impossible to implement as all the 3301 mutagenized plants (https://mvd.iaea.org/), and any other deriving from a cross with them, would have to go through the burdensome GMO approval process.

[6] https://ec.europa.eu/food/safety/rasff_en (visited on June 27, 2019).

same regulation as GMO,[7] may have been aware of this. But, as consumer choice is driven by affect rather than deliberate reasoning (Aerni, 2011; Stasi et al., 2018), retailers tend to make use of GMO-free labeling strategies that cultivate consumer fears rather than point at the long safety record of GMOs for human consumption (Ray and Wilkie, 1970; Laros and Steenkamp, 2004; Schurman, 2004; Aerni et al., 2011; Russo, 2015).

But, maybe, it is neither the EKAH, ECJ, the anti-GMO activists, nor the retailers that are to blame for the widespread disregard of the facts about modern biotechnology. Instead, it is the old Directive 2001/18/EC and its definition of a GMO. In Article 2, GMO is defined as "an organism, with the exception of human being, in which the genetic material has been altered in a way that does not occur naturally by mating and/or natural recombination." If that definition would be followed with the ECJ to its extremes, then consumer choice in supermarkets would probably shrink to a tiny number of wild fruits, vegetables, and cereals. In return, it has also been shown that many of the common types of alterations introduced by NBT could also potentially occur naturally, if "occurring naturally" includes conventional breeding too, as the Directive 2001/18/EC seems to imply (Custers et al., 2018).

The inconsistent use of the term GMO and, with it, NBT in Europe (Ammann, 2014; Tagliabue, 2016) may eventually lead to prohibitive regulation in many other countries and thus become a serious obstacle to the Agenda 2030, the implementation plan to achieve the Sustainable Development Goals (SDGs). The SDGs were approved by the United Nations General Assembly in fall 2015 with the purpose of creating a more inclusive and sustainable global community by 2030. At the core of the SDGs is global agriculture. It will have to increase the quantity and the quality of food production in order to ensure greater access to healthy diets. Simultaneously, agriculture must become more sustainable by reducing the use of fertilizer and means of plant protection. The combination of objectives can only be achieved by means of sustainable intensification, which includes the genetic improvement of plants so that they become more tolerant to biotic and abiotic stress factors, make better use of photosynthesis and soil nutrients, and enhance the nutritional value of basic food crops. Conventional plant breeding may still be able to address some of these challenges, but it is time-consuming and cannot be tailored well to local preferences, which results in low adoption rates (Aerni, 2006). New breeding techniques have the potential to address these drawbacks. In this context, the PP, based on the Commission's own definition (EC (European Commission), 2000), would be obliged to also assess the risk of nonaction (Aerni et al., 2016). This is also the view of the Group of Chief Scientific Advisors of the EC, which challenges the ruling of the ECJ in a statement published in November 2018 in which it regards the old GMO Directive as no longer fit for purpose (GCSA (Group of Chief Scientific Advisors), 2018).

However, as long as the current process-based regulation continues to be defended by leading European stakeholders from an ethical, legal, and retail business perspective and in disregard of scientific expertise, Europe will be unable to meet its own ambitions to contribute to the numerous SDGs through the creation of a sustainable bioeconomy (Aerni, 2018; EC, 2018).

[7] https://www.ohnegentechnik.org/fileadmin/ohne-gentechnik/presse/p_180710_Offener_Brief_EU_Kommission_180710.pdf (visited on July 29, 2019).

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