10 years of frontiers in plant science

Edited by
Yunde Zhao and Joshua L. Heazlewood

Published in
Frontiers in Plant Science

About Frontiers

Frontiers is more than just an open access publisher of scholarly articles: it is a pioneering approach to the world of academia, radically improving the way scholarly research is managed. The grand vision of Frontiers is a world where all people have an equal opportunity to seek, share and generate knowledge. Frontiers provides immediate and permanent online open access to all its publications, but this alone is not enough to realize our grand goals.

Frontiers journal series

The Frontiers journal series is a multi-tier and interdisciplinary set of open-access, online journals, promising a paradigm shift from the current review, selection and dissemination processes in academic publishing. All Frontiers journals are driven by researchers for researchers; therefore, they constitute a service to the scholarly community. At the same time, the *Frontiers journal series* operates on a revolutionary invention, the tiered publishing system, initially addressing specific communities of scholars, and gradually climbing up to broader public understanding, thus serving the interests of the lay society, too.

Dedication to quality

Each Frontiers article is a landmark of the highest quality, thanks to genuinely collaborative interactions between authors and review editors, who include some of the world's best academicians. Research must be certified by peers before entering a stream of knowledge that may eventually reach the public - and shape society; therefore, Frontiers only applies the most rigorous and unbiased reviews. Frontiers revolutionizes research publishing by freely delivering the most outstanding research, evaluated with no bias from both the academic and social point of view. By applying the most advanced information technologies, Frontiers is catapulting scholarly publishing into a new generation.

What are Frontiers Research Topics?

Frontiers Research Topics are very popular trademarks of the *Frontiers journals series*: they are collections of at least ten articles, all centered on a particular subject. With their unique mix of varied contributions from Original Research to Review Articles, Frontiers Research Topics unify the most influential researchers, the latest key findings and historical advances in a hot research area.

Find out more on how to host your own Frontiers Research Topic or contribute to one as an author by contacting the Frontiers editorial office: frontiersin.org/about/contact

10 years of frontiers in plant science

Table of
contents

Check for updates

Improving Crops for a Changing World

James R. Lloyd* and Jens Kossmann

Department of Genetics, Institute for Plant Biotechnology, University of Stellenbosch, Stellenbosch, South Africa

Plant biotechnology has been fundamental to the development of human civilisation. The domestication of plants helped increase food production, allowing the sustenance of populations in large settlements and they provide most calories in the human diet alongside being used as fodder for farm animals. They are also good sources of both therapeutic drugs and industrial feed stocks, while more recently they have been used to produce pharmaceutical proteins and biofuels. There are nevertheless many areas where plants can be improved through genetic manipulation and there are pressing reasons why this needs to be accomplished.

INCREASING YIELD

The need for increasing crop yields to provide food for a burgeoning world population has been recognised for centuries, since at least the time of Thomas Malthus. His "An Essay on the Principle of Population" (Malthus, 1798) led to the concept of the Malthusian trap where increasing populations become starved when they outstrip growth in food production. Even though the world population has increased approximately eight-fold since 1800 to almost 8 billion in 2019 (United Nations, Department of Economic and Social Affairs, Population Division, 2019), plant breeders have managed to increase yields to keep pace with this growth and this should be celebrated as a triumph of agricultural biotechnology.

Notwithstanding this historical increase in food production there are still many issues that need to be addressed. Despite the relatively high yields of modern plant varieties, estimates indicate that 800 million people still suffer from calorie deficit (Global Nutrition Report, 2016) and this is a growing problem as the world's population is expected to reach approximately 11 billion by the end of this century (United Nations, Department of Economic and Social Affairs, Population Division, 2019). This means that agricultural yields need to continue to increase, but rates of yield increases produced by plant breeders are declining to levels that are insufficient to cope with population growth (Ray et al., 2013). In addition, anthropomorphic climate change means that the plants will have to survive with increased levels of abiotic stresses (Ray et al., 2019).

Pests are thought to be responsible for some of the highest yield decreases in crops, ranging between 25 and 40%. The highest potential losses come from weeds (34%), while insects (18%) and microbial diseases (16%) also lead to significant losses (Oerke, 2006). Some of the first commercially released transgenic crops were engineered to be resistant to broad spectrum herbicides—such as glyphosate or glufosinate ammonium—to help overcome competition. Since then their widespread use has led to the development of herbicide resistant plants with almost 40 glyphosate resistant weeds having been identified worldwide since 1996 (Heap and Duke, 2018). Although stacking herbicide resistant traits in crop plants will help reduce the development of resistance, there is still a need to identify novel herbicides alongside corresponding resistance mechanisms that can be used to expand this portfolio.

Resistance to attack by both insect and microbes can be introduced by conventional plant breeding, but loci leading to resistance can be difficult to identify and time consuming to incorporate into elite lines. Therefore, transgenic technologies are often used, especially when

it comes to resisting insect predation. Expression of genes encoding insecticidal proteins from *Bacillus thuringiensis* have become the mainstay of resisting insect attack in many transgenic plants. Field developed resistance by insect pests to either Cry1 or Cry3 proteins has, however, been reported in the literature (Tabashnik, 2015) meaning that the development of crops containing improved insecticidal proteins is needed to overcome this threat to food security.

A few transgenic traits leading to resistance to microbial diseases have been commercialised, and many more have been demonstrated in model plants (Dong and Ronald, 2019). The best example of the use of transgenic technology in this respect is the Rainbow papaya which has largely replaced the conventional crop in Hawaii due to its resistance to papaya ringspot virus (Hamim et al., 2018). No natural resistance to this disease has been identified, so transgenic technology is the best method of protecting this crop. Although several other transgenic plants resistant to diseases have been approved for growth in the United States, they are not currently used. For many crops introducing genetic variation leading to disease resistance will be vital to help reduce yield penalties from microbial pathogens.

Abiotic stresses also cause major losses in crops, with estimates of more than 50%. The first transgenic crop with improved resistance to drought stress was released in 2011 and since then similar technologies have been engineered into other crops to improve abiotic stress tolerance. Tolerance in other crops is also being developed using a mixture of conventional breeding as well as transgenic and genome editing techniques and these improved plants are urgently needed given predictions of climate change leading to increased periods of abiotic stress, especially drought (McKersie, 2015).

Although alleviating crop losses by reducing biotic and abiotic stresses will greatly help increase productivity, there are other ways that yields can be increased. Many large-scale international projects are currently ongoing to accomplish this, for example the introduction of C4 type photosynthesis in rice to decrease inefficiencies caused by photorespiration (Ermakova et al., 2020) and this project, alongside others involving the rational manipulation of plant metabolism, will likely prove important in increasing food production.

HEALTHIER PLANTS

While increasing yield is important for food security, making plants healthier will help overcome nutritional deficiencies. Approximately 2 billion people currently suffer from micronutrient deficiency which can lead to stunted growth in children (Global Nutrition Report, 2020). In addition, several chronic diseases—such as type II diabetes—are influenced by the types of food that we eat.

Many biofortified plants are being developed (Garg et al., 2018) to overcome these issues through a combination of conventional breeding and transgenesis. For example, some plants contain low amounts in specific types of amino acids and populations that rely on these as staple foods can suffer from deficiencies. The development of high lysine maize by conventional plant breeding has led to large potential improvements to child health in some parts of Africa (Gunaratna et al., 2010), but increasing levels of this amino acid further would still be helpful. In addition, raising vitamin levels could help drastically improve health benefits and the recent approval of golden rice in several countries (Stokstad, 2019) is a welcome development in the fight against vitamin A deficiency that still leads to the deaths of 670,000 children per annum. Altering plants to contain increased levels of health promoting compounds such as carotenoids or omega-3 fatty acids, or to engineer the presence of compounds that act as prebiotics may also lead to health benefits.

Plants can also help in producing healthier lifestyles through the production of pharmaceutical chemicals or proteins (Chin et al., 2006; Schillberg et al., 2019). Many of the world's drugs were discovered in plants as they contain a much wider range of metabolites that many other organisms due to the specialised metabolism that they contain. Bioprospecting to identify novel pharmaceuticals, engineering genomes to increase amounts of these metabolites in plants or synthetic biology approaches to introduce such pathways in other organisms are all important methodologies that can help produce increased amounts of novel pharmaceutical. Other protein-based pharmaceuticals—such as plantibodies or vaccine epitopes—can be produced very efficiently in plants and their development and commercialisation can have a major beneficial effect on diseases in both humans and animals.

INDUSTRIAL USES OF PLANTS

Plants produce a number of products—such as starch and cell wall material—that can be used in large scale industrial processes. They often have to be modified before use and their modification *in planta* can, therefore, help make them more useful to for industrial uses (Zeeman et al., 2010; Loqué et al., 2015). In addition, the increase in atmospheric CO_2 over the past century caused by the burning of fossil fuel is well-known and biofuels can help to lower the rate of increase. Currently many biofuels are produced from sugars or oils harvested from plants that could be used for food or feed and developing plants into second generation biofuel feedstocks is, therefore, imperative to try and reverse this. Second generation biofuel production is currently not economically viable due to inefficiencies in degrading plant biomass to fermentable sugars (Bhatia et al., 2017), meaning that the development of plants with more easily digested cell walls is needed. There may also be plants that are suitable for production of biofuels that can grow on non-arable land and the development of such biofuel crops is needed. The use of algae in this regard is especially interesting and the manipulation of algae metabolism to increase oil accumulation for biodiesel, or to grow faster, will likely be of great importance for future biofuels (Behera et al., 2015). Engineering plants may also

allow for carbon sequestration to reverse increasing atmospheric carbon dioxide.

PLANT BIOTECHNOLOGY IN NON-INDUSTRIALISED COUNTRIES

Most commercial GM plants have been manufactured in industrialised countries and may not be suitable for growth in other parts of the world. Two examples demonstrating this come from Africa. Firstly, bollworm resistant bt cotton is no longer grown in Burkina Faso as the cotton quality from these plants was not as good as conventional local cotton varieties (Luna and Dowd-Uribe, 2020). Secondly, a study in South Africa demonstrated that locally produced maize varieties outcompeted insect resistant GM maize when there was little insect infestation (Fischer et al., 2015). These examples indicate that low- and middle-income countries will potentially miss out on the benefits of GM plants unless there is sufficient commercial incentive to engineer local varieties with this technology. This can happen either through partnerships with large agricultural biotechnology companies and/or academic institutions, or through local production.

Most calories in the human diet come from a small number of plants mainly the cereals maize, rice, wheat, millet, sorghum and some tuberous crops such as potato. There are many other plants that could act as crops if more research was applied to them and these are known as orphan crops (Ye and Fan, 2021). Examples of these include tef, yams, cassava, finger millet, pigeon pea and groundnut. These may well be more suitable for growth in non-industrialised countries, especially by small scale farmers and the development of such orphan crops would help food security.

CHANGING TECHNOLOGIES AND THEIR REGULATION

Plant improvement encompasses several types of technologies. Until the 1990s this was dominated by marker assisted breeding, but since then transgenic and cisgenic technologies have made valuable contributions to increasing productivity (Areal et al., 2012; Klümper and Qaim, 2014). More recently various genome editing techniques have been established that can directly alter nuclear DNA (Arora and Narula, 2017; Jaganathan et al., 2018; Manghwar et al., 2019). These allow precise editing of genomes

through the introduction of knockout or missense mutations at targeted loci or even the introduction of epigenetic changes. This is a fast-changing field, but the introduction of such techniques into all crop plants will be hugely helpful in allowing crop development.

Given the importance of these novel technologies to improve plant yield, regulatory processes are essential to allow development of improved crops. Many parts of the world have well-established frameworks, albeit often based on differing principles (Turnbull et al., 2021). While some parts of the world focus of trait-based approaches, others use the precautionary principle. Most developing countries, however, lack any framework which hampers their access to novel plant varieties and this is leading to a divide between industrialised and non-industrialised countries potentially increasing inequality (He and Krainer, 2021). The convergence—or further divergence—of regulatory frameworks will be critical in influencing progress of biotechnologically altered plants.

Ultimately the solution to improving crop yield will lie in a combination of different technologies including plant breeding, transgenesis, cisgenesis, genome editing, improved systems to monitor crops to assess whether they are stressed as well as better agricultural machinery. Many of these technologies have been integral in improving plant yields over the past century, while others will become increasingly important to improving plants in the future. Multidisciplinarity will be key to the successful development of these technologies to produce the crops that we will need.

The Chief Editors of the Plant Biotechnology section at Frontiers in Plant Science wish to use the section to help applied plant scientists develop improved crops. We expect that the majority of papers submitted to the Plant Biotechnology section will utilise transgenic, cisgenic or genome editing technologies and we welcome papers developing improved crop plants by those techniques as well as studies examining novel ways to improve agronomic traits in model plants. Finally, we wish to help regulatory regimes by welcoming papers discussing varying approaches to this in different parts of the world.

AUTHOR CONTRIBUTIONS

REFERENCES

Areal, F., Riesgo, L., and Rodriguez-Cerezo, E. (2012). Economic and agronomic impact of commercialized GM crops: a meta-analysis. *J. Agric. Sci.* 151, 7–33. doi: 10.1017/S0021859612000111

Arora, L., and Narula, A. (2017). Gene editing and crop improvement using CRISPR-cas9 system. *Front. Plant Sci.* 8:1932. doi: 10.3389/fpls.2017.01932

Behera, S., Singh, R., Arora, R., Sharma, N. K., Shukla, M., and Kumar, S. (2015). Scope of algae as third generation biofuels. *Front. Bioeng. Biotechnol.* 2:90. doi: 10.3389/fbioe.2014.00090

Bhatia, S. K., Kim, S. H., Yoon, J. J., and Yang, Y. H. (2017). Current status and strategies for second generation biofuel production using microbial systems. *Energy Convers. Manag.* 148, 1142–1156. doi: 10.1016/j.enconman.2017.06.073

Chin, Y. W., Balunas, M. J., Chai, H. B., and Kinghorn, A. D. (2006). Drug discovery from natural sources. *AAPS J.* 8, E239–E253. doi: 10.1007/BF02854894

Dong, O. X., and Ronald, P. C. (2019). Genetic engineering for disease resistance in plants: recent progress and future perspectives. *Plant Physiol.* 180, 26–38. doi: 10.1104/pp.18.01224

Ermakova, M., Danila, F. R., Furbank, R. T., and von Caemmerer, S. (2020). On the road to C4 rice: advances and perspectives. *Plant J.* 101, 940–950. doi: 10.1111/tpj.14562

Fischer, K., Van Den Berg, J., and Mutengwa, C. (2015). Is Bt maize effective in improving South African smallholder agriculture? *S. Afr. J. Sci.* 111, 1–2. doi: 10.17159/sajs.2015/a0092

Garg, M., Sharma, N., Sharma, S., Kapoor, P., Kumar, A., Chunduri, V., et al. (2018). Biofortified crops generated by breeding, agronomy, and transgenic approaches are improving lives of millions of people around the world. *Front. Nutr.* 5:12. doi: 10.3389/fnut.2018.00012

Global Nutrition Report (2016). *From Promise to Impact: Ending Malnutrition by 2030.* Washington, DC: IFPRI Books.

Global Nutrition Report (2020). "Inequalities in the global burden of malnutrition," in *Action on Equity to End Malnutrition* (Bristol), 32–60. Available online at: www.globalnutritionreport.org/reports/2020-global-nutrition-report/

Gunaratna, N. S., De Groote, H., Nestel, P., Pixley, K. V., and McCabe, G. P. (2010). A meta-analysis of community-based studies on quality protein maize. *Food Policy* 35, 202–210. doi: 10.1016/j.foodpol.2009.11.003

Hamim, I., Borth, W. B., Marquez, J., Green, J. C., Melzer, M. J., and Hu, J. S. (2018). Transgene-mediated resistance to Papaya ringspot virus: challenges and solutions. *Phytoparasitica* 46, 1–18. doi: 10.1007/s12600-017-0636-4

He, S., and Krainer, K. M. C. (2021). The inequity of biotechnological impact. *Mol. Plant* 14, 1–2. doi: 10.1016/j.molp.2020.12.011

Heap, I., and Duke, S. O. (2018). Overview of glyphosate-resistant weeds worldwide. *Pest Manag. Sci.* 74, 1040–1049. doi: 10.1002/ps.4760

Jaganathan, D., Ramasamy, K., Sellamuthu, G., Jayabalan, S., and Venkataraman, G. (2018). CRISPR for crop improvement: an update review. *Front. Plant Sci.* 9:985. doi: 10.3389/fpls.2018.00985

Klümper, W., and Qaim, M. (2014). A meta-analysis of the impacts of genetically modified crops. *PLoS ONE* 9:e111629. doi: 10.1371/journal.pone.0111629

Loqué, D., Scheller, H. V., and Pauly, M. (2015). Engineering of plant cell walls for enhanced biofuel production. *Curr. Opin. Plant Biol.* 25, 151–161. doi: 10.1016/j.pbi.2015.05.018

Luna, J. K., and Dowd-Uribe, B. (2020). Knowledge politics and the Bt cotton success narrative in Burkina Faso. *World Dev.* 136:105127. doi: 10.1016/j.worlddev.2020.105127

Malthus, T. (1798). *An essay on the Principle of Population.* London: J. Johnson. Available online at: www.esp.org/books/malthus/population/malthus.pdf

Manghwar, H., Lindsey, K., Zhang, X., and Jin, S. (2019). CRISPR/Cas System: recent advances and future prospects for genome editing. *Trends Plant Sci.* 24, 1102–1125. doi: 10.1016/j.tplants.2019.09.006

McKersie, B. (2015). Planning for food security in a changing climate. *J. Exp. Bot.* 66, 3435–3450. doi: 10.1093/jxb/eru547

Oerke, E. C. (2006). Crop losses to pests. *J. Agric. Sci.* 144, 31–43. doi: 10.1017/S0021859605005708

Ray, D. K., Mueller, N. D., West, P. C., and Foley, J. A. (2013). Yield trends are insufficient to double global crop production by 2050. *PLoS ONE* 8:e66428. doi: 10.1371/journal.pone.0066428

Ray, D. K., West, P. C., Clark, M., Gerber, J. S., Prishchepov, A. V., and Chatterjee, S. (2019). Climate change has likely already affected global food production. *PLoS ONE* 14:e0217148. doi: 10.1371/journal.pone.0217148

Schillberg, S., Raven, N., Spiegel, H., Rasche, S., and Buntru, M. (2019). Critical analysis of the commercial potential of plants for the production of recombinant proteins. *Front. Plant Sci.* 10:720. doi: 10.3389/fpls.2019.00720

Stokstad, E. (2019). After 20 years, Golden Rice nears approval. *Science* 366:934. doi: 10.1126/science.366.6468.934

Tabashnik, B. E. (2015). ABCs of insect resistance to Bt. *PLoS Genet.* 11:e1005646. doi: 10.1371/journal.pgen.1005646

Turnbull, C., Lillemo, M., and Hvoslef-Eide, T. A. K. (2021). Global regulation of genetically modified crops amid the gene edited crop boom – A review. *Front. Plant Sci.* 12:630396. doi: 10.3389/fpls.2021.630396

United Nations, Department of Economic and Social Affairs, Population Division (2019). *World Population Prospects 2019: Highlights. ST/ESA/SER.A/423.* Available online at: population.un.org/wpp/Publications/Files/WPP2019_Highlights.pdf

Ye, C. Y., and Fan, L. (2021). Orphan crops and their wild relatives in the genomic era. *Mol. Plant* 14, 27–39. doi: 10.1016/j.molp.2020.12.013

Zeeman, S. C., Kossmann, J., and Smith, A. M. (2010). Starch: Its metabolism, evolution, and biotechnological modification in plants. *Annu. Rev. Plant Biol.* 61, 209–234. doi: 10.1146/annurev-arplant-042809-112301

Check for
updates

Grand Challenges in Microalgae Domestication

Eric Maréchal*

Laboratoire de Physiologie Cellulaire et Végétale, CNRS, CEA, INRAE, Université Grenoble Alpes, IRIG, CEA Grenoble, Grenoble, France

INTRODUCTION

The term "microalgae" is a practical designation for photosynthetic unicellular cells, embracing organisms from two domains of life, i.e., Bacteria (Cyanobacteria) and various clades of Eukaryota deriving from primary (Archaeplastida) or secondary (e.g., Stramenopile) endosymbiosis events. In spite of this dispersed taxonomic distribution, microalgae share features that make them somehow "alike". Oxygenic photosynthesis derives from a common origin and makes microalgae prominent in trophic networks, as primary producers. They are unicellular or form very small colonies, and their cultivation rely on common methods, with provision of light, CO_2, water, and nutrients. Microalgae produce valuable molecules, like glycans, lipids, pigments, proteins, etc. Thus, although inappropriate in botanical or taxonomic sense, the term "microalgae" takes its legitimate meaning in ecology and human industry. This is both a weakness, when trying to transfer knowledge from one organism to another, and a strength, when addressing similar biotechnological questions. The development of a microalgae-based industry has become a societal challenge in the past decade. The climatic emergency and pressure on arable lands make the need for novel carbon-free and sustainable productions each day more urgent. Applications range from food, health, green chemistry to biofuels, with the promise of biomolecules produced from CO_2 captured from the atmosphere or carbon-emitting industries. In this context, an "algae sector" has emerged, gathering actors specialized in algae cultivation, harvesting, extraction processes, and biorefinery.

Turning a wild algal strain into an "algal crop", i.e., "domesticating" a microalga, represents a grail, because initial traits of interest may be present, like a relatively high level of oil, carbohydrates, pigments, etc., but the path to an enhanced, reproducible and up-scalable yield is extremely challenging.

Some lessons can be learnt from agriculture and give a novel stimulus to research in the microalgae sector. When one walks in nature, does he or she find wild plants resembling wheat, maize, tomato, sunflower, rapeseed, etc? Crop plants look giant and obese, when compared to their wild counterparts. Furthermore, after harvest, it is rare that cultivated seeds escape and invade uncultivated areas. Plant domestication is therefore focused on productivity and quality, but not on fitness in competition with wild communities. The strong difference between wild and domesticated plants illustrates that gains in yield should be obtainable in other branches of life, keeping in mind that cultivated plants are diploid, whereas the majority of currently cultivated microalgae are haploid.

BASIC LESSONS FROM AGRICULTURE

The domestication of crop plants started during the Neolithic Period and can be reconstructed based on population genetics studies, the analysis of archeological artifacts and the experimental

validation of reasonable hypotheses. In this first period of agriculture, the purification of strains by selecting traits of interest and the control of breeding techniques were consciously or unconsciously the strategies of choice.

If we focus on "gene targets" governing traits of interest, a well-known example is the domestication of teosinte into the crop maize. It involved "domestication genes" controlling the growth of lateral stems or formation of naked kernels (Chen et al., 2021). With genetic and agronomic strategies developed in the last century, additional mutations allowed the cultivation of maize virtually in all places in the world, with remarkably high yields. Targeting genes can now be much faster using CRISPR/Cas9-based methods (Lemmon et al., 2018). It is also feasible to produce plants devoid of any foreign DNA by methods collectively called next-generation or new breeding techniques (NBTs) (Holme et al., 2019). Some of the NBTs target genes with a Cas9-coding DNA vector who does not integrate into the plant genome, or even a microinjected Cas9 protein and gRNAs, whereas other NBTs are based on non-targeted random mutations (Holme et al., 2019; Anders et al., 2021). The concept of "gene target" proved determinant in the understanding of some domesticated organisms. The "*de novo*" domestication of tomato could thus be reconstructed experimentally gene-by-gene (Lemmon et al., 2018). This being stated, when transferred to microalgae, it would be naïve to limit the understanding of domestication to the modification of well-selected genes. Some traits depend on complex interactions, and strategies should also consider regulatory genes controlling broad metabolic, physiological, or developmental processes, combinations of genes and non-targeted multiple random mutations.

Plant domestication also highlights the benefits of features, which cannot be reduced to a gene target approach. In schematic terms, improved traits can emerge from specific "rearrangements" or "duplications of chromosomes". In the simplest example in maize, the crossing of homozygous parent lines can give rise to an heterozygous "hybrid" with superior robustness and productivity, a phenomenon called "heterosis" (Srivastava et al., 2020; Chen et al., 2021). Polyploidy can also "improve" patterns of gene expression (Schaart et al., 2021). On the one hand, it can be a high level of "autopolyploidy", which can be induced by a variety of techniques (Chen et al., 2020). On the other hand, "allopolyploids" deriving from the crossing of very close but distinct species, can give rise to progenies retaining the parent chromosomes and exhibiting higher productivity and robustness. Rapeseed (*Brassica napus*) is an allotetraploid, containing chromosomes from *B. rapa* and *B. olearacea* parents (Mason and Snowdon, 2016). Likewise, common wheat (*Triticum aestivun*) is a hexaploid combining chromosomes from diverse *Triticum* and *Aegilops* species (Parisod and Badaeva, 2020). The improvement of algal strains by developing hybrids, autopolyploids or allopolyploids is currently barely explored.

The last lesson from plant domestication may be that growth, productivity, resistance to diseases, etc., can be addressed by domestication strategies, but the response to abiotic environmental stressors, like drought or nutrient scarcity are more difficult questions (Li et al., 2021).

CHALLENGES IN MICROALGAE DOMESTICATION

With the notable exceptions of yeast and some fungi, or *Chlamydomonas*, genetics is poorly developed in microbiology. Due to their unicellular nature, microalgae populate environments (oceans, rivers, soils, snow, ice, extreme habitats, etc.) primarily by mitotic divisions, either in haploid or diploid forms. The majority of microbial organisms are not cultivable (Ding et al., 2014). Strains collected in nature can be purified using a cell sorter or by serial cultivation on petri dishes until obtaining clonal axenic lines. Sometimes it is not possible to separate a microalga from its companion bacteria (Lupette et al., 2016). Only in response to environmental or physiological triggers, do gametogenesis, sexual reproduction and meiosis occur (Lopez et al., 2015). Whereas, breeding and shuffling of natural alleles are major drivers of diversity in plants and animals, they do not seem to play this role in microalgae. Genetic diversity relies on genomic mutation rates and transfers of genetic material within and between species. A bias in the microbiological approach of domestication, compared to agriculture, is that the majority of strategies aims at developing an improved clonal strain, and disregard more global approaches with multiple series of mutant lines, which could be crossed, combined and improved.

A first challenge is to pursue the exploration of biodiversity. This includes efforts to resolve the question of non-cultivable strains. Some qualitative traits like the capacity to produce a biomolecule of interest may serve as a starting point for domestication attempts; nevertheless, the capacity to grow fast and produce biomass should be considered an important initial property for further consideration.

A second challenge is the lack of sequenced genomes and the difficulty to genetically transform many non-model and emerging model species. Let us cover the biodiversity of microalgae pointing to some examples of current efforts on "algal crop" models.

- In Cyanobacteria, spirulina (*Arthrospira platensis*) is "technically" haploid. It contains a rich equipment of restriction enzymes, and methods to efficiently transform this popular Cyanobacterial crop have been made available only recently (Jeamton et al., 2017; Dehghani et al., 2018).
- In Eukaryota, the Archaeplastida comprise three lineages: Green Algae, Red Algae, and Glaucophyta. The Green Alga genetic model is *Chlamydomonas reinhardtii*, with a genome sequenced two decades ago (Blaby et al., 2014; Lopez et al., 2015). Its life cycle relies mainly on haploid asexual divisions; sexual reproduction can be controlled *in vitro* (Wilson, 2008). Nuclear and chloroplastic transformations are possible and multiple methods have been developed for gene editing (Ghribi et al., 2020). It is thus considered as a model for synthetic biology (Scaife et al., 2015). In spite of these advantages, its limited biomass and productivity does not make *C. reinhardtii* a real crop (Butler et al., 2020). *Chlorella* and *Dunaliella* species have life cycles close to that of *C.*

reinhardtii. Chlorella are maintained in haploid form, and it is difficult to know whether sexual reproduction could be obtained. Genomic data have been made available recently for a few strains (Wu et al., 2019) and transformation mediated by *Agrobacterium tumefaciens* is possible (Cha et al., 2012; Sharma et al., 2021). *Dunalliella* has a main haploid cycle, but sexual reproduction is known. Draft genomic data are available (Polle et al., 2017) and nuclear and chloroplastic transformations have been validated recently (Dehghani et al., 2017; Norzagaray-Valenzuela et al., 2018; Bahador et al., 2019; Song et al., 2019). Technically both *Chlorella* and *Dunaliella* could be amenable to intensive genetic engineering. *Scenedesmus* and *Haematococcus* species are Green Algae producing pluri-nuclear cells, with four to eight haploid nuclei, in the course of cell fission. Genomes of *S. obliquus* (Nag Dasgupta et al., 2018; Astafyeva et al., 2020) and *H. pluvialis* (Luo et al., 2019; Morimoto et al., 2020) are available. Transformation of *S. obliquus* via *A. tumefasciens* is feasible (Suttangkakul et al., 2019). Both nuclear and chloroplastic transformations have been described for *H. pluvialis* (Yuan et al., 2019; Wang et al., 2020; Cui et al., 2021). Finally, Charophyta are Green Algae close to Embryophyta, propagating by haploid asexual division; sexual reproduction is known. Genomic data are available for the Charophyta model *Klebsormidium* (Hori et al., 2014), and transformation and gene editing have been obtained in a *Closterium* species (Abe et al., 2011). In Red Algae, *Galdieria sulphuraria* is considered for its cultivation at high temperature and acidity. Its small genome has been known for nearly two decades (Barbier et al., 2005), and genetic engineering is possible (Fujiwara et al., 2019).

- In photosynthetic Stramenopiles, a branch of Eukaryota deriving from a secondary endosymbiosis, the diatom *Phaeodactylum* and the eustigmatophytes *Nannochloropsis/Microchloropsis* species are crop models studied by multiple groups worldwide. Robust genomic data (Bowler et al., 2008; Vieler et al., 2012) and tools for genetic engineering are available; multiple examples show the power of gene editing to improve traits and domesticate these lines (Siaut et al., 2007; De Riso et al., 2009; Kilian et al., 2011; Cao et al., 2012; Daboussi et al., 2014; Dolch et al., 2017; Poliner et al., 2018a; Nobusawa et al., 2019; Billey et al., 2021). Remarkably, whereas eustigmatophytes are maintained in haploid form, the vegetative cells of diatoms are diploid.

In the above listed microalgae, strategies developed on multiple gene targets rely on recent technological developments. Progresses in non-GMO plant domestication also inspire the search for methods allowing the transient expression of Cas9 and gRNAs, for instance, by an episomal DNA, removed after lifting the vector selection pressure, to generate strains without any foreign DNA (Poliner et al., 2018b; Sharma et al., 2018; Moosburner et al., 2020).

A third challenge is to control meiosis, gametogenesis, and sexual reproduction. Conventional genetics could boost targeted strategies, by allowing allelic rearrangements. Major breakthroughs could be anticipated if sexual reproduction could be controlled in routine in *Chlorella*, *Dunaliella*, *Scenedesmus*, *Klebsormidium*, *Phaeodactylum* etc. We need to keep in mind that in some algal species, the existence of mating types may limit the capacity to develop homozygous diploids requiring the development of self-mating lines (Kariyawasam et al., 2019).

A fourth challenge lies in the development of systems allowing the control of chromosomic combinations and duplication. Heterosis relies directly on the capacity to obtain heterozygous hybrids from homozygous lines. Diatoms have diploid vegetative cells allowing the exploration of this property. The development of autopolyploids from haploid cells has been attempted in pioneering experiments by treatments of *Chlamydomonas* with molecules blocking cell division, like colchicine (Wetherell and Krauss, 1956) or colcemide (Kwak et al., 2017; Kariyawasam et al., 2019). In diploid *Chlamydomonas* lines, the lipid yield was improved (Kwak et al., 2017). Concerning allopolyploidy, sexual reproduction between distinct parental species needs to occur. The genome of the diatom *Fistullifera solaris* derives from two distinct parental species (Tanaka et al., 2015). *F. solaris* is oleaginous, suggesting that allopolyploidy may have a positive impact on productivity in diatoms as well. Future work on cell fusion may help circumventing the need for a sexual reproduction, within or between species, to further explore this potential.

Eventually, a last challenge concerns random mutagenesis and *in vivo* directed evolution approaches (Crook et al., 2016) as a way to domesticate microalgae (Pourmir and Johannes, 2012; Rossoni and Weber, 2019). Such strategies require excellent screening methods for selection of improved lines. They also need sequencing efforts to characterize mutants and comprehend the relation between genomic changes and gained properties.

In conclusion, microalgae domestication is in its infancy. The exploration of biodiversity needs to be pursued. Given the development of enabling methods in an increasing number of microalgal groups, there is no doubt that the coming decade will be marked by fascinating results.

AUTHOR CONTRIBUTIONS

EM has conceived and written the manuscript.

FUNDING

REFERENCES

Abe, J., Hori, S., Tsuchikane, Y., Kitao, N., Kato, M., and Sekimoto, H. (2011). Stable nuclear transformation of the closterium peracerosum-strigosum-littorale complex. *Plant Cell Physiol.* 52, 1676–1685. doi: 10.1093/pcp/pcr103

Anders, S., Cowling, W., Pareek, A., Gupta, K. J., Singla-Pareek, S. L., and Foyer, C. H. (2021). Gaining acceptance of novel plant breeding technologies. *Trends Plant Sci.* 26, 575–587. doi: 10.1016/j.tplants.2021.03.004

Astafyeva, Y., Alawi, M., Indenbirken, D., Danso, D., Grundhoff, A., Hanelt, D., et al. (2020). Draft genome sequence of the green alga *Scenedesmus acuminatus* SAG 38.81. *Microbiol. Resour. Announc.* 9:e01278-19. doi: 10.1128/MRA.01278-19

Bahador, E., Einali, A., Azizian-Shermeh, O., and Sangtarash, M. H. (2019). Metabolic responses of the green microalga *Dunaliella salina* to silver nanoparticles-induced oxidative stress in the presence of salicylic acid treatment. *Aquat. Toxicol.* 217:105356. doi: 10.1016/j.aquatox.2019.105356

Barbier, G., Oesterhelt, C., Larson, M. D., Halgren, R. G., Wilkerson, C., Garavito, R. M., et al. (2005). Comparative genomics of two closely related unicellular thermo-acidophilic red algae, *Galdieria sulphuraria* and *Cyanidioschyzon merolae*, reveals the molecular basis of the metabolic flexibility of *Galdieria sulphuraria* and significant differences in carbohydrate metabolism of both algae. *Plant Physiol.* 137, 460–474. doi: 10.1104/pp.104.051169

Billey, E., Magnesini, L., Leterme, S., Bedhomme, M., Andres-Robin, A., Poulet, L., et al. (2021). Characterization of the bubblegum acyl-CoA synthetase of *Microchloropsis gaditana*. *Plant Physiol.* 185, 815–835. doi: 10.1093/plphys/kiaa110

Blaby, I. K., Blaby-Haas, C. E., Tourasse, N., Hom, E. F., Lopez, D., Aksoy, M., et al. (2014). The chlamydomonas genome project: a decade on. *Trends Plant Sci.* 19, 672–680. doi: 10.1016/j.tplants.2014.05.008

Bowler, C., Allen, A. E., Badger, J. H., Grimwood, J., Jabbari, K., Kuo, A., et al. (2008). The phaeodactylum genome reveals the evolutionary history of diatom genomes. *Nature* 456, 239–244. doi: 10.1038/nature07410

Butler, T., Kapoore, R. V., and Vaidyanathan, S. (2020). *Phaeodactylum tricornutum*: a diatom cell factory. *Trends Biotechnol.* 38, 606–622. doi: 10.1016/j.tibtech.2019.12.023

Cao, S., Zhang, X., Ye, N., Fan, X., Mou, S., Xu, D., et al. (2012). Evaluation of putative internal reference genes for gene expression normalization in Nannochloropsis sp. by quantitative real-time RT-PCR. *Biochem. Biophys. Res. Commun.* 424, 118–123. doi: 10.1016/j.bbrc.2012.06.086

Cha, T. S., Yee, W., and Aziz, A. (2012). Assessment of factors affecting Agrobacterium-mediated genetic transformation of the unicellular green alga, *Chlorella vulgaris*. *World J. Microbiol. Biotechnol.* 28, 1771–1779. doi: 10.1007/s11274-011-0991-0

Chen, J. T., Coate, J. E., and Meru, G. (2020). Editorial: artificial polyploidy in plants. *Front. Plant Sci.* 11:621849. doi: 10.3389/fpls.2020.621849

Chen, Q., Li, W., Tan, L., and Tian, F. (2021). Harnessing knowledge from maize and rice domestication for new crop breeding. *Mol. Plant* 14, 9–26. doi: 10.1016/j.molp.2020.12.006

Crook, N., Abatemarco, J., Sun, J., Wagner, J. M., Schmitz, A., and Alper, H. S. (2016). *In vivo* continuous evolution of genes and pathways in yeast. *Nat. Commun.* 7:13051. doi: 10.1038/ncomms13051

Cui, H., Zhao, C., Xu, W., Zhang, H., Hang, W., Zhu, X., et al. (2021). Characterization of type-2 diacylglycerol acyltransferases in *Haematococcus lacustris* reveals their functions and engineering potential in triacylglycerol biosynthesis. *BMC Plant Biol.* 21:20. doi: 10.1186/s12870-020-02794-6

Daboussi, F., Leduc, S., Marechal, A., Dubois, G., Guyot, V., Perez-Michaut, C., et al. (2014). Genome engineering empowers the diatom *Phaeodactylum tricornutum* for biotechnology. *Nat. Commun.* 5:3831. doi: 10.1038/ncomms4831

De Riso, V., Raniello, R., Maumus, F., Rogato, A., Bowler, C., and Falciatore, A. (2009). Gene silencing in the marine diatom *Phaeodactylum tricornutum*. *Nucleic Acids Res.* 37:e96. doi: 10.1093/nar/gkp448

Dehghani, J., Adibkia, K., Movafeghi, A., Barzegari, A., Pourseif, M. M., Maleki Kakelar, H., et al. (2018). Stable transformation of *Spirulina (Arthrospira) platensis*: a promising microalga for production of edible vaccines. *Appl. Microbiol. Biotechnol.* 102, 9267–9278. doi: 10.1007/s00253-018-9296-7

Dehghani, J., Movafeghi, A., Barzegari, A., and Barar, J. (2017). Efficient and stable transformation of *Dunaliella pseudosalina* by 3 strains of *Agrobacterium tumefaciens*. *Bioimpacts* 7, 247–254. doi: 10.15171/bi.2017.29

Ding, X., Cao, C. C., and Sun, X. (2014). Intrinsic correlation of oligonucleotides: a novel genomic signature for metagenome analysis. *J. Theor. Biol.* 353, 9–18. doi: 10.1016/j.jtbi.2014.02.039

Dolch, L. J., Rak, C., Perin, G., Tourcier, G., Broughton, R., Leterrier, M., et al. (2017). A palmitic acid elongase affects eicosapentaenoic acid and plastidial monogalactosyldiacylglycerol levels in nannochloropsis. *Plant Physiol.* 173, 742–759. doi: 10.1104/pp.16.01420

Fujiwara, T., Hirooka, S., Mukai, M., Ohbayashi, R., Kanesaki, Y., Watanabe, S., et al. (2019). Integration of a *Galdieria* plasma membrane sugar transporter enables heterotrophic growth of the obligate photoautotrophic red alga *Cynanidioschyzon merolae*. *Plant Direct* 3:e00134. doi: 10.1002/pld3.134

Ghribi, M., Nouemssi, S. B., Meddeb-Mouelhi, F., and Desgagne-Penix, I. (2020). Genome editing by CRISPR-Cas: a game change in the genetic manipulation of *Chlamydomonas*. *Life* 10:295. doi: 10.3390/life10110295

Holme, I. B., Gregersen, P. L., and Brinch-Pedersen, H. (2019). Induced genetic variation in crop plants by random or targeted mutagenesis: convergence and differences. *Front. Plant Sci.* 10:1468. doi: 10.3389/fpls.2019.01468

Hori, K., Maruyama, F., Fujisawa, T., Togashi, T., Yamamoto, N., Seo, M., et al. (2014). *Klebsormidium flaccidum* genome reveals primary factors for plant terrestrial adaptation. *Nat. Commun.* 5:3978. doi: 10.1038/ncomms4978

Jeamton, W., Dulsawat, S., Tanticharoen, M., Vonshak, A., and Cheevadhanarak, S. (2017). Overcoming intrinsic restriction enzyme barriers enhances transformation efficiency in *Arthrospira platensis* C1. *Plant Cell Physiol.* 58, 822–830. doi: 10.1093/pcp/pcx016

Kariyawasam, T., Joo, S., Goodenough, U., and Lee, J. H. (2019). Novel approaches for generating and manipulating diploid strains of *Chlamydomonas reinhardtii*. *Algae* 34, 35–43. doi: 10.4490/algae.2019.34.2.25

Kilian, O., Benemann, C. S., Niyogi, K. K., and Vick, B. (2011). High-efficiency homologous recombination in the oil-producing alga *Nannochloropsis* sp. *Proc. Natl. Acad. Sci. U.S.A.* 108, 21265–21269. doi: 10.1073/pnas.1105861108

Kwak, M., Park, W. K., Shin, S. E., Koh, H. G., Lee, B., Jeong, B., et al. (2017). Improvement of biomass and lipid yield under stress conditions by using diploid strains of *Chlamydomonas reinhardtii*. *Algal Res. Biomass Biofuels Bioproducts* 26, 180–189. doi: 10.1016/j.algal.2017.07.027

Lemmon, Z. H., Reem, N. T., Dalrymple, J., Soyk, S., Swartwood, K. E., Rodriguez-Leal, D., et al. (2018). Rapid improvement of domestication traits in an orphan crop by genome editing. *Nat. Plants* 4, 766–770. doi: 10.1038/s41477-018-0259-x

Li, S., Zhang, C., Li, J., Yan, L., Wang, N., and Xia, L. (2021). Present and future prospects for wheat improvement through genome editing and advanced technologies. *Plant Commun.* 2:100211. doi: 10.1016/j.xplc.2021.100211

Lopez, D., Hamaji, T., Kropat, J., De Hoff, P., Morselli, M., Rubbi, L., et al. (2015). Dynamic changes in the transcriptome and methylome of *Chlamydomonas reinhardtii* throughout its life cycle. *Plant Physiol.* 169, 2730–2743. doi: 10.1104/pp.15.00861

Luo, Q., Bian, C., Tao, M., Huang, Y., Zheng, Y., Lv, Y., et al. (2019). Genome and transcriptome sequencing of the astaxanthin-producing green microalga, *Haematococcus pluvialis*. *Genome Biol. Evol.* 11, 166–173. doi: 10.1093/gbe/evy263

Lupette, J., Lami, R., Krasovec, M., Grimsley, N., Moreau, H., Piganeau, G., et al. (2016). *Marinobacter* dominates the bacterial community of the *Ostreococcus tauri* phycosphere in culture. *Front. Microbiol.* 7:1414. doi: 10.3389/fmicb.2016.01414

Mason, A. S., and Snowdon, R. J. (2016). Oilseed rape: learning about ancient and recent polyploid evolution from a recent crop species. *Plant Biol.* 18, 883–892. doi: 10.1111/plb.12462

Moosburner, M. A., Gholami, P., Mccarthy, J. K., Tan, M., Bielinski, V. A., and Allen, A. E. (2020). Multiplexed knockouts in the model diatom phaeodactylum by episomal delivery of a selectable Cas9. *Front. Microbiol.* 11:5. doi: 10.3389/fmicb.2020.00005

Morimoto, D., Yoshida, T., and Sawayama, S. (2020). Draft genome sequence of the astaxanthin-producing microalga *Haematococcus lacustris* strain NIES-144. *Microbiol. Resour. Announc.* 9:e00128-20. doi: 10.1128/MRA.00128-20

Nag Dasgupta, C., Nayaka, S., Toppo, K., Singh, A. K., Deshpande, U., and Mohapatra, A. (2018). Draft genome sequence and detailed characterization

of biofuel production by oleaginous microalga *Scenedesmus quadricauda* LWG002611. *Biotechnol. Biofuels* 11, 308. doi: 10.1186/s13068-018-1308-4

Nobusawa, T., Yamakawa-Ayukawa, K., Saito, F., Nomura, S., Takami, A., and Ohta, H. (2019). A homolog of Arabidopsis SDP1 lipase in *Nannochloropsis* is involved in degradation of de novo-synthesized triacylglycerols in the endoplasmic reticulum. *Biochim. Biophys. Acta* 1864, 1185–1193. doi: 10.1016/j.bbalip.2019.05.013

Norzagaray-Valenzuela, C. D., German-Baez, L. J., Valdez-Flores, M. A., Hernandez-Verdugo, S., Shelton, L. M., and Valdez-Ortiz, A. (2018). Establishment of an efficient genetic transformation method in *Dunaliella tertiolecta* mediated by *Agrobacterium tumefaciens*. *J. Microbiol. Methods* 150, 9–17. doi: 10.1016/j.mimet.2018.05.010

Parisod, C., and Badaeva, E. D. (2020). Chromosome restructuring among hybridizing wild wheats. *New Phytol.* 226, 1263–1273. doi: 10.1111/nph.16415

Poliner, E., Farre, E. M., and Benning, C. (2018a). Advanced genetic tools enable synthetic biology in the oleaginous microalgae *Nannochloropsis* sp. *Plant Cell Rep.* 37, 1383–1399. doi: 10.1007/s00299-018-2270-0

Poliner, E., Takeuchi, T., Du, Z. Y., Benning, C., and Farre, E. M. (2018b). Non-transgenic marker-free gene disruption by an episomal CRISPR system in the *Oleaginous Microalga*, *Nannochloropsis oceanica* CCMP1779. *ACS Synth. Biol.* 7, 962–968. doi: 10.1021/acssynbio.7b00362

Polle, J. E. W., Barry, K., Cushman, J., Schmutz, J., Tran, D., Hathwaik, L. T., et al. (2017). Draft nuclear genome sequence of the halophilic and beta-carotene-accumulating green alga *Dunaliella salina* strain CCAP19/18. *Genome Announc.* 5:e01105-17. doi: 10.1128/genomeA.01105-17

Pourmir, A., and Johannes, T. W. (2012). Directed evolution: selection of the host organism. *Comput. Struct. Biotechnol. J.* 2:e201209012. doi: 10.5936/csbj.201209012

Rossoni, A. W., and Weber, A. P. M. (2019). Systems biology of cold adaptation in the polyextremophilic red alga *Galdieria sulphuraria*. *Front. Microbiol.* 10:927. doi: 10.3389/fmicb.2019.00927

Scaife, M. A., Nguyen, G., Rico, J., Lambert, D., Helliwell, K. E., and Smith, A. G. (2015). Establishing *Chlamydomonas reinhardtii* as an industrial biotechnology host. *Plant J.* 82, 532–546. doi: 10.1111/tpj.12781

Schaart, J. G., Van De Wiel, C. C. M., and Smulders, M. J. M. (2021). Genome editing of polyploid crops: prospects, achievements and bottlenecks. *Transgenic Res.* 30, 337–351. doi: 10.1007/s11248-021-00251-0

Sharma, A. K., Nymark, M., Sparstad, T., Bones, A. M., and Winge, P. (2018). Transgene-free genome editing in marine algae by bacterial conjugation—comparison with biolistic CRISPR/Cas9 transformation. *Sci. Rep.* 8:14401. doi: 10.1038/s41598-018-32342-0

Sharma, P. K., Goud, V. V., Yamamoto, Y., and Sahoo, L. (2021). Efficient *Agrobacterium tumefaciens*-mediated stable genetic transformation of green microalgae, *Chlorella sorokiniana*. *3 Biotech* 11:196. doi: 10.1007/s13205-021-02750-7

Siaut, M., Heijde, M., Mangogna, M., Montsant, A., Coesel, S., Allen, A., et al. (2007). Molecular toolbox for studying diatom biology in *Phaeodactylum tricornutum*. *Gene* 406, 23–35. doi: 10.1016/j.gene.2007.05.022

Song, G., Wang, W., Hu, L., Liu, Y., Li, A., Du, J., et al. (2019). An exploration of the rapid transformation method for *Dunaliella salina* system. *AMB Express* 9:181. doi: 10.1186/s13568-019-0905-3

Srivastava, R. K., Bollam, S., Pujarula, V., Pusuluri, M., Singh, R. B., Potupureddi, G., et al. (2020). Exploitation of heterosis in pearl millet: a review. *Plants* 9:807. doi: 10.3390/plants9070807

Suttangkakul, A., Sirikhachornkit, A., Juntawong, P., Puangtame, W., Chomtong, T., Srifa, S., et al. (2019). Evaluation of strategies for improving the transgene expression in an oleaginous microalga *Scenedesmus acutus*. *BMC Biotechnol.* 19:4. doi: 10.1186/s12896-018-0497-z

Tanaka, T., Maeda, Y., Veluchamy, A., Tanaka, M., Abida, H., Marechal, E., et al. (2015). Oil accumulation by the oleaginous diatom *Fistulifera solaris* as revealed by the genome and transcriptome. *Plant Cell* 27, 162–176. doi: 10.1105/tpc.114.135194

Vieler, A., Wu, G., Tsai, C. H., Bullard, B., Cornish, A. J., Harvey, C., et al. (2012). Genome, functional gene annotation, and nuclear transformation of the heterokont oleaginous alga *Nannochloropsis oceanica* CCMP1779. *PLoS Genet.* 8:e1003064. doi: 10.1371/journal.pgen.1003064

Wang, K., Cui, Y., Wang, Y., Gao, Z., Liu, T., Meng, C., et al. (2020). Chloroplast genetic engineering of a unicellular green alga *Haematococcus pluvialis* with expression of an antimicrobial peptide. *Mar. Biotechnol.* 22, 572–580. doi: 10.1007/s10126-020-09978-z

Wetherell, D. F., and Krauss, R. W. (1956). Colchicine-induced polyploidy in *Chlamydomonas*. *Science* 124, 25–26. doi: 10.1126/science.124.3210.25

Wilson, N. F. (2008). Gametic cell adhesion and fusion in the unicellular alga *Chlamydomonas*. *Methods Mol. Biol.* 475, 39–51. doi: 10.1007/978-1-59745-250-2_3

Wu, T., Li, L., Jiang, X., Yang, Y., Song, Y., Chen, L., et al. (2019). Sequencing and comparative analysis of three Chlorella genomes provide insights into strain-specific adaptation to wastewater. *Sci. Rep.* 9:9514. doi: 10.1038/s41598-019-45511-6

Yuan, G., Xu, X., Zhang, W., Zhang, W., Cui, Y., Qin, S., et al. (2019). Biolistic Transformation of *Haematococcus pluvialis* with constructs based on the flanking sequences of its endogenous alpha tubulin gene. *Front. Microbiol.* 10:1749. doi: 10.3389/fmicb.2019.01749

Check for updates

Plant Biology Research: What Is Next?

Anna N. Stepanova*

Department of Plant and Microbial Biology, Program in Genetics, North Carolina State University, Raleigh, NC, United States

Plant biology is a key area of science that bears major weight in the mankind's ongoing and future efforts to combat the consequences of global warming, climate change, pollution, and population growth. An in-depth understanding of plant physiology is paramount to our ability to optimize current agricultural practices, to develop new crop varieties, or to implement biotechnological innovations in agriculture. The next-generation cultivars would have to withstand environmental contamination and a wider range of growth temperatures, soil nutrients and moisture levels and effectively deal with growing pathogen pressures to continue to yield well in even suboptimal conditions.

What are the next big questions in plant physiology, and plant biology in general, and what avenues of research should we be investigating and training students in for the next decade? As a plant scientist surrounded by like-minded individuals, I hear a lot of ideas that over time turn into buzz words, such as plant resilience, genotype-to-phenotype, data science, systems biology, biosensing, synthetic biology, neural networks, robustness, interdisciplinary training, new tool development, modeling, etc. What does it all mean and what are the main challenges that we should all be working on solving? Herein, I present my personal perspective on what the immediate questions and the biggest longer-term issues in plant science are. I suggest some themes and directions for future research in plant biology, some relatively obvious and some potentially unique, having been shaped by my own professional interests, experiences and the background in plant molecular genetics and physiology.

INTEGRATION, PACKAGING, VISUALIZATION AND INTERPRETATION OF EXISTING OMICS AND GENETIC DATA

For the past three decades, a lot of emphasis has been made on a small set of plant model organisms, primarily on Arabidopsis. There is no other plant on earth we know as much about as we do about this mustard weed. One clear need in the area of plant sciences is to make sense of the vast amount of descriptive phenotypic data that have been generated for this species and a handful of others—the transcriptome, metabolome, proteome, phenome, interactome, etc.— and the amazing genetic resources that have been built: mutants, transgenic lines and natural accession germplasm collections, tools and protocols, genomic sequences and other resources (Koorneef and Meinke, 2010). Now, how do we organize these data into a series of integrated, comprehensive, user-friendly, cross-communicating databases that are easily accessible, searchable, trackable, and visual, with data that are downloadable and compatible with comparative analyses? How do we display the available data at a variety of scales, from the subcellular to the organismal and population level—think Google Earth but for an ecosystem or an agricultural field that allows you to zoom in and out to see the overview and the closeup—perhaps, by integrating and expanding existing initiative likes Plant Cell Atlas and ePlant (Waese et al., 2017; Rhee et al., 2019)? With the genome sequences of these select organisms in hand, often of multiple accessions of each, what can we learn about the genotype-to-phenotype relations? How can we use that knowledge

to extrapolate the rules or patterns we discover in model organisms to species for which we have no experimental data beyond possibly a draft-quality genomic sequence and a few fragmentary phenotypic datasets? In other words, can the data obtained in reference organisms be leveraged to infer useful information relevant to a wide range of species of agricultural, ecological or, perhaps, ethnobotanical importance? Let's look into some examples of that.

TRANSLATIONAL RESEARCH: MOVING FOUNDATIONAL DISCOVERIES FROM MODELS TO CROPS

It comes as no surprise that for the past 10–20 years the emphasis has been gradually shifting from Arabidopsis to non-model organisms, including crops and rare plant species. The key reason for that is the pressing need to move fast on crop improvement and plant conservation in light of the worlds' fast-growing population, climate change, pollution, habitat and agricultural land loss, and ever-increasing pathogen pressures. This shift of research focus is also steered by changing governmental policies and funders' priorities. To make the transition to studying crops and other non-models as smooth as possible, robust computational pipelines are needed that produce high-quality genome assemblies from combinations of short- and long-read sequences. In this regard, tackling the much more complex genomes of polyploid species presents an even greater challenge. With the genome sequences and high-quality assembles on hand, orthologous genes that have previously been studied only in reference organisms need to be tested for function in candidate processes in the non-model species of interest to determine what aspects of their function are conserved and what features are divergent. The key bottleneck in this process is, of course, the recalcitrance of many non-models to genetic transformation and plant regeneration (Anjanappa and Gruissem, 2021). Thus, a major effort would need to be invested into new method development to improve the plant *in vitro* culturing, genetic transformation and regeneration pipelines, with the ectopic activation of morphogenesis genes like *BABY BOOM*, *WUSCHEL*, *LEAFY COTYLEDON1* and *2*, and several others holding major promise for boosting the regeneration efficiency of otherwise recalcitrant plant species and cultivars (Gordon-Kamm et al., 2019). Further optimization of genome editing technologies, including classical gene disruption through indels as well as more targeted gene edits *via* base- and prime-editing or homologous-recombination-based methods, should enable highly tailored manipulation of genes of interest. The foundational knowledge gained in both model and non-model organisms can then be leveraged by applied plant biologists and environmentalists in crop improvement and plant conservation.

INTERPRETING THE CODE

One aspect of experimental research we have become good at over the past 10 years is genome and transcriptome sequencing. The current challenge is to learn to infer what the sequence tells us about what a gene does and how it is regulated based on the code alone. Can we look at gene's genomic sequence and infer not only the gene function, but also the different levels of gene regulation, all from just the sequence without any additional experimentation? To elaborate on that distinction between function and regulation, we can already infer the likely function of an orthologous gene in a crop (previously studied in another species) based on the degree of conservation of its genomic sequence, and deduce, for instance, an enzymatic reaction a protein may catalyze, or a DNA element a transcription factor may bind, or a specific ion the channel may transport, or an array of ligands or other molecules a protein may interact with. What we cannot yet reliably do is to predict based on the gene sequence alone when and where the gene is transcribed and what environmental or developmental stimuli alter its expression, how stable its transcript is, what splicing patterns the transcript has in specific cell types or conditions, or what factors dictate these patterns, or how well the transcript is translated, how the protein folds, where in the cell the protein is targeted, what its half-life is, and so on. Can we someday look at the gene sequence and predict whether the gene is essential or what organ or tissues will be affected in the loss- or gain-of-function mutant, and what phenotype the mutant will show, all without having to run an experiment? Once we learn to do that for a diploid model plant, can the knowledge be translated to polyploids that may have a greater level of gene redundancy and potentially more cases of neofunctionalization? How do we gain that extraordinary power?

One of the critical components of the inferring-the-function or genotype-to-phenotype challenge will involve machine learning and neural network models, with the size and quality of the training datasets presenting as the likely bottleneck that would determine the accuracy of neural networks' predictions (Ching et al., 2018). While the role of computational biologists in this endeavor would be to develop new algorithms or adapt existing pipelines and test the models, the irreplaceable function of experimental plant biologists in this effort will be to generate the most complete and robust datasets for model training. This inevitably brings us to the next big theme, data quality.

DATA QUALITY: STANDARDIZATION, RELIABILITY, ROBUSTNESS AND TRACKING

As experimental scientists, most if not all of us have had the negative experience of not being able to reproduce an important result (sometimes even our own) or confirm the identity of a material someone has shared with us (e.g., a strain, a plasmid, or a seed stock from a colleague or another lab). Issues with biological variation (e.g., differences in germination between seed batches), small sample size (due to prohibitive cost, time or material constraints, or other limitations), human error (suboptimal labeling nomenclature, poor tracking, inadequate record keeping, substandard experimental design, miscalculation, personnel changes, or outright sloppiness) or malfunctioning instrumentation (in many cases, due to the lack of funding or time to upkeep or upgrade the equipment) can

all contribute to the limited reproducibility of experimental data or sample mix-up. Rarely is the wrongdoing intentional, but the consequences of these errors can be enormous. What can we do to minimize mistakes, standardize internal lab protocols and record keeping, and ultimately improve the reproducibility of published data? I would support a universal funder's mandate for detailed electronic note keeping (much like private companies require), automatic data backups and regular equipment upgrades, meticulous planning before an experiment is run (including developing a comprehensive sample labeling nomenclature, beyond the common 1, 2, 3), inclusion of universal controls (e.g., Arabidopsis Columbia accession included in every Arabidopsis experiment irrespective of what other germplasm is being tested), extensive sample replication, validation of the results at multiple steps in the process (like Sanger sequencing of construct intermediates), and other common-sense but often time-consuming practices (such as regrowing all genotypes side by side and using fresh seed stocks in an experiment to minimize seed batch effects, or resequencing every construct before donating it to the stock center or sharing it with others).

A different yet related constraint we often encounter in plant sciences is the inability to track and/or obtain the materials or datasets reported by other research groups or oftentimes even by prior members of one's own lab. To ensure the long-term availability and unrestricted access to published constructs, germplasm, omics datasets and other resources generated by the public sector, funding agencies should make it mandatory for all materials and data to be deposited in relevant stock centers, sequence repositories, etc. immediately upon publication. I often wonder whether this practice could be encouraged if one's scientific productivity and impact were to be evaluated not only by the number of papers published, but also by the number of stocks or datasets deposited and their usage by the community (e.g., the frequency of stock orders or data downloads). Publishers, on the other hand, should fully enforce the old rules that all submitted manuscripts must adhere to the established guidelines for proper scientific nomenclature (e.g., gene accession numbers, mutant names, or chemical structures) and include community access codes (e.g., gene identifiers, mutant stock numbers, Genbank accession codes, etc.) and detailed annotations for all materials and data utilized or generated in a study, with the compliance being a prerequisite for publication. These simple steps would reduce ambiguities, facilitate resource tracking, and make published materials and datasets universally available.

The extra effort invested into careful experiment planning, execution, record keeping, and making published materials and datasets trackable and accessible will undoubtedly lead to fewer but higher-quality research papers being published and ultimately save time and resources down the road. Of course, an external mandate for greater rigor and accountability would also mean the need for funding agencies to financially support the extra effort and develop ways to monitor the labs' adherence to the new stricter rigor and dissemination practices, but it is commonsense that in the long run it is cheaper to do the experiment right the first time around than waste years trying to reproduce or follow up on erroneous data or remaking the resource that has been generated previously.

SYNTHETIC BIOLOGY

An exciting and highly promising area of sciences that plant biologists are starting to embrace more widely is synthetic biology. First, what is synthetic biology? To a plant biologist, it is a useful extension of classical molecular genetics that integrates basic engineering principles and aims to rebuild biology from the ground up. Traditionally, classically trained biologists approach learning about nature from top to bottom, much like a curious child trying to break a toy apart to see what it is made of. Synthetic biologists, vice versa, try to rebuild a functional system from its pieces to understand what its minimal required components are. In plant biology, we are still very far from being able to rebuild entire plants or plant cells from scratch, but we can reconstitute the pathways, e.g., those that we have previously studied in their native context, in a heterologous host cell, aka the chassis, or introduce simple gene regulatory circuits we have artificially built. Why would we want to do that? For one, to see if we can recreate the native behavior to ensure that we fully understand the pathway or the mechanism of regulation. In addition, this can be a useful endeavor from a practical perspective, as is the case in metabolic engineering, where a native or semi-synthetic biosynthetic pathway is expressed in a heterologous host (an intact plant or a cell suspension) to produce a valuable metabolite (Lu et al., 2016; Birchfield and McIntosh, 2020), or in biosensing, where a synthetic genetic construct is introduced to turn the host into a bio-detector for a particular stimulus or ligand of interest, e.g., a metabolite (Garagounis et al., 2021).

We do not fully comprehend what we cannot ourselves recreate. We may know, for example, that a gene is induced, for example, by heat stress, but that observation does not tell us anything about the developmental regulation of that gene, or what other biotic or abiotic factors control this gene's expression. An illustrative example of how limited our current knowledge is and how synthetic biology can help us to bypass the lack of comprehensive understanding is to try the following mental exercise. How would one go about conferring a desired pattern of expression to a gene of interest, so that the gene is transcribed, for example, only in a flower, in the anthers at a particular stage of flower development, and only in response to heat stress? If we are talking about a model organism, we can scavenge available transcriptomic data in hopes of finding a native gene with such a pattern, but chances are that most anther-enriched genes will be expressed elsewhere and/or will be regulated by stimuli other than the heat stress. With the vast amount of transcriptomic data and limited ChIP-seq, DAP-seq and chromatin availability data (ATAC-seq, DNase-seq, etc.), we still have no reliable ways to infer transcription patterns of a native gene across all tissues and conditions. A combination of bioinformatic analysis (to identify putative transcription factor binding sites based on sequence conservation) (Zemlyanskaya et al., 2021), classical transgene promoter bashing (that involves building a series of transgenes with chunks of the promoter deleted or replaced in an effort

to characterize the effect of these targeted DNA modifications on the expression of a reporter gene in a systematic manner) (Andersson and Sandelin, 2020), and/or more recently, *in planta* promoter bashing via genome editing (i.e., generating targeted promoter modifications directly in the native genomic context) (Pandiarajan and Grover, 2018) are often relied upon to identify regulatory *cis*-elements in the promoters of interest. However, these approaches will not be enough to identify the full array of the DNA *cis*-elements that dictate the spatiotemporal regulation of a gene of interest, but these strategies may be helpful at pinpointing some candidate *cis*-elements and experimentally validating which elements are required.

If a particular DNA element is experimentally shown to be necessary, let's say, for heat stress upregulation, the next step is to test if the element is sufficient. This could be done by building a tandem of these elements, making a synthetic proximal promoter and placing it upstream of a well-characterized core promoter like that of *35S* to drive a reporter (Ali and Kim, 2019). In the best-case scenario, if we are successful with finding an element that can confer heat-inducible expression to the reporter, we have no easy way of restricting this heat-activated expression to just the anthers, let alone at a specific stage of anther development. Even if we had another DNA element at hand that confers tissue-specific expression (in this example, in anthers), we have no straightforward way of implementing what computer scientists would view as the Boolean AND logic— to combine these DNA elements (e.g., in a single proximal promoter) in a manner that the transcription of the gene will now only be triggered specifically in anthers in response to heat, but not in any other conditions or tissues. Synthetic biology makes the implementation of that AND logic (and other types of Boolean logic gates) possible, e.g., through the use of heterodimeric transcription factors, with one monomer active in anthers (through the use of an anther-specific promoter) and another monomer expressed only in response to heat stress (through the use of a heat-regulated promoter) (**Figure 1**). In this scenario, the full heterodimeric transcription factor would only be reconstituted in the anthers of heat-treated plants and will activate its target genes only in those flower tissues specifically under heat stress.

Thus, synthetic biology enables us to build genetic devices capable of controlling specific processes of interest despite the lack of the full mechanistic understanding of all the moving parts in those processes. In the near future, more and more plant biologists will adopt synthetic biology as a powerful way to bypass some of the technical bottlenecks in plant sciences. Who knows, someday futuristic concepts of a minimal plant genome and a minimal plant cell (Yang et al., 2020) may even become a reality. How soon will we have a thorough enough understanding of plant molecular genetics and physiology, so that we can determine the minimal set of genes to make a functional plant that can stay alive in a single stable (optimal) environment? What would we need to add to the minimal system to make the plant now capable of responding to stress and thriving in less-than-optimal conditions? Although one would agree that we have a very long way before we can get there, it is not too early to start thinking about those more ambitious projects,
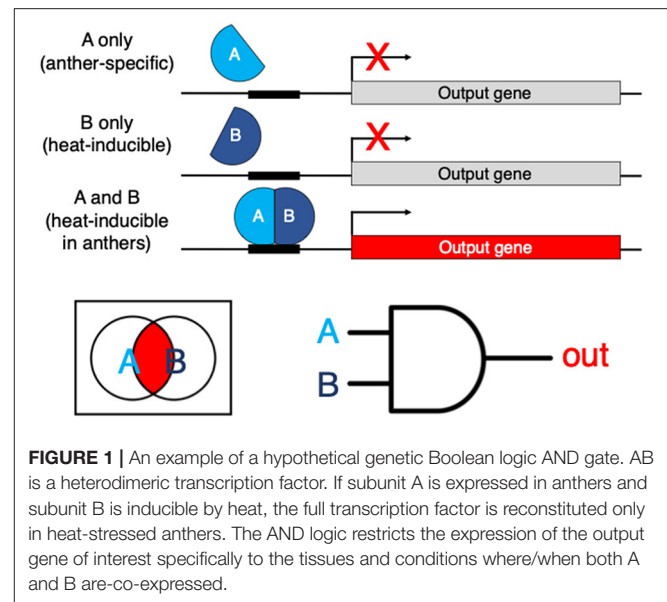
FIGURE 1 | An example of a hypothetical genetic Boolean logic AND gate. AB is a heterodimeric transcription factor. If subunit A is expressed in anthers and subunit B is inducible by heat, the full transcription factor is reconstituted only in heat-stressed anthers. The AND logic restricts the expression of the output gene of interest specifically to the tissues and conditions where/when both A and B are co-expressed.

while working on still very difficult but more achievable shorter-term goals where synthetic biology will play a central role, such as developing nitrogen-fixing cereal crops (Bloch et al., 2020) or C4 rice (Ermakova et al., 2020).

OTHER DIRECTIONS AND CONCLUDING REMARKS

Several other areas relevant to plant sciences will have paramount importance to our ability to propel plant biology research forward. Advanced automated high-throughput imaging and phenotyping will provide a more systematic, robust way to collect reliable morphometric data on a diversity of plant species in the lab, the greenhouse, and the field. New computational tool development and the implementation of novel experimental methods, along with the optimization and streamlining of existing tools and protocols, will remain the main driver of research progress, with single-cell omics approaches likely taking center stage for the next few years. Data science will play an even more predominant role given the vast amount of new data being generated and the need to handle and make sense of all that information. Systems-level approaches, mathematical modeling and machine learning will become a more integral part of plant biology research, enabling scientists to systematize and prioritize complex data and provide plant researchers with experimentally testable predictions.

If we want to see the breakthroughs we are making at the bench or in the field implemented in real-life products, we also need to work on shifting the public perception of biotechnologies. Critical steps toward rebuilding public trust in science include a greater understanding of the societal impacts of proposed innovations through collaboration with social scientists, the engagement of researchers with the science policy making process, and the active participation of all scientists

(students, postdocs, technicians, faculty, industry professionals, etc.) in community outreach programs to make our work—and its implications—accessible to the general public. Lastly, one essential factor that would make the scientific advancements sustainable in the long run is a generous investment into the robust, trans-disciplinary training of the next generation of plant scientists. Our ability to create a welcoming environment for trainees from all backgrounds and paths of life would allow these students and postdocs to feel that their research team is their second family. Today's trainees are the ones who will be solving the world's pressing issues for years to come. Our ability to provide young scientists with the solid knowledge base and diverse skills would ensure that they are well equipped to take on the next big challenge.

Looking ahead, fundamental research on model organisms, applied work on crops, and conservation studies on rare plants will all continue to be of vital importance to modern plant biology. High-throughput inquiries and gene-specific projects done by mega-groups and small labs in state-of-the-art facilities or traditional field labs will all remain indispensable to the progress of plant sciences. In the end, addressing pressing societal issues like feeding the world's growing population and mitigating climate change ultimately rests on our ability as scientists to come together and harness the power of plants. Plant biology research is positioned to play a central role in this critical endeavor. It is an exciting and urgent time to be—or become—a plant scientist.

AUTHOR CONTRIBUTIONS

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

FUNDING

REFERENCES

Ali, S., and Kim, W. C. (2019). A fruitful decade using synthetic promoters in the improvement of transgenic plants. *Front. Plant. Sci.* 10:1433. doi: 10.3389/fpls.2019.01433

Andersson, R., and Sandelin, A. (2020). Determinants of enhancer and promoter activities of regulatory elements. *Nat. Rev. Genet.* 21, 71–87. doi: 10.1038/s41576-019-0173-8

Anjanappa, R. B., and Gruissem, W. (2021). Current progress and challenges in crop genetic transformation. *J. Plant Physiol.* 261:153411. doi: 10.1016/j.jplph.2021.153411

Birchfield, A. S., and McIntosh, C. A. (2020). Metabolic engineering and synthetic biology of plant natural products—a minireview. *Curr. Plant Biol.* 24:100163. doi: 10.1016/j.cpb.2020.100163

Bloch, S. E., Ryu, M. H., Ozaydin, B., and Broglie, R. (2020). Harnessing atmospheric nitrogen for cereal crop production. *Curr. Opin. Biotechnol.* 62, 181–188 doi: 10.1016/j.copbio.2019.09.024

Ching, T., Himmelstein, D. S., Beaulieu-Jones, B. K., Kalinin, A. A., Do, B. T., Way, G. P., et al. (2018). Opportunities and obstacles for deep learning in biology and medicine. *J. R. Soc. Interface* 15:20170387. doi: 10.1098/rsif.2017.0387

Ermakova, M., Danila, F. R., Furbank, R. T., and von Caemmerer, S. (2020). On the road to C4 rice: advances and perspectives. *Plant J.* 101, 940–950. doi: 10.1111/tpj.14562

Garagounis, C., Delkis, N., and Papadopoulou, K. K. (2021). Unraveling the roles of plant specialized metabolites: using synthetic biology to design molecular biosensors. *New Phytol.* 231, 1338–1352. doi: 10.1111/nph.17470

Gordon-Kamm, B., Sardesai, N., Arling, M., Lowe, K., Hoerster, G., Betts, S., et al. (2019). Using morphogenic genes to improve recovery and regeneration of transgenic plants. *Plants (Basel)* 8. doi: 10.3390/plants8020038

Koorneef, M., and Meinke, D. (2010). The development of Arabidopsis as a model plant. *Plant J.* 61, 909–921. doi: 10.1111/j.1365-313X.2009.04086.x

Lu, X., Tang, K., and Li, P. (2016). Plant metabolic engineering strategies for the production of pharmaceutical terpenoids. *Front. Plant. Sci.* 7:1647. doi: 10.3389/fpls.2016.01647

Pandiarajan, R., and Grover, A. (2018). In vivo promoter engineering in plants: are we ready? *Plant Sci.* 277, 132–138. doi: 10.1016/j.plantsci.2018.10.011

Rhee, S., Birnbaum, K. D., and Ehrhardt, D. W. (2019). Towards building a plant cell atlas. *Trends Plant Sci.* 24, 303–310. doi: 10.1016/j.tplants.2019.01.006

Waese, J., Fan, J., Pasha, A., Yu, H., Fucile, J., Shi, R., et al. (2017). ePlant: visualizing and exploring multiple levels of data for hypothesis generation in plant biology. *Plant Cell* 29, 1806–1821. doi: 10.1105/tpc.17.00073

Yang, X., Medford, J. I., Markel, M., Shih, P. M., De Paoli, H. C., Trinh, C. T., et al. (2020). Plant biosystems design research roadmap 1.0. *BioDesign Res.* 2020:8051764. doi: 10.34133/2020/8051764

Zemlyanskaya, E. V., Dolgikh, V. A., Levitsky, V. G., and Mironova, V. (2021). Transcriptional regulation in plants: using omics data to crack the cis-regulatory code. *Curr. Opin. Plant Biol.* 63:102058. doi: 10.1016/j.pbi.2021.102058

Check for
updates

A Retro-Perspective on Auxin Transport

Markus M. Geisler*

Department of Biology, University of Fribourg, Fribourg, Switzerland

INTRODUCTION

The transport of the plant hormone auxin has been a hotspot in plant biology since its discovery (Darwin and Darwin, 1880; Zazimalova et al., 2010; Friml, 2021; Hammes et al., 2021). After its identification and verification as IAA (3-indolyl acetic acid; Went and Thimann, 1937), auxin gained high interest and fascination in the plant community but also in society because it allowed us to explain daily-seen phenomena, such as phototropism, gravitropism, patterning, and development (Christie and Murphy, 2013; Geisler et al., 2014; Morohashi et al., 2017; Konstantinova et al., 2021).

The mid twentieth century saw the emergence in the use of artificial and natural auxins as growth regulators and herbicides, and led to advances in reduced tillage agriculture as well as widespread military use of "auxinic" defoliants, such as 2,4-D (Friml and Palme, 2002). This first major wave of auxin research characterized by a predominantly biochemical characterization of auxin action in respect to growth lasted until the early 1990's and resulted in fascinating concepts, including the "chemiosmotic model of auxin transport" (Rubery and Sheldrake, 1973, 1974; Raven, 1975; Goldsmith, 1977; see **Figure 1**) and the "auxin canalization theory" (Sachs, 2000; Bennett et al., 2014; Ravichandran et al., 2020).

Then in the mid-1990's, the tools of molecular biology and the use of Arabidopsis as a model system provided the framework for breathtakingly rapid advancements that unwrapped many of the secrets underlying polar auxin transport and its role in plant development. This period allowed for the identification and characterization of multiple auxin transporter families (for details, see below) and the principal verification of the major theories. Excellent science led to a flow of beautiful publications that promoted auxin as the rising star of plant biology (Benjamins and Scheres, 2008).

If one undertakes a PUBMED key word search for "auxin transport", the server returns more than 4.000 refereed publications from the period 1995–2021. Looking back, plant science in general has been influenced fundamentally by auxin transport research. At the same time, the community was also selling the fascinating cell-to-cell or polar transport of IAA as an auxin-specific and plant-unique phenomenon. However, as was the case with much research from the twentieth century, this period has not been without controversy, and some important publications from this period represent wrong turns that required retractions.

Under this light, this grand challenge article is not meant to provide an all-encompassing overview on auxin transport; for the interested reader, I refer to the many excellent reviews that have already been written and are cited below. Instead of providing another "lexicon of auxin transport," the idea of this "retro-perspective" is to provide a brief overview on key aspects of auxin transport and use this opportunity to point out crucial misunderstandings and misconceptions, outline formal gaps and make concrete suggestions for urgent future work. The intention is to limit this article to a few arbitrarily selected aspects that are important for an understanding of the fascinating cell-to-cell or polar transport of IAA, the major native auxin.
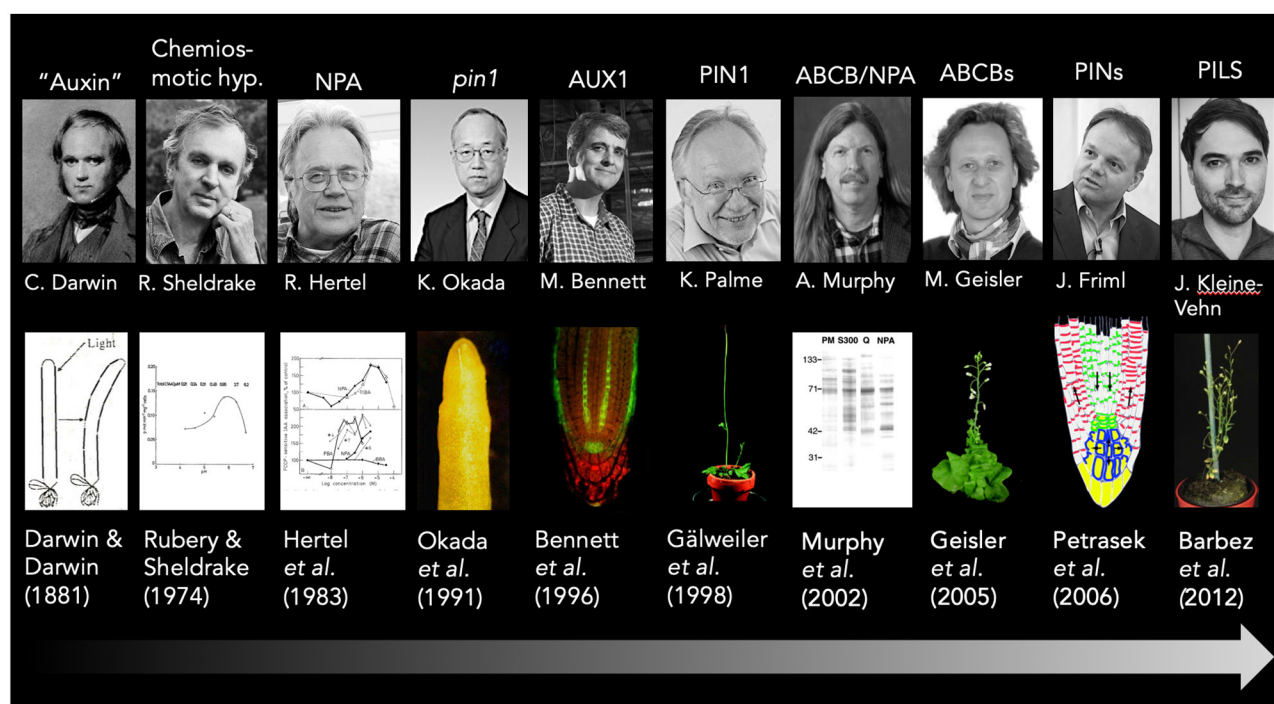
FIGURE 1 | A short timeline of the auxin transport history. Key findings in auxin transport research that are discussed in this article are highlighted and correlated with the main responsible researcher and key publications; exemplary pictures are added for illustration purposes. Please note that due to space restrictions, some topics are only assigned to a single person, and acknowledge that major findings were conducted by several groups. This holds true for the formulation of the chemiosmotic hypothesis that was suggested independently by at least two groups Rubery and Sheldrake (1973), Raven (1975), and summarized later by Goldsmith (1977). Sincere apologies to all colleagues from the auxin transport field that contributed and are not included here. The *abcb1 abcb19* mutant picture is taken from Bailly et al. (2006), while the PIN reflux model is taken from Wabnik et al. (2011).

POLAR AUXIN TRANSPORT

In the 1960's, polar transport of radiolabeled auxin was definitively shown in pea stems and maize coleoptiles (Briggs, 1960; Leopold, 1964) and the hunt for the underlying mechanisms began in earnest. In the 1970's, auxin transport experiments combined with inhibitor studies (Rubery and Sheldrake, 1973, 1974; Katekar and Geissler, 1977) led to the formulation of the chemiosmotic hypothesis of auxin transport (Rubery and Sheldrake, 1973, 1974; Raven, 1975; Goldsmith, 1977) describing how IAA could move from cell to cell. Its basis is formed by the chemical nature of IAA, which as a weak acid ($pK_a = 4.85$) and can partially cross the plasma membrane from the apoplast (pH 5.5) but not from the neutral cytoplasm which requires an export system (Zazimalova et al., 2010). It was thus postulated that auxin is transported into and out of the cell through the action of specific carrier proteins (Rubery and Sheldrake, 1973, 1974; Raven, 1975; Goldsmith, 1977). It was also proposed that the strictly controlled directionality of auxin flow may be the result of an asymmetric cellular localization of auxin efflux carriers (Goldsmith, 1977; Martin et al., 1990).

However, several biochemical observations indicated that the simple concept of chemiosmotic auxin transport required further elaboration. In the apoplast at pH 5.5, only ∼16% of IAA is protonated (Zazimalova et al., 2010). Studies in membrane vesicles and intact tissues predicted the presence of an auxin

uptake symporter (Hertel et al., 1983; Lomax et al., 1985). The identification of ATPase activity and auxin binding sites on the plasma membrane predicted the presence of a vanadate-sensitive ATPase activity apart from the plasma membrane ATPases that contributed to auxin transport (Jacobs and Hertel, 1978; Jacobs and Taiz, 1980).

In the 1990's, the chemiosmotic hypothesis gained significant support from genetic and cell biology studies in *Arabidopsis thaliana* and led to the identification and characterization of auxin efflux and influx transporters of the plasma membrane belonging to the AUXIN-RESISTANT1/LIKE AUX1 (AUX1/LAX), the PIN-FORMED (PIN), and the B subgroup of ABC transporter (ABCB) families (Bennett et al., 1996; Galweiler et al., 1998; Luschnig et al., 1998). For the Arabidopsis root tip, a "reverse fountain model" was proposed based on transporter locations and mutant phenotype analyses in order to explain an auxin (signaling) maximum in the quiescent center (Swarup and Bennett, 2003). Computer models supported a self-sustaining "auxin reflux loop" that is thought to function as an "auxin capacitor" (Friml, 2003; Kepinski and Leyser, 2005; Benjamins and Scheres, 2008). These auxin reflux loops created by the combined action of multiple auxin transporters are thought to establish local auxin maxima and minima allowing auxin to act as versatile physiological and developmental switch (Vanneste and Friml, 2009). In that respect the mode of auxin action is eventually closer to a morphogen

(Berleth and Sachs, 2001; Dubrovsky et al., 2008) rather than to a classical hormone.

Grand Challenges: The above outlined scenario defines transmembrane auxin transport over the plasma membrane as a major driving force for the establishment of local auxin gradients (Robert and Friml, 2009; Vanneste and Friml, 2009; Geisler et al., 2014). However, all steps in between, like apoplastic and cytoplasmic diffusion as well as vacuolar and ER compartmentalization, are still black boxes. While apoplastic IAA diffusion might simply follow a concentration-gradient provided by export and uptake systems, this might be slightly trickier for a cytoplasmic passage. This holds true especially for mature epidermal and cortical cells in the differentiation zone of the root tip, where the cytoplasm is limited to a small cytoplasmic strip. Also, it is unclear to what extent IAA metabolism, such as oxidation (Peer et al., 2013), and conjugation (Woodward and Bartel, 2005), as well as IAA compartmentalization into organelles (Sauer and Kleine-Vehn, 2019; Salazar-Iribe and De-la-Pena, 2020) has an effect on the polar auxin transport.

Connected to this, alternative concepts that are based on continuous, cytoplasmic auxin gradients over many cells that are inter-connected *via* a network of plasmodesmata might be worth considering. In such a model, auxin transporters would solely provide local auxin sinks at plasma membrane subdomains needed for cytoplasmic diffusion. As a support of such a highly speculative concept, recently several studies have revealed that plasmodesmata contribute to auxin distribution, and that a manipulation of these transport pathways alters auxin-related phenotypes (Band, 2021; Winnicki et al., 2021).

Another relevant question arises, is such a cell-to-cell delivery limited to auxin? In fact, there are now multiple reports on the transport of other hormones, such as cytokinins, abscisic acid (ABA) and gibberellic acid (GA), where short-distance delivery for the creation of gradients have been described (Geisler, 2018; Anfang and Shani, 2021). Obviously, for ABA ($pK_a = 4.74$) and GA ($pK_a = 4.04$), which are also weak acids, cellular compartmentalization dependent on pH can be assumed.

Another grand challenge is to view auxin transport in real-time. Real-time imaging of auxin flows has been limited by the absence of a dynamic auxin sensor because transcriptional and degron-based auxin reporters are excellent tools, but simply too slow to allow for *in vivo* imaging of auxin transport in real time (Geisler, 2018; Walia et al., 2018). With the recent development of the FRET-based auxin sensor, AuxSen (Herud-Sikimic et al., 2021), the auxin transport field has no excuses anymore to tackle these questions. A major advantage of AuxSen is the usage of heterologous proteins as binding domains (here: the bacterial Trp repressor) over plant endogenous proteins because they are unlikely to interfere with plant signaling pathways. In the case where a sole auxin sensor with a fixed IAA affinity is not suitable to report auxin gradients over several cell files with different local auxin concentrations, multiparametric imaging could be achieved by expressing multiple auxin sensors. Recently, a 2-in-1 genetically encoded fluorescence indicator fused *via* a 14-amino-acid linker was established (Waadt et al., 2020). In addition, these auxin sensors might be combined with auxin transport activity sensors (Isoda et al., 2021) that could provide an indirect read-out

for auxin transport. Pioneering work in animal systems has enabled the multiplexing and simultaneous recording of many processes, in part through differential subcellular targeting and in part through the development of fluorescence-lifetime imaging (FLIM) sensors (Greenwald et al., 2018; Isoda et al., 2021).

AUXIN TRANSPORTERS

In light of the fact that auxin, and thus also auxin transport, is involved directly or indirectly in so many if not all physiological and developmental processes in higher plants, it is not that surprising that plants have recruited a plethora of auxin transporters from different transport families (Zazimalova et al., 2010). Currently, the four main auxin transporter families are comprised of the AUX1/LAX (with 4 isoforms in Arabidopsis), the PIN (8), the ABCB (11), and the PIN-LIKES (PILS; 7) families [reviewed in Zazimalova et al. (2010) and Hammes et al. (2021)]. While most members of the former three families reside as expected on the plasma membrane, short PIN (Mravec et al., 2009; Ding et al., 2012) and PILS proteins (Barbez et al., 2012) are found predominantly at the ER, where they contribute primarily to auxin homeostasis (Schwuchow et al., 2001; Barbez and Kleine-Vehn, 2013). AUX1/LAX isoforms were shown to function as importers (Yang et al., 2006), long PINs are thought to export auxin, while ABCBs mainly export, however, import directionalities were also reported (Geisler et al., 2005; Santelia et al., 2005; Terasaka et al., 2005; Kamimoto et al., 2012; Ofori et al., 2018; Zhang et al., 2018).

ABCBs were initially a challenge for the auxin transport community because their substrate specificity was equated with human ABCB-type multi-drug transporters. However, transport experiments demonstrated that the plant transporters exhibited a remarkable specificity to auxin (Geisler et al., 2005). Moreover, unlike for PIN, AUX1/LAX, and PILS proteins, not all ABCB isoforms are auxin transporters (Park et al., 2017; Ogasawara et al., 2020). Furthermore, a gene duplication event in the ABC transporter family (Ogasawara et al., 2020) hindered their identification in classical genetic screens (Zhang et al., 2018). In between, based on the identification of a signature D/E-P motif for auxin transporting ABCBs (ATAs) it was suggested that 11 of the 22 full-size ABCBs are ATAs (Geisler and Hegedus, 2020; Hao et al., 2020). In between, functional redundancy between similar ATA isoforms could be solved by using clade-specific gene silencing (Zhang et al., 2018).

Interestingly, AUX1, PIN1, and ABCB1/PGP1 were already identified in the mid 1990's (Bennett et al., 1996; Galweiler et al., 1998; Sidler et al., 1998; see **Figure 1**), however, it took nearly another decade until their auxin transport activities were verified by whole-cell transport studies (Geisler et al., 2005; Petrasek et al., 2006; Yang et al., 2006). Today it is clear that the choice of whole-cell assays enabled confident measuring of auxin transport by reducing IAA diffusion due to a more favorable surface-to-volume ratio compared to smaller microsomal vesicles. A major drawback of whole-cell export assays is that it only permits a semi-quantitative analysis of export capacities because they require an uncontrolled loading step. The assay only offers

measuring uptake kinetics for importers as shown for AUX1 (Yang et al., 2006).

Beside these four major classes of transporter, there is an increasing number of new putative auxin transporters from other transporter families that, based on the fact that they were originally assigned to other substrates, were recently called "moonlighting" auxin transporters (Hammes et al., 2021). In my view, this assignment is not fully correct because *moonlighting proteins* are defined by a second, unrelated function. The transporters NRT1.1/ NPF6.3/ CHL1 (Beeckman and Friml, 2010; Krouk et al., 2010; Wang et al., 2020) or WAT1/UmamiT5 (Ranocha et al., 2013) are more likely to have dual (or multiple) substrate specificities. For most of these transporters, despite having convincing auxin-related phenotype, clear-cut auxin transport activity awaits confirmation.

An excellent review on auxin transporters has raised the slightly provocative question "Auxin transporters—Why so many?" (Zazimalova et al., 2010). At the time only 15 auxin transporters were described in Arabidopsis. The community is now confronted with a minimum of 30 Arabidopsis auxin transporters from three major families that are all energized differently. The generally accepted chemiosmotic model of auxin transport initially pointed to AUX1/LAX proteins rather than to PINs and ABCBs, which are driven by electrochemical gradients and ATP hydrolyses, respectively. However, the same transporter profile is also found in other essential signaling molecules, such as the secondary messenger Ca^{2+}, that employs calcium channels, Ca^{2+}/H^+ antiporters, and Ca^{2+}ATPases of the P-type (Geisler et al., 2000). In principle the same *modi operandi* are used by PINs/PILSs, AUX1/LAXs, and ABCBs where auxin is moved by electro-chemical gradients, H^+ symport or ATP hydrolysis. As it stands, evolution apparently favored the availability of multiple, energetically distinct transport systems for essential signaling molecules.

On the other hand, the high number of auxin transporters might not come as a big surprise because the over-representation of transport systems is a general plant strategy and is considered an adaptation to its sessile life style (Kang et al., 2011; Kretzschmar et al., 2011; Park et al., 2017; Anfang and Shani, 2021). Also, the *chemiosmotic model* "might have gotten something wrong" by predicting mainly auxin exporters on the plant plasma membrane: in Arabidopsis roughly half (14 out of 30) of the auxin transporters are plasma membrane exporters, while six out of the 30 are cellular importers, while 10 are internal importers. These simple numbers might suggest that the role of auxin uptake and homeostasis for plant performance are slightly underestimated.

Grand Challenges: In the near future, we urgently need a thorough biochemical characterization of key auxin transporters to enable us to assign their role in PAT. As explained above this is currently hindered by the use of whole-cell transport systems as a concession toward IAA diffusion. A way forward could be to use synthetic auxin analogs that ideally had similar kinetic properties but reduced diffusion rates.

Connected to this, another important milestone is to investigate the suggested interplay between auxin transporters, such as PIN-ABCB pairs (Bandyopadhyay et al., 2007;

Blakeslee et al., 2007; Mravec et al., 2008; Teale et al., 2021). Previous studies point to a functional interaction between these transporter classes influencing transport capacities, directionalities and inhibitor sensitivities (see below; Blakeslee et al., 2007). However, this exciting concept is far from being understood and was indirectly questioned recently (Teale et al., 2021). The way forward is probably difficult and would require protein purification and reconstitution in a cell-free system.

As a spin-off from this protein work, structure-function analyses should be envisaged for key members of all auxin transporter families. Remarkably, no crystal or cryo-EM structure of any auxin transporter exists, and considering its importance as a signaling molecule, slightly embarrassing for the auxin community. Symporter and ABCB structures from different non-plant sources are available and it would be informative to assess structural differences to non-auxin transporting orthologs. Of special interest are evolutionary conserved differences in putative substrate (auxin) binding domains, which could be easily identified by co-crystallization. Of importance are also PIN protein structures, less in respect to their transport mechanisms but in their regulation by loop phosphorylation (Hammes et al., 2021). One should also not forget that, in contrast to ABCBs and AUX1/LAX proteins, PINs form a plant-specific subgroup of MFS transporters, and therefore a structure would be of special interest.

In principle, all transporter locations align well with known auxin streams in the root tip and mutant phenotypes in Arabidopsis, however, one should not forget that the latter were also mainly deduced from transporter expression, which is only a very indirect proxy for substrate streams at best (Geisler, 2018). However, assignment of a specific auxin transporters in this complicated auxin transport network at the plant level seems to have reached its limitations through the use of classical genetics and biochemistry. The reason lies in the redundant and the interactive action of the many auxin transporters from different families. Another level of complication is added by the fact that we are facing a mobile signal. Therefore, the successful methods of first defining auxin transport streams and then to assign transporters to these streams (Kuhlemeier, 2007) may have reached its limitation. An alternative route is offered by mathematical modeling, which can integrate multiple transporters from different transporter families (Kramer, 2008; Band et al., 2014; Middleton et al., 2018). This has already been done for PIN export and AUX1/LAX influx carriers, respectively (Band et al., 2014; Middleton et al., 2018). Especially convincing was a recent combination of mathematical modeling that included PIN locations and auxin maxima deduced from experimental (confocal) data (Band et al., 2014). An extension of such work on ABCBs and even a combination of transporters from distinct transporter families should be very informative. Such studies should also include the different turnover numbers for transporters of the different subclasses; currently they are considered to transport equally. The advantage is that *via* mathematical modeling a high number of transporters and transporter combinations can be tried, this would enable testing a near unlimited number of hypotheses.

AUXIN TRANSPORTER EVOLUTION

The recent evo-devo (evolutionary developmental biology) wave has not stopped at auxin either (Friedman, 2009; Finet and Jaillais, 2012; O'Connor et al., 2017). While evolutionary analyses on nuclear auxin signaling components have been done (Kato et al., 2018; Blazquez et al., 2020), this unfortunately cannot be said for auxin transporters. This is a pity because sequence and expression data covering lower plants and algae are becoming publicly available and would allow some urgent questions to be addressed (outlined above). For example, the identification of "old" transporter families in an evolutionary sense and the assignment of other transporter classes to key developmental innovations would allow us to make predictions on the origin of auxin transport and at the same time to assign specific roles to these transporter families. On the other hand, such analyses have been hampered by the fact that, unlike for other transporters or auxin signaling components, it is "nearly" impossible to confidently predict auxin transport specificity simply by sequence homology. This is especially the case in this type of analysis as homology decreases drastically with phylogenetic distances.

Only a few studies using different approaches at different quality levels have addressed auxin transporter evolution so far and those have limited their attention to the green lineage (*Viridiplantae*) comprising chlorophytes and streptophytes (Viaene et al., 2014; Skokan et al., 2019; Zhang et al., 2019; Vosolsobe et al., 2020). The unified current picture that emerges is that ABCBs (virtually found in all domains of life) and PILS are ancient auxin transporters, while PINs and AUX1/LAXs are more recent lineages (Vosolsobe et al., 2020). Despite being found in most charophytes, PINs can be less frequently identified in chlorophytes (Vosolsobe et al., 2020). The different origins of PIN and PILS proteins is somewhat surprising as PILS were originally identified based on sequence homology (Barbez et al., 2012) and as such both contain a diagnostic *Auxin efflux carrier component 2* (IPR033526) motif. However, recently good evidence for an independent evolution was provided (Feraru et al., 2012). The origin of AUX1/LAX transporters showing a fragmentary distribution over charophytes and chlorophytes (Vosolsobe et al., 2020) is less clear.

A recent thorough analysis (Vosolsobe et al., 2020) pointed out several remarkable surprises: First, in some basal charophytes, such as *Chlorokybophyceae*, all secondary auxin transporters (PIN, AUX1/LAX, and PILS) are secondarily lost, meaning that these algae mainly rely on ABCBs. Second, the most complex algae, *Chara*, showing a nearly plant-like stature and clear evidence for PAT, contains only PIN and ABCB-type auxin transporters.

In summary, it appears that all four transporter classes have evolved independently and are usually not present in any single algae, with the exception of *Klebsormidium sp.* (Vosolsobe et al., 2020). The previous view that PINs have arisen with the presence of a vasculature and thus with the water-land-transition is apparently not true (Galvan-Ampudia and Offringa, 2007; Vosolsobe et al., 2020). This does not exclude that PINs, generally thought to provide a high degree of developmental plasticity, might be needed for the newly established sessile lifestyle where new physiological requirements (such as gravitropism and phototropism) play an important role (Bennett, 2015). But this role of PINs is most likely attributed to their diversification in land plants (Bennett, 2015). However, in this context it should also be kept in mind that gravitropism is not a strict requirement for the establishment of auxin gradients as they are known to exist in space (Ferl and Paul, 2016). In light of these findings, the previous concept that ER-based auxin homeostasis instead of plasma membrane export is the ancient auxin transport system (Viaene et al., 2013) is probably off the table. Finally, despite original predictions that auxin transporter polarity seems to be a newly acquired it is not essential for PAT.

Grand Challenges: In the next few years, the community urgently needs to enhance our knowledge on auxin transporter evolution because this might offer an understanding of auxin action as a signaling molecule *per se*. An interesting venue may be provided by understanding why unicellular organisms, such as green algae, need an auxin export system at all. This may originally have represented an excretion system liberating the cells of toxic by-products of metabolism [like in mammalian tumor cells or during some human diseases (Chanclud and Lacombe, 2017)] or allow the cells to export IAA as a signaling molecule allowing for intercellular communication during intraspecific quorum sensing (Chanclud and Lacombe, 2017; Vosolsobe et al., 2020). Another idea is that in unicellular organisms there might a need for auxin gradients permitting physiological reactions, such as growth promotion. For the unicellular moss, *Ceratodon purpureus,* it was shown that disruption of auxin export by NPA interferes with unicellular gravitropism of the protonema (Schwuchow et al., 2001).

In order to do so, we need more genomes from underrepresented algae lineages and evolutionary analyses need to be carried out more thoroughly, like done for the auxin signaling components (Blazquez et al., 2020). Analyses based on sequence homology that include key elements defining substrate specificity or regulation [such as the D/E-P motif for ABCBs (Hao et al., 2020) or the *Auxin efflux carrier component 2* (IPR033526) motif for PINs and PILS (Feraru et al., 2012)] might be the way to go. While current analyses have focused for good reason around the water-land transition and thus on the green lineage, this scrutiny must urgently be extended to other algae and non-Arabidopsis plants, especially crop plants. Of special interest will be brown algae for that developmental effects caused by IAA are reported (Bogaert et al., 2019).

A further grand challenge is the co-evolutionary analysis of auxin transporters and regulatory components, such as kinases and chaperones. This has been partially initiated for PINs and members of the AGC kinase family that seem to have co-evolved (Galvan-Ampudia and Offringa, 2007). Such an analysis is of interest because prominent members of this family, such as PINOID and phot1, were also shown to regulate ABCB transport activity (Christie et al., 2011; Henrichs et al., 2012; Christie and Murphy, 2013), which would suggest that these functional interactions were acquired secondarily.

In any case, it will be essential to tie-up any conclusion from evolutionary analyses of transport studies to prove predicted auxin transport activities and substrate specificities.

This is because homology-based predictions have their pitfalls. Additionally, such transport studies should be confirmed through functional complementation of auxin transporter mutants in Arabidopsis as has been recently started for ancient PIN isoforms (Skokan et al., 2019). Interestingly, the most primitive *PIN* gene known to date from the basal *Streptophyte* green alga *Klebsormidium flaccidum* was unable to rescue the defects in root gravitropism in the *pin2* mutant (Zhang et al., 2019), although it was shown to be a functional auxin transporter (Skokan et al., 2019). Finally, there is an urgent need to establish algal models to enable direct auxin transport measuring and genetic access.

AUXIN TRANSPORTER REGULATION

As can be expected for an essential signaling molecule, like auxin, its transmembrane distribution by auxin transporter proteins is tightly regulated at the transcriptional and post-transcriptional level (Benjamins et al., 2005; Robert and Offringa, 2008; Geisler et al., 2016, 2017; Hammes et al., 2021). Over the last decades for the different transporter families, different depths of understanding toward their regulation have been provided but it is probably safe to predict that auxin transporters (like most other transporters) are regulated at all known aspects of post-transcriptional regulation, including transport activity, membrane trafficking, and protein stability.

For a long time, the auxin community mainly focused on the establishment and maintenance of transporter polarity, with a special emphasis on the trafficking routes of PIN proteins (Rakusova et al., 2015; Zhou and Luo, 2018; Han et al., 2021). In short, PINs are constitutively internalized on clathrin-coated vesicles (Kleine-Vehn and Friml, 2008; Kleine-Vehn et al., 2008) and recycled back to the plasma membrane. These processes are regulated by a wealth of regulatory factors, including various GTPases, ARF-GEFs, and ARF-GAPs (Chen and Friml, 2014; Adamowski and Friml, 2015; Friml, 2021; Han et al., 2021). Another regulatory module orchestrating PIN polarity is formed by the interplay of AGC kinases and protein phosphatase 2A which regulate the phosphorylation status of cytoplasmic PIN loops (for details, see below; Michniewicz et al., 2007; Robert and Offringa, 2008; Huang et al., 2010; Offringa and Huang, 2013). This focus drove the prediction of the chemiosmotic model but also integrated auxin transporter networks into the main physiological read-outs of root gravitropism and shoot phototropism. On the other hand, for many years advances in PIN biochemistry were stuck because all attempts to demonstrate auxin transport for PIN proteins failed due to technical reasons.

As of today, a key concept is promoted that is partially based on the chemiosmotic model. This concept emphasizes transporter polarity as the basis for the polar distribution of auxin (Wisniewska et al., 2006), however this has not yet been verified. Along the same lines, dynamic transporter cycling has been suggested as a strict requirement for transporter polarities and both criteria together have served as a benchmark for auxin transporters. Thus, a central question for the future is to what extent is transporter polarity (and transporter dynamics) a requirement for polar transport. This is important as any uniformly, localized transporter can be activated on polar subdomains by local regulatory events, like protein phosphorylation (Christie and Murphy, 2013).

This brings us to a developing field that has demonstrated that auxin transport depends on the activity of a subgroup of plant-specific serine/threonine kinases, the so called AGC kinases (Galvan-Ampudia and Offringa, 2007; Rademacher and Offringa, 2012). Members of the AGC kinase subclade VIII were shown to phosphorylate PINs and ABCBs on their cytoplasmic loops leading to activation of long PINs (Zourelidou et al., 2009, 2014; Barbosa and Schwechheimer, 2014; Hammes et al., 2021). For ABCB1 and ABCB19, the activating and inhibiting effects on auxin transport by the AGCVIII kinases (PINOID and phot1), result in defects in gravitropism and phototropism (Christie et al., 2011; Henrichs et al., 2012; Christie and Murphy, 2013). Opposite effects on ABCBs by AGC kinase phosphorylation were discussed to be caused by interaction between the ABCBs and the immunophilin-like FKBP42, Twisted Dwarf1 (TWD1), which is thought to recruit the AGC kinases (Christie and Murphy, 2013; Geisler et al., 2016). Overall, the developmental phenotypes reported for AGCVIII kinase mutants align well with those of the respective kinase substrate mutants, which is probably best illustrated by the phenotypes of the *pinoid* and the *pin1* mutants, showing overlapping degrees of pin-shaped inflorescences (Benjamins et al., 2001; Friml et al., 2004).

An interesting finding is that some kinases of the AGC3 and AGC4 subcluster, such as PINOID and phot1, phosphorylate auxin transporters from different subclasses, like PIN1/ABCB1 and PIN3/ABCB19, respectively (Geisler et al., 2016). Remarkably and also puzzling is that AGC1 and AGC3 kinases target the same phosphorylation sites of PIN proteins but that AGC3 kinases (unlike AGC1 kinases) were initially found to regulate PIN polarity (Hammes et al., 2021). This has caused debates in the community mainly because the two major "factions" insisted on a exclusivity claim for their findings, while widely ignoring the option that both are not mutually exclusive. Indeed, a clean dissection of both events is technically challenging because both an increase of transporter polarity and transporter activity would lead to enhanced transport, which in the context of auxin canalization would be self-amplifying.

Finally, auxin transporter folding by PPIases (*cis-trans* peptidylprolyl isomerases) seem to have both an effect on PIN and ABCB transport activity and trafficking (Geisler and Bailly, 2007; Geisler et al., 2016). TWD1 was shown to function as chaperone during early ABCB biogenesis based on the finding that ABCB1,4,19, unlike PINs, are retained and degraded at the ER in the *twd1* mutant (Wu et al., 2010; Wang et al., 2013). As a result, *abcb1 abcb19* plants resemble the *twd1* mutant and show similar PAT defects (Geisler et al., 2003; see **Figure 1**). However, auxin-transporting ABCBs (ATAs) contain an essential proline as part of a diagnostic D/E-P motif in their C-terminal nucleotide-binding folds that is essential for auxin transport but not for trafficking (Geisler and Hegedus, 2020; Hao et al., 2020). Thus, TWD1 might have a dual role in ABCB activation and secretion, respectively, which is an analogy to human FKBP38 (Geisler and Hegedus, 2020).

Similarly, PIN1 was shown to be folded and regulated by the parvulin, PIN1At, known to fold proline residues preceding phosphorylation sites (Xi et al., 2016). However, it is not entirely clear if these events lead to altered transport activity or transporter polarity or both. ABCB1 contains a series of prolines in the vicinity of putative phosphorylation sites in its regulatory linker (Henrichs et al., 2012), however it is unknown if folding and phosphorylation events are interconnected.

Grand Challenges: Future grand challenges include a proper dissection of regulatory events on auxin transporters from different classes. This is indeed important because it is currently not yet clear whether protein phosphorylation leads to transport activation on the transport or polarity level. Moreover, a thorough investigation of overlapping kinase activities on members of different transporter subclasses is essential. Both can be addressed by *in vitro* and *in vivo* biochemistry (Jones et al., 2013; Geisler, 2018). The latter requires an integration of regulatory components, like kinases, chaperones, etc., and their effect on protein stabilities and transport activities, which will allow for a prediction of fluxes over time. The techniques to image kinase or transporter activities (by usage of transport activity sensor and SPARK (Separation of Phases-based Activity Reporter of Kinase) assays) and transporter-regulator interaction (by using FRET) are available and need to be transferred or optimized to the plant field (Geisler, 2018).

NPA

As for other disciplines, the identification of pharmacological inhibitors was extremely helpful for auxin transport research. In the late 1950's, a series of phtalamic acid derivates were reported to inhibit tropic bending, coining the name *phytotropin* (Morgan and Söding, 1958). Since the work in the 1980's on maize coleoptile segments (Hertel and Flory, 1968) and vesicles (Hertel et al., 1983), we know that NPA is a non-competitive inhibitor of auxin efflux but not of growth. What is less recognized is that NPA differentially inhibits the export of IAA and synthetic auxin, such as 1-NAA and 2.4-D (Delbarre et al., 1996). Also overlooked is that NPA, like other phytotropins, is thought to bind to the same receptor, through which it performs its physiological responses (Katekar and Geissler, 1975; Geissler et al., 1985; Michalke et al., 1992). This has led to speculation that the exporter might own a transceptor-like function (Hossel et al., 2005).

In the 1990's, different groups invested an enormous effort in characterizing the number, affinities and identities of putative plasma membrane-based NPA targets (Michalke et al., 1992; Cox and Muday, 1994; Bernasconi et al., 1996; Dixon et al., 1996; Butler et al., 1998; Teale and Palme, 2018). The overall outcome as reviewed in (Teale and Palme, 2018) revealed a very complex, partially contradicting picture with respect to the number and nature of the targets (Teale and Palme, 2018).

A route to the identification of an NPA target was provided by the isolation of the mutant allele *pin-formed1* (*pin1*) that resembles plants grown on NPA (Okada et al., 1991; see

Figure 1). Consecutively, the *PIN1* gene was cloned and PIN1 was identified as a member of the major facilitator superfamily with a striking polar localization (Galweiler et al., 1998). This correlation served as a quasi-accepted proof that PINs in general are NPA-sensitive auxin exporters, which was finally demonstrated in tobacco BY2 cells (Petrasek et al., 2006). For some time, a puzzling finding for the community was that PIN1 was inactive in heterologous non-plant systems, such as yeast or oocytes, shedding some doubt on its direct function as a transporter. However, also this missing detail was solved by the finding that PIN1-mediated transport is strictly dependent on phosphorylation, which was provided either by AGC kinase co-expression or phospho-mimicry (Zourelidou et al., 2009; Wang et al., 2012). Recently, two independent reports using oocytes and Arabidopsis protoplasts further validated PINs as direct targets of NPA (Abas et al., 2021; Hammes et al., 2021; Teale et al., 2021). Interestingly, one provided evidence that PIN1 inhibition by NPA does not involve classical allosteric inhibition but acts *via* an induction of PIN homo- and heterodimers, which is counteracted by PIN1 phosphorylation and IAA (Teale et al., 2021).

Another line of NPA inhibition of auxin exporters was developed by the identification of ABCB transporters and TWD1 by NPA-affinity chromatography (Noh et al., 2001; Murphy et al., 2002). Consequently, ABCBs and TWD1 were confirmed to bind NPA (Geisler et al., 2003; Kim et al., 2010; Zhu et al., 2016) and ABCB-mediated export was found to be NPA-sensitive (Geisler et al., 2005; Bouchard et al., 2006; Kim et al., 2010). NPA, like different flavonols, was able to disrupt ABCB-TWD1 interaction suggesting that NPA might bind at their interface (Bailly et al., 2008). During this time, the NPA binding site on the so-called FK506-binding domain (FKBD) of TWD1 had been precisely mapped by NMR and chemical density mapping was verified by mutational analyses (Zhu et al., 2016). Based on *in silico* docking and again verified by mutagenesis, NPA is thought to bind to the C-terminal nucleotide binding fold (NBD2) of ABCB1 (Kim et al., 2010), which is both in agreement with the finding that the interaction is provided by FKBD and NBD2 of TWD1 and ABCB1, respectively (Geisler et al., 2003).

Using chemical-genetic screens, the NPA analog, BUM (2-[4-(diethylamino)-2-hydroxybenzoyl]benzoic acid), was identified and shown to have an IC_{50} value that is roughly a factor 30 lower (Kim et al., 2010). Physiological analysis and binding assays identified ABCBs, primarily ABCB1, as key targets of BUM, whereas PIN proteins were shown to be not be directly affected (Kim et al., 2010). TWD1 seems to own a second function on auxin transport that involves bundling of the actin cytoskeleton (Zhu and Geisler, 2015; Zhu et al., 2016). TWD1 is required for NPA-mediated actin remodeling that seems to involve ACTIN7, which itself is responsible for proper plasma membrane trafficking of PINs and ABCBs (Zhu and Geisler, 2015; Zhu et al., 2016). Interestingly, both the epidermal twisting in *abcb1 abcb19* and *twd1* can be partially rescued by NPA treatments (Wang et al., 2013), indicating that NPA targets beside TWD1 and ABCBs might be involved. Another promising outcome of the initial NPA-affinity chromatography (Murphy

et al., 2000, 2002) was the aminopeptidase, APM1, that was characterized as a low-affinity NPA-binding protein. The *apm1* mutant has reduced PAT and PIN and ABCB delocalization (Peer et al., 2009).

Grand Challenges: Overall it seems that 60 years after its first description, we now have a slightly better understanding of NPA action and it is good to see that initial predictions that NPA interferes primarily with the efflux complex seem to hold true. It is now clear that the path to understand NPA was heavily complicated by the fact that there are multiple NPA targets in plants, each with different binding affinities that partially interact with each other. On top it was shown that some of these interactions, such as between PINs and ABCBs, can influence the binding affinities of these complexes (Blakeslee et al., 2007). Another level of complication is caused by the fact that NPA seems to interfere with transporter phosphorylation. This is highlighted by the finding that the protein phosphatase subunit 2A, called *Roots Curl under NPA1* (*RCN1*), a regulator of PIN transcytosis (Michniewicz et al., 2007), was identified in chemical genetic screens under NPA (Garbers et al., 1996; Deruere et al., 1999). Further, NPA was also suspected to alter auto-phosphorylation of PINOID by direct binding (Henrichs et al., 2012). Finally, there are reports that NPA might directly interfere with actin bundling in an action that is independent of TWD1 (Dhonukshe et al., 2008; Zhu and Geisler, 2015), which could alter auxin transporter trafficking directly.

It is remarkable that our understanding of the mechanism of such an important research tool used in so many labs around the world is still incomplete. Priority must be given to the biochemical characterization of NPA binding sites on known targets (such as PINs and ABCBs) by NMR, SPR (or similar), and NPA co-crystallization. At the next level, a systematic *in planta* dissection of NPA-sensitivities of auxin transport complexes must be achieved using suitable approaches, such as quantitative proximity ligation assays (PLA; Teale et al., 2021). Having the protein targets in hand, would allow for the development of specific (efflux) inhibitors that are more selective toward a certain transporter class.

Moreover, it will be essential to completely understand the overlapping *pin-formed* phenotype that is thought to be caused by genetic (*pin1*, *pinoid*) or pharmacological inhibition (NPA, BUM) of PAT that has branded the *PIN* subfamily. Despite our progress, it is still noteworthy that until today a plausible explanation for the inflorescence defects in *pin1* is still missing, especially in light of the fact that auxin levels in these tissues are not different to wild-type (Jones et al., 2005). Furthermore, one should not forget that growth on NPA (or BUM) likely leads to a saturated inhibition of all NPA targets in the plant making pin-formed inflorescences most-likely a pleiotropic phenotype. The finding that such as a phenotype is copied by single *pin1* or *pid* mutations suggests that PIN1 and/or PID most likely interfere with an overlapping subset of multiple downstream targets. That PIN1 was recently found to form complexes with multiple proteins, including other PIN isoforms, supports this overall concept (Blakeslee et al., 2007; Teale et al., 2021).

Finally, a continuously open question is the existence of a native NPA analog, which was originally assigned to flavonol derivates based on their ability to compete out NPA in binding assays and their ability to inhibit PAT (Murphy et al., 2000; Brown et al., 2001; Peer et al., 2001; Teale and Palme, 2018). For a while these were discarded (Peer and Murphy, 2007; Teale and Palme, 2018), however, recent work showing that they inhibit PIN transport by dimerization in analogy to NPA might place them back on the table (Teale et al., 2021). However, in this respect it might be important to recall that this effect (like the one for NPA) could be also simply caused by inhibition of kinases involved in PIN phosphorylation that would lead to a similar result.

THE REAL GRAND CHALLENGE

In the last few years, the auxin transport community wasted a lot of energy on discussions about which auxin transporter family or regulatory component or concept is more "important" for auxin transport. While the usage of "importance" is a rather volatile term in evolution, the criteria for such a ranking were remarkably unscientific, being more personal and arbitrary in nature. In a trial to promote their "own" family or concept of auxin transport, simplistic and generalist assignments were created that sometimes did not reflect the truth and lacked experimental proof. These ideas persist today in the community and are thus very difficult to revise.

This created an atmosphere that was built on doubt and ignorance, and did not promote scientific progress. In that respect, I would like to suggest a reset and that we should become again interested in differences between auxin transporters with respect to their polarity, their mode of energization, plasma membrane stability or NPA sensitivity. We should see differences in auxin transport data more like a challenge than a flaw, which is in general probably a good mindset.

Throughout this perspective article, I have summarized and critically evaluated current knowledge as well as the many inconsistencies in the field. I have considered what could be done if energies and resources were fostered. In my eyes, the perspectives are clear but will require a better and more neutral, meaning a less self-centered, approach. Such a change in attitude might represent the biggest future challenge for the community. But it is worth trying as it has the potential to assist us to refocus on the essentials, which is after all the beauty of auxin transport. As a positive, it will help us to regain lost trust inside the plant community.

In addition to the grand challenges for basic research of auxin transport, we urgently need to better integrate with the applied sciences. Considering the important roles that auxin transport plays for plant development, we should keep an eye to the future of life on the planet. This focus might include the production of food, forage, fiber, fuel and pharmaceuticals as well as ecosystem services. We need to apply our basic research to societal questions, like feeding our children's children, environmental questions, like growing plants in climates where we already see changes that negatively impact quantity and quality of plant products and species diversity.

AUTHOR CONTRIBUTIONS

FUNDING

ACKNOWLEDGMENTS

REFERENCES

Abas, L., Kolb, M., Stadlmann, J., Janacek, D. P., Lukic, K., Schwechheimer, C., et al. (2021). Naphthylphthalamic acid associates with and inhibits PIN auxin transporters. *Proc. Natl. Acad. Sci. U. S. A.* 2020:118. doi: 10.1073/pnas.2020857118

Adamowski, M., and Friml, J. (2015). PIN-dependent auxin transport: action, regulation, and evolution. *Plant Cell* 27, 20–32. doi: 10.1105/tpc.114.134874

Anfang, M., and Shani, E. (2021). Transport mechanisms of plant hormones. *Curr. Opin. Plant Biol.* 63:102055. doi: 10.1016/j.pbi.2021.102055

Bailly, A., Sovero, V., and Geisler, M. (2006). The twisted Dwarf's ABC: how immunophilins regulate auxin transport. *Plant Signal Behav.* 1, 277–280. doi: 10.4161/psb.1.6.3531

Bailly, A., Sovero, V., Vincenzetti, V., Santelia, D., Bartnik, D., Koenig, B. W., et al. (2008). Modulation of P-glycoproteins by auxin transport inhibitors is mediated by interaction with immunophilins. *J. Biol. Chem.* 283, 21817–21826. doi: 10.1074/jbc.M709655200

Band, L. R. (2021). Auxin fluxes through plasmodesmata. *New Phytol.* 2021:17517. doi: 10.1111/nph.17517

Band, L. R., Wells, D. M., Fozard, J. A., Ghetiu, T., French, A. P., Pound, M. P., et al. (2014). Systems analysis of auxin transport in the Arabidopsis root apex. *Plant Cell* 26, 862–875. doi: 10.1105/tpc.113.119495

Bandyopadhyay, A., Blakeslee, J. J., Lee, O. R., Mravec, J., Sauer, M., Titapiwatanakun, B., et al. (2007). Interactions of PIN and PGP auxin transport mechanisms. *Biochem. Soc. Trans.* 35, 137–141. doi: 10.1042/BST0350137

Barbez, E., and Kleine-Vehn, J. (2013). Divide Et Impera–cellular auxin compartmentalization. *Curr. Opin. Plant Biol.* 16, 78–84. doi: 10.1016/j.pbi.2012.10.005

Barbez, E., Kubes, M., Rolcik, J., Beziat, C., Pencik, A., Wang, B., et al. (2012). A novel putative auxin carrier family regulates intracellular auxin homeostasis in plants. *Nature* 485, 119–122. doi: 10.1038/nature11001

Barbosa, I. C., and Schwechheimer, C. (2014). Dynamic control of auxin transport-dependent growth by AGCVIII protein kinases. *Curr. Opin. Plant Biol.* 22, 108–115. doi: 10.1016/j.pbi.2014.09.010

Beeckman, T., and Friml, J. (2010). Nitrate contra auxin: nutrient sensing by roots. *Dev. Cell.* 18, 877–878. doi: 10.1016/j.devcel.2010.05.020

Benjamins, R., Malenica, N., and Luschnig, C. (2005). Regulating the regulator: the control of auxin transport. *Bioessays* 27, 1246–1255. doi: 10.1002/bies.20322

Benjamins, R., Quint, A., Weijers, D., Hooykaas, P., and Offringa, R. (2001). The PINOID protein kinase regulates organ development in Arabidopsis by enhancing polar auxin transport. *Development* 128, 4057–4067. doi: 10.1242/dev.128.20.4057

Benjamins, R., and Scheres, B. (2008). Auxin: the looping star in plant development. *Annu. Rev. Plant Biol.* 59, 443–465. doi: 10.1146/annurev.arplant.58.032806.103805

Bennett, M. J., Marchant, A., Green, H. G., May, S. T., Ward, S. P., Millner, P. A., et al. (1996). Arabidopsis AUX1 gene: a permease-like regulator of root gravitropism. *Science* 273, 948–950. doi: 10.1126/science.273.5277.948

Bennett, T. (2015). PIN proteins and the evolution of plant development. *Trends Plant Sci.* 20, 498–507. doi: 10.1016/j.tplants.2015.05.005

Bennett, T., Hines, G., and Leyser, O. (2014). Canalization: what the flux? *Trends Genet.* 30, 41–48. doi: 10.1016/j.tig.2013.11.001

Berleth, T., and Sachs, T. (2001). Plant morphogenesis: long-distance coordination and local patterning. *Curr. Opin. Plant Biol.* 4, 57–62. doi: 10.1016/S1369-5266(00)00136-9

Bernasconi, P., Patel, B. C., Reagan, J. D., and Subramanian, M. V. (1996). The N-1-naphthylphthalamic acid-binding protein is an integral membrane protein. *Plant Physiol.* 111, 427–432. doi: 10.1104/pp.111.2.427

Blakeslee, J. J., Bandyopadhyay, A., Lee, O. R., Mravec, J., Titapiwatanakun, B., Sauer, M., et al. (2007). Interactions among PIN-FORMED and P-glycoprotein auxin transporters in Arabidopsis. *Plant Cell.* 19, 131–147. doi: 10.1105/tpc.106.040782

Blazquez, M. A., Nelson, D. C., and Weijers, D. (2020). Evolution of plant hormone response pathways. *Annu. Rev. Plant Biol.* 71, 327–353. doi: 10.1146/annurev-arplant-050718-100309

Bogaert, K. A., Blommaert, L., Ljung, K., Beeckman, T., and De Clerck, O. (2019). Auxin function in the brown alga *Dictyota dichotoma. Plant Physiol.* 179, 280–299. doi: 10.1104/pp.18.01041

Bouchard, R., Bailly, A., Blakeslee, J. J., Oehring, S. C., Vincenzetti, V., Lee, O. R., et al. (2006). Immunophilin-like TWISTED DWARF1 modulates auxin efflux activities of Arabidopsis P-glycoproteins. *J. Biol. Chem.* 281, 30603–30612. doi: 10.1074/jbc.M604604200

Briggs, W. R. (1960). Light dosage and phototropic responses of corn and oat coleoptiles. *Plant Physiol.* 35, 951–962. doi: 10.1104/pp.35.6.951

Brown, D. E., Rashotte, A. M., Murphy, A. S., Normanly, J., Tague, B. W., Peer, W. A., et al. (2001). Flavonoids act as negative regulators of auxin transport *in vivo* in arabidopsis. *Plant Physiol.* 126, 524–535. doi: 10.1104/pp.126.2.524

Butler, J. H., Hu, S., Brady, S. R., Dixon, M. W., and Muday, G. K. (1998). *In vitro* and *in vivo* evidence for actin association of the naphthylphthalamic acid-binding protein from zucchini hypocotyls. *Plant J.* 13, 291–301. doi: 10.1046/j.1365-313X.1998.00017.x

Chanclud, E., and Lacombe, B. (2017). Plant hormones: key players in gut microbiota and human diseases? *Trends Plant Sci.* 22, 754–758. doi: 10.1016/j.tplants.2017.07.003

Chen, X., and Friml, J. (2014). Rho-GTPase-regulated vesicle trafficking in plant cell polarity. *Biochem. Soc. Trans.* 42, 212–218. doi: 10.1042/BST20130269

Christie, J. M., and Murphy, A. S. (2013). Shoot phototropism in higher plants: new light through old concepts. *Am. J. Bot.* 100, 35–46. doi: 10.3732/ajb.1200340

Christie, J. M., Yang, H., Richter, G. L., Sullivan, S., Thomson, C. E., Lin, J., et al. (2011). phot1 inhibition of ABCB19 primes lateral auxin fluxes in the shoot apex required for phototropism. *PLoS Biol.* 9:e1001076. doi: 10.1371/journal.pbio.1001076

Cox, D. N., and Muday, G. K. (1994). NPA binding activity is peripheral to the plasma membrane and is associated with the cytoskeleton. *Plant Cell* 6, 1941–1953. doi: 10.1105/tpc.6.12.1941

Darwin, C., and Darwin, F. (1880). *The Power of Movements in Plants*. London: Murray. doi: 10.5962/bhl.title.102319

Delbarre, A., Muller, P., Imhoff, V., and Guern, J. (1996). Comparison of mechanisms controlling uptake and accumulation of 2,4-dichlorophenoxy acetic acid, naphthalene-1-acetic acid, and indole-3-acetic acid in suspension-cultured tobacco cells. *Planta* 198, 532–541. doi: 10.1007/BF00262639

Deruere, J., Jackson, K., Garbers, C., Soll, D., and Delong, A. (1999). The RCN1-encoded A subunit of protein phosphatase 2A increases phosphatase activity *in vivo. Plant J.* 20, 389–399. doi: 10.1046/j.1365-313x.1999.00607.x

Dhonukshe, P., Grigoriev, I., Fischer, R., Tominaga, M., Robinson, D. G., Hasek, J., et al. (2008). Auxin transport inhibitors impair vesicle motility and actin cytoskeleton dynamics in diverse eukaryotes. *Proc. Natl. Acad. Sci. U. S. A.* 105, 4489–4494. doi: 10.1073/pnas.0711414105

Ding, Z., Wang, B., Moreno, I., Duplakova, N., Simon, S., Carraro, N., et al. (2012). ER-localized auxin transporter PIN8 regulates auxin homeostasis

and male gametophyte development in Arabidopsis. *Nat. Commun.* 3:941. doi: 10.1038/ncomms1941

Dixon, M. W., Jacobson, J. A., Cady, C. T., and Muday, G. K. (1996). Cytoplasmic orientation of the naphthylphthalamic acid-binding protein in zucchini plasma membrane vesicles. *Plant Physiol.* 112, 421–432. doi: 10.1104/pp.112.1.421

Dubrovsky, J. G., Sauer, M., S., Napsucialy-Mendivil, Ivanchenko, M. G., Friml, J., Shishkova, S., et al. (2008). Auxin acts as a local morphogenetic trigger to specify lateral root founder cells. *Proc. Natl. Acad. Sci. U. S. A.* 105, 8790–8794. doi: 10.1073/pnas.0712307105

Feraru, E., Vosolsobe, S., Feraru, M. I., Petrasek, J., and Kleine-Vehn, J. (2012). Evolution and structural diversification of PILS putative auxin carriers in plants. *Front. Plant Sci.* 3:227. doi: 10.3389/fpls.2012.00227

Ferl, R. J., and Paul, A. L. (2016). The effect of spaceflight on the gravity-sensing auxin gradient of roots: GFP reporter gene microscopy on orbit. *NPJ Microgravity* 2:15023. doi: 10.1038/npjmgrav.2015.23

Finet, C., and Jaillais, Y. (2012). Auxology: when auxin meets plant evo-devo. *Dev. Biol.* 369, 19–31. doi: 10.1016/j.ydbio.2012.05.039

Friedman, W. E. (2009). Evolution. Auxin at the evo-devo intersection. *Science* 324, 1652–1653. doi: 10.1126/science.1176526

Friml, J. (2003). Auxin transport - shaping the plant. *Curr. Opin. Plant Biol.* 6, 7–12. doi: 10.1016/S1369526602000031

Friml, J. (2021). Fourteen stations of auxin. *Cold Spring Harb Perspect Biol.* 16:a039859. doi: 10.1101/cshperspect.a039859

Friml, J., and Palme, K. (2002). Polar auxin transport–old questions and new concepts? *Plant Mol. Biol.* 49, 273–284. doi: 10.1007/978-94-010-0377-3_2

Friml, J., Yang, X., Michniewicz, M., Weijers, D., Quint, A., Tietz, O., et al. (2004). A PINOID-dependent binary switch in apical-basal PIN polar targeting directs auxin efflux. *Science* 306, 862–865. doi: 10.1126/science.1100618

Galvan-Ampudia, C. S., and Offringa, R. (2007). Plant evolution: AGC kinases tell the auxin tale. *Trends Plant Sci.* 12, 541–547. doi: 10.1016/j.tplants.2007.10.004

Galweiler, L., Guan, C. H., Muller, A., Wisman, E., Mendgen, K., Yephremov, A., et al. (1998). Regulation of polar auxin transport by AtPIN1 in Arabidopsis vascular tissue. *Science* 282, 2226–2230. doi: 10.1126/science.282.5397.2226

Garbers, C., DeLong, A., Deruere, J., Bernasconi, P., and Soll, D. (1996). A mutation in protein phosphatase 2A regulatory subunit A affects auxin transport in Arabidopsis. *EMBO J.* 15, 2115–2124. doi: 10.1002/j.1460-2075.1996.tb00565.x

Geisler, M. (2018). Seeing is better than believing: visualization of membrane transport in plants. *Curr. Opin. Plant Biol.* 46, 104–112. doi: 10.1016/j.pbi.2018.09.005

Geisler, M., Aryal, B., di Donato, M., and Hao, P. (2017). A critical view on ABC transporters and their interacting partners in auxin transport. *Plant Cell Physiol.* 58, 1601–1614. doi: 10.1093/pcp/pcx104

Geisler, M., Axelsen, K. B., Harper, J. F., and Palmgren, M. G. (2000). Molecular aspects of higher plant P-type Ca(2+)-ATPases. *Biochim. Biophys. Acta* 1465, 52–78. doi: 10.1016/S0005-2736(00)00131-0

Geisler, M., and Bailly, A. (2007). Tete-a-tete: the function of FKBPs in plant development. *Trends Plant Sci.* 12, 465–473. doi: 10.1016/j.tplants.2007.08.015

Geisler, M., Bailly, A., and Ivanchenko, M. (2016). Master and servant: regulation of auxin transporters by FKBPs and cyclophilins. *Plant Sci.* 245, 1–10. doi: 10.1016/j.plantsci.2015.12.004

Geisler, M., Blakeslee, J. J., Bouchard, R., Lee, O. R., Vincenzetti, V., Bandyopadhyay, A., et al. (2005). Cellular efflux of auxin catalyzed by the Arabidopsis MDR/PGP transporter AtPGP1. *Plant J.* 44, 179–194. doi: 10.1111/j.1365-313X.2005.02519.x

Geisler, M., and Hegedus, T. (2020). A twist in the ABC: regulation of ABC transporter trafficking and transport by FK506-binding proteins. *FEBS Lett.* 594, 3986–4000. doi: 10.1002/1873-3468.13983

Geisler, M., Kolukisaoglu, H. U., Bouchard, R., Billion, K., Berger, J., Saal, B., et al. (2003). TWISTED DWARF1, a unique plasma membrane-anchored immunophilin-like protein, interacts with Arabidopsis multidrug resistance-like transporters AtPGP1 and AtPGP19. *Mol. Biol. Cell.* 14, 4238–4249. doi: 10.1091/mbc.e02-10-0698

Geisler, M., Wang, B., and Zhu, J. (2014). Auxin transport during root gravitropism: transporters and techniques. *Plant Biol.* 16, 50–57. doi: 10.1111/plb.12030

Geissler, A. E., Pilet, P. E., and Katekar, G. F. (1985). Growth and gravireaction of maize roots treated with a phytotropin. *J. Plant Physiol.* 119, 25–34. doi: 10.1016/S0176-1617(85)80212-1

Goldsmith, M. H. M. (1977). Polar transport of auxin. *Ann. Rev. Plant Physiol. Plant Mol. Biol.* 28, 439–478. doi: 10.1146/annurev.pp.28.060177.002255

Greenwald, E. C., Mehta, S., and Zhang, J. (2018). Genetically encoded fluorescent biosensors illuminate the spatiotemporal regulation of signaling networks. *Chem. Rev.* 118, 11707–11794. doi: 10.1021/acs.chemrev.8b00333

Hammes, U. Z., Murphy, A. S., and Schwechheimer, C. (2021). Auxin transporters-a biochemical view. *Cold Spring Harb. Perspect. Biol.* 2021:a039875. doi: 10.1101/cshperspect.a039875

Han, H., Adamowski, M., Qi, L., Alotaibi, S. S., and Friml, J. (2021). PIN-mediated polar auxin transport regulations in plant tropic responses. *New Phytol.* 2021:17617. doi: 10.1111/nph.17617

Hao, P., Xia, J., Liu, J., M., Di Donato, Pakula, K., Bailly, A., et al. (2020). Auxin-transporting ABC transporters are defined by a conserved D/E-P motif regulated by a prolylisomerase. *J. Biol. Chem.* 295, 13094–13105. doi: 10.1074/jbc.RA120.014104

Henrichs, S., Wang, B., Fukao, Y., Zhu, J., Charrier, L., Bailly, A., et al. (2012). Regulation of ABCB1/PGP1-catalysed auxin transport by linker phosphorylation. *EMBO J.* 31, 2965–2980. doi: 10.1038/emboj.2012.120

Hertel, R., and Flory, R. (1968). Auxin movement in corn coleoptiles. *Planta* 82, 123–144. doi: 10.1007/BF01305716

Hertel, R., Lomax, T. L., and Briggs, W. R. (1983). Auxin transport in membrane vesicles from *Cucurbita pepo* L. *Planta* 157, 193–201. doi: 10.1007/BF00405182

Herud-Sikimic, O., Stiel, A. C., Kolb, M., Shanmugaratnam, S., Berendzen, K. W., Feldhaus, C., et al. (2021). A biosensor for the direct visualization of auxin. *Nature* 592, 768–772. doi: 10.1038/s41586-021-03425-2

Hossel, D., Schmeiser, C., and Hertel, R. (2005). Specificity patterns indicate that auxin exporters and receptors are the same proteins. *Plant Biol.* 7, 41–48. doi: 10.1055/s-2004-830475

Huang, F., Zago, M. K., Abas, L., van Marion, A., Galvan-Ampudia, C. S., and Offringa, R. (2010). Phosphorylation of conserved PIN motifs directs Arabidopsis PIN1 polarity and auxin transport. *Plant Cell* 22, 1129–1142. doi: 10.1105/tpc.109.072678

Isoda, R., Yoshinari, A., Ishikawa, Y., Sadoine, M., Simon, R., Frommer, W. B., et al. (2021). Sensors for the quantification, localization and analysis of the dynamics of plant hormones. *Plant J.* 105, 542–557. doi: 10.1111/tpj.15096

Jacobs, M., and Hertel, R. (1978). Auxin binding to subcellular fractions from *Cucurbita hypocotyls*: *in vitro* evidence for an auxin transport carrier. *Planta* 142, 1–10. doi: 10.1007/BF00385113

Jacobs, M., and Taiz, L. (1980). Vanadate inhibition of auxin-enhanced H secretion and elongation in pea epicotyls and oat coleoptiles. *Proc. Natl. Acad. Sci. U. S. A.* 77, 7242–7246. doi: 10.1073/pnas.77.12.7242

Jones, A. M., Grossmann, G., Danielson, J. A., Sosso, D., Chen, L. Q., Ho, C. H., et al. (2013). *In vivo* biochemistry: applications for small molecule biosensors in plant biology. *Curr. Opin. Plant Biol.* 16, 389–395. doi: 10.1016/j.pbi.2013.02.010

Jones, S. E., Demeo, J. S., Davies, N. W., Noonan, S. E., and Ross, J. J. (2005). Stems of the Arabidopsis pin1–1 mutant are not deficient in free indole-3-acetic acid. *Planta* 222, 530–534. doi: 10.1007/s00425-005-1561-1

Kamimoto, Y., Terasaka, K., Hamamoto, M., Takanashi, K., Fukuda, S., Shitan, N., et al. (2012). Arabidopsis ABCB21 is a facultative auxin importer/exporter regulated by cytoplasmic auxin concentration. *Plant Cell Physiol.* 53, 2090–2100. doi: 10.1093/pcp/pcs149

Kang, J., Park, J., Choi, H., Burla, B., Kretzschmar, T., Lee, Y., et al. (2011). Plant ABC transporters. *Arabidopsis Book* 9:e0153. doi: 10.1199/tab.0153

Katekar, G. F., and Geissler, A. E. (1975). Auxin transport inhibitors: fluorescein and related compounds. *Plant Physiol.* 56, 645–646. doi: 10.1104/pp.56.5.645

Katekar, G. F., and Geissler, A. E. (1977). Auxin transport inhibitors: III. Chemical requirements of a class of auxin transport inhibitors. *Plant Physiol.* 60, 826–829. doi: 10.1104/pp.60.6.826

Kato, H., Nishihama, R., Weijers, D., and Kohchi, T. (2018). Evolution of nuclear auxin signaling: lessons from genetic studies with basal land plants. *J. Exp. Bot.* 69, 291–301. doi: 10.1093/jxb/erx267

Kepinski, S., and Leyser, O. (2005). Plant development: auxin in loops. *Curr. Biol.* 15, R208–R210. doi: 10.1016/j.cub.2005.03.012

Kim, J. Y., Henrichs, S., Bailly, A., Vincenzetti, V., Sovero, V., Mancuso, S., et al. (2010). Identification of an ABCB/P-glycoprotein-specific inhibitor of auxin transport by chemical genomics. *J. Biol. Chem.* 285, 23309–23317. doi: 10.1074/jbc.M110.105981

Kleine-Vehn, J., and Friml, J. (2008). Polar targeting and endocytic recycling in auxin-dependent plant development. *Annu. Rev. Cell Dev. Biol.* 24, 447–473. doi: 10.1146/annurev.cellbio.24.110707.175254

Kleine-Vehn, J., Langowski, L., Wisniewska, J., Dhonukshe, P., Brewer, P. B., and Friml, J. (2008). Cellular and molecular requirements for polar PIN targeting and transcytosis in plants. *Mol. Plant.* 1, 1056–1066. doi: 10.1093/mp/ssn062

Konstantinova, N., Korbei, B., and Luschnig, C. (2021). Auxin and root gravitropism: addressing basic cellular processes by exploiting a defined growth response. *Int. J. Mol. Sci.* 22:52749. doi: 10.3390/ijms22052749

Kramer, E. M. (2008). Computer models of auxin transport: a review and commentary. *J. Exp. Bot.* 59, 45–53. doi: 10.1093/jxb/erm060

Kretzschmar, T., Burla, B., Lee, Y., Martinoia, E., and Nagy, R. (2011). Functions of ABC transporters in plants. *Essays Biochem.* 50, 145–160. doi: 10.1042/bse0500145

Krouk, G., Lacombe, B., Bielach, A., F., Perrine-Walker, Malinska, K., Mounier, E., et al. (2010). Nitrate-regulated auxin transport by NRT1.1 defines a mechanism for nutrient sensing in plants. *Dev. Cell.* 18, 927–937. doi: 10.1016/j.devcel.2010.05.008

Kuhlemeier, C. (2007). Phyllotaxis. *Trends Plant Sci.* 12, 143–150. doi: 10.1016/j.tplants.2007.03.004

Leopold, A. C. (1964). The polarity of auxin transport. *Brookhaven Symp. Biol.* 16, 218–234.

Lomax, T. L., Mehlhorn, R. J., and Briggs, W. R. (1985). Active auxin uptake by zucchini membrane vesicles: quantitation using ESR volume and delta pH determinations. *Proc. Natl. Acad. Sci. U. S. A.* 82, 6541–6545. doi: 10.1073/pnas.82.19.6541

Luschnig, C., Gaxiola, R. A., Grisafi, P., and Fink, G. R. (1998). EIR1, a root-specific protein involved in auxin transport, is required for gravitropism in *Arabidopsis thaliana*. *Genes Dev.* 12, 2175–2187. doi: 10.1101/gad.12.14.2175

Martin, M. H., Goldsmith, M. H., and Goldsmith, T. H. (1990). On polar auxin transport in plant cells. *J. Math. Biol.* 28, 197–223. doi: 10.1007/BF00163145

Michalke, W., Katekar, G. F., and Geissler, A. E. (1992). Phytotropin-binding sites and auxin transport in *Cucurbita pepo*: evidence for two recognition sites. *Planta* 187, 254–260. doi: 10.1007/BF00201948

Michniewicz, M., Zago, M. K., Abas, L., Weijers, D., Schweighofer, A., Meskiene, I., et al. (2007). Antagonistic regulation of PIN phosphorylation by PP2A and PINOID directs auxin flux. *Cell* 130, 1044–1056. doi: 10.1016/j.cell.2007.07.033

Middleton, A. M., Dal Bosco, C., Chlap, P., Bensch, R., Harz, H., Ren, F., et al. (2018). Data-driven modeling of intracellular auxin fluxes indicates a dominant role of the ER in controlling nuclear auxin uptake. *Cell Rep.* 22, 3044–3057. doi: 10.1016/j.celrep.2018.02.074

Morgan, D. G., and Söding, H. (1958). Über die Wirkungsweise von Phthalsäuremono-α-Naphthylamid (PNA) auf das Wachstum der Haferkoleoptile. *Planta* 52, 235–249. doi: 10.1007/BF01911712

Morohashi, K., Okamoto, M., Yamazaki, C., Fujii, N., Miyazawa, Y., Kamada, M., et al. (2017). Gravitropism interferes with hydrotropism *via* counteracting auxin dynamics in cucumber roots: clinorotation and spaceflight experiments. *New Phytol.* 215, 1476–1489. doi: 10.1111/nph.14689

Mravec, J., Kubes, M., Bielach, A., Gaykova, V., Petrasek, J., Skupa, P., et al. (2008). Interaction of PIN and PGP transport mechanisms in auxin distribution-dependent development. *Development* 135, 3345–3354. doi: 10.1242/dev.021071

Mravec, J., Skupa, P., Bailly, A., Hoyerova, K., Krecek, P., Bielach, A., et al. (2009). Subcellular homeostasis of phytohormone auxin is mediated by the ER-localized PIN5 transporter. *Nature* 459, 1136–1140. doi: 10.1038/nature08066

Murphy, A., Peer, W. A., and Taiz, L. (2000). Regulation of auxin transport by aminopeptidases and endogenous flavonoids. *Planta* 211, 315–324. doi: 10.1007/s004250000300

Murphy, A. S., Hoogner, K. R., Peer, W. A., and Taiz, L. (2002). Identification, purification, and molecular cloning of N-1-naphthylphthalmic acid-binding plasma membrane-associated aminopeptidases from Arabidopsis. *Plant Physiol.* 128, 935–950. doi: 10.1104/pp.010519

Noh, B., Murphy, A. S., and Spalding, E. P. (2001). Multidrug resistance-like genes of Arabidopsis required for auxin transport and auxin-mediated development. *Plant Cell* 13, 2441–2454. doi: 10.1105/tpc.010350

O'Connor, D. L., Elton, S., Ticchiarelli, F., Hsia, M. M., Vogel, J. P., and Leyser, O. (2017). Cross-species functional diversity within the PIN auxin efflux protein family. *Elife* 6:36. doi: 10.7554/eLife.31804.036

Offringa, R., and Huang, F. (2013). Phosphorylation-dependent trafficking of plasma membrane proteins in animal and plant cells. *J. Integr. Plant Biol.* 55, 789–808. doi: 10.1111/jipb.12096

Ofori, P. A., Geisler, M., M. di Donato Pengchao, H., Otagaki, S., Matsumoto, S., and Shiratake, K. (2018). Tomato ATP-binding cassette transporter SlABCB4 is involved in auxin transport in the developing fruit. *Plants* 7:30065. doi: 10.3390/plants7030065

Ogasawara, F., Kodan, A., and Ueda, K. (2020). ABC proteins in evolution. *FEBS Lett.* 2020:13945. doi: 10.1002/1873-3468.13945

Okada, K., Ueda, J., Komaki, M. K., Bell, C. J., and Shimura, Y. (1991). Requirement of the auxin polar transport system in early stages of arabidopsis floral bud formation. *Plant Cell* 3, 677–684. doi: 10.2307/3869249

Park, J., Lee, Y., Martinoia, E., and Geisler, M. (2017). Plant hormone transporters: what we know and what we would like to know. *BMC Biol.* 15:93. doi: 10.1186/s12915-017-0443-x

Peer, W. A., Brown, D. E., Tague, B. W., Muday, G. K., Taiz, L., and Murphy, A. S. (2001). Flavonoid accumulation patterns of transparent testa mutants of arabidopsis. *Plant Physiol.* 126, 536–548. doi: 10.1104/pp.126.2.536

Peer, W. A., Cheng, Y., and Murphy, A. S. (2013). Evidence of oxidative attenuation of auxin signalling. *J. Exp. Bot.* 64, 2629–2639. doi: 10.1093/jxb/ert152

Peer, W. A., Hosein, F. N., Bandyopadhyay, A., Makam, S. N., Otegui, M. S., Lee, G. J., et al. (2009). Mutation of the membrane-associated M1 protease APM1 results in distinct embryonic and seedling developmental defects in Arabidopsis. *Plant Cell* 21, 1693–1721. doi: 10.1105/tpc.108.059634

Peer, W. A., and Murphy, A. S. (2007). Flavonoids and auxin transport: modulators or regulators? *Trends Plant Sci.* 12, 556–563. doi: 10.1016/j.tplants.2007.10.003

Petrasek, J., Mravec, J., Bouchard, R., Blakeslee, J. J., Abas, M., Seifertova, D., et al. (2006). PIN proteins perform a rate-limiting function in cellular auxin efflux. *Science* 312, 914–918. doi: 10.1126/science.1123542

Rademacher, E. H., and Offringa, R. (2012). Evolutionary adaptations of plant AGC kinases: from light signaling to cell polarity regulation. *Front. Plant Sci.* 3:250. doi: 10.3389/fpls.2012.00250

Rakusova, H., Fendrych, M., and Friml, J. (2015). Intracellular trafficking and PIN-mediated cell polarity during tropic responses in plants. *Curr. Opin. Plant Biol.* 23, 116–123. doi: 10.1016/j.pbi.2014.12.002

Ranocha, P., Dima, O., Nagy, R., Felten, J., C., Corratge-Faillie, Novak, O., et al. (2013). Arabidopsis WAT1 is a vacuolar auxin transport facilitator required for auxin homoeostasis. *Nat. Commun.* 4:2625. doi: 10.1038/ncomms3625

Raven, J. A. (1975). Transport of indoleacetic-acid in plant-cells in relation to Ph and electrical potential gradients, and its significance for polar iaa transport. *New Phytol.* 74, 163–172. doi: 10.1111/j.1469-8137.1975.tb02602.x

Ravichandran, S. J., Linh, N. M., and Scarpella, E. (2020). The canalization hypothesis - challenges and alternatives. *New Phytol.* 227, 1051–1059. doi: 10.1111/nph.16605

Robert, H. S., and Friml, J. (2009). Auxin and other signals on the move in plants. *Nat. Chem. Biol.* 5, 325–332. doi: 10.1038/nchembio.170

Robert, H. S., and Offringa, R. (2008). Regulation of auxin transport polarity by AGC kinases. *Curr. Opin. Plant Biol.* 11, 495–502. doi: 10.1016/j.pbi.2008.06.004

Rubery, P. H., and Sheldrake, A. R. (1973). Effect of pH and surface charge on cell uptake of auxin. *Nat. New Biol.* 244, 285–288. doi: 10.1038/newbio244285a,0

Rubery, P. H., and Sheldrake, A. R. (1974). Carrier-mediated auxin transport. *Planta* 118, 101–121. doi: 10.1007/BF00388387

Sachs, T. (2000). Integrating cellular and organismic aspects of vascular differentiation. *Plant Cell Physiol.* 41, 649–56. doi: 10.1093/pcp/41.6.649

Salazar-Iribe, A., and De-la-Pena, C. (2020). Auxins, the hidden player in chloroplast development. *Plant Cell Rep.* 39, 1595–1608. doi: 10.1007/s00299-020-02596-y

Santelia, D., Vincenzetti, V., Azzarello, E., Bovet, L., Fukao, Y., Duchtig, P., et al. (2005). MDR-like ABC transporter AtPGP4 is involved in auxin-mediated lateral root and root hair development. *FEBS Lett.* 579, 5399–5406. doi: 10.1016/j.febslet.2005.08.061

Sauer, M., and Kleine-Vehn, J. (2019). PIN-FORMED and PIN-LIKES auxin transport facilitators. *Development* 146:168088. doi: 10.1242/dev.168088

Schwuchow, J., Michalke, W., and Hertel, R. (2001). An auxin transport inhibitor interferes with unicellular gravitropism in protonemata of the moss *Ceratodon purpureus*. *Plant Biol*. 3, 357–363. doi: 10.1055/s-200 1-16459

Sidler, M., Hassa, P., Hasan, S., Ringli, C., and Dudler, R. (1998). Involvement of an ABC transporter in a developmental pathway regulating hypocotyl cell elongation in the light. *Plant Cell* 10, 1623–1636. doi: 10.1105/tpc.10.10.1623

Skokan, R., Medvecka, E., Viaene, T., Vosolsobe, S., Zwiewka, M., Muller, K., et al. (2019). PIN-driven auxin transport emerged early in streptophyte evolution. *Nat. Plants* 5, 1114–1119. doi: 10.1038/s41477-019-0542-5

Swarup, R., and Bennett, M. (2003). Auxin transport: the fountain of life in plants? *Dev. Cell* 5, 824–826. doi: 10.1016/S1534-5807(03)00370-8

Teale, W., and Palme, K. (2018). Naphthylphthalamic acid and the mechanism of polar auxin transport. *J. Exp. Bot*. 69, 303–312. doi: 10.1093/jxb/erx323

Teale, W. D., Pasternak, T., C., Dal Bosco, Dovzhenko, A., Kratzat, K., Bildl, W., et al. (2021). Flavonol-mediated stabilization of PIN efflux complexes regulates polar auxin transport. *EMBO J*. 40:e104416. doi: 10.15252/embj.2020104416

Terasaka, K., Blakeslee, J. J., Titapiwatanakun, B., Peer, W. A., Bandyopadhyay, A., Makam, S. N., et al. (2005). PGP4, an ATP binding cassette P-glycoprotein, catalyzes auxin transport in *Arabidopsis thaliana* roots. *Plant Cell* 17, 2922–2939. doi: 10.1105/tpc.105.035816

Vanneste, S., and Friml, J. (2009). Auxin: a trigger for change in plant development. *Cell* 136, 1005–1016. doi: 10.1016/j.cell.2009.03.001

Viaene, T., Delwiche, C. F., Rensing, S. A., and Friml, J. (2013). Origin and evolution of PIN auxin transporters in the green lineage. *Trends Plant Sci*. 18, 5–10. doi: 10.1016/j.tplants.2012.08.009

Viaene, T., Landberg, K., Thelander, M., Medvecka, E., Pederson, E., Feraru, E., et al. (2014). Directional auxin transport mechanisms in early diverging land plants. *Curr. Biol*. 24, 2786–2791. doi: 10.1016/j.cub.2014.09.056

Vosolsobe, S., Skokan, R., and Petrasek, J. (2020). The evolutionary origins of auxin transport: what we know and what we need to know. *J. Exp. Bot*. 71, 3287–3295. doi: 10.1093/jxb/eraa169

Waadt, R., Koster, P., Andres, Z., Waadt, C., Bradamante, G., Lampou, K., et al. (2020). Dual-reporting transcriptionally linked genetically encoded fluorescent indicators resolve the spatiotemporal coordination of cytosolic abscisic acid and second messenger dynamics in Arabidopsis. *Plant Cell* 32, 2582–2601. doi: 10.1105/tpc.19.00892

Wabnik, K., Govaerts, W., Friml, J., and Kleine-Vehn, J. (2011). Feedback models for polarized auxin transport: an emerging trend. *Mol. Biosyst*. 7, 2352–2359. doi: 10.1039/c1mb05109a

Walia, A., Waadt, R., and Jones, A. M. (2018). Genetically encoded biosensors in plants: pathways to discovery. *Annu. Rev. Plant Biol*. 69, 497–524. doi: 10.1146/annurev-arplant-042817-040104

Wang, B., Bailly, A., Zwiewka, M., Henrichs, S., Azzarello, E., Mancuso, S., et al. (2013). Arabidopsis TWISTED DWARF1 functionally interacts with auxin exporter ABCB1 on the root plasma membrane. *Plant Cell* 25, 202–214. doi: 10.1105/tpc.112.105999

Wang, B., Henrichs, S., and Geisler, M. (2012). The AGC kinase, PINOID, blocks interactive ABCB/PIN auxin transport. *Plant Sig. Behav*. 7, 1515–1517. doi: 10.4161/psb.22093

Wang, W., Hu, B., Li, A., and Chu, C. (2020). NRT1.1s in plants: functions beyond nitrate transport. *J. Exp. Bot*. 71, 4373–4379. doi: 10.1093/jxb/erz554

Went, F. W., and Thimann, K. V. (1937). *Phytohormones*. New York, NY: Macmillan.

Winnicki, K., Polit, J. T., Zabka, A., and Maszewski, J. (2021). Do plasmodesmata play a prominent role in regulation of auxin-dependent genes at early stages of embryogenesis? *Cells* 10:40733. doi: 10.3390/cells10040733

Wisniewska, J., Xu, J., Seifertova, D., Brewer, P. B., Ruzicka, K., Blilou, I., et al. (2006). Polar PIN localization directs auxin flow in plants. *Science* 312:883. doi: 10.1126/science.1121356

Woodward, A. W., and Bartel, B. (2005). Auxin: regulation, action, and interaction. *Ann. Bot*. 95, 707–735. doi: 10.1093/aob/mci083

Wu, G., Otegui, M. S., and Spalding, E. P. (2010). The ER-localized TWD1 immunophilin is necessary for localization of multidrug resistance-like proteins required for polar auxin transport in Arabidopsis roots. *Plant Cell* 22, 3295–3304. doi: 10.1105/tpc.110.078360

Xi, W., Gong, X., Yang, Q., Yu, H., and Liou, Y. C. (2016). Pin1At regulates PIN1 polar localization and root gravitropism. *Nat. Commun*. 7:10430. doi: 10.1038/ncomms10430

Yang, Y. D., Hammes, U. Z., Taylor, C. G., Schachtman, D. P., and Nielsen, E. (2006). High-affinity auxin transport by the AUX1 influx carrier protein. *Curr. Biol*. 16, 1123–1127. doi: 10.1016/j.cub.2006.04.029

Zazimalova, E., Murphy, A. S., Yang, H., Hoyerova, K., and Hosek, P. (2010). Auxin transporters–why so many? *Cold Spring Harb. Perspect. Biol*. 2:a001552. doi: 10.1101/cshperspect.a001552

Zhang, Y., Nasser, V., Pisanty, O., Omary, M., Wulff, N., M., et al. (2018). A transportome-scale amiRNA-based screen identifies redundant roles of Arabidopsis ABCB6 and ABCB20 in auxin transport. *Nat. Commun*. 9:4204. doi: 10.1038/s41467-018-06410-y

Zhang, Y., Xiao, G., Wang, X., Zhang, X., and Friml, J. (2019). Evolution of fast root gravitropism in seed plants. *Nat. Commun*. 10:3480. doi: 10.1038/s41467-019-11471-8

Zhou, J. J., and Luo, J. (2018). The PIN-FORMED auxin efflux carriers in plants. *Int. J. Mol. Sci*. 19:92759. doi: 10.3390/ijms19092759

Zhu, J., Bailly, A., Zwiewka, M., Sovero, V., Di Donato, M., Ge, P., et al. (2016). TWISTED DWARF1 mediates the action of auxin transport inhibitors on actin cytoskeleton dynamics. *Plant Cell* 28, 930–948. doi: 10.1105/tpc.15.00726

Zhu, J., and Geisler, M. (2015). Keeping it all together: auxin-actin crosstalk in plant development. *J. Exp. Bot*. 66, 4983–4998. doi: 10.1093/jxb/erv308

Zourelidou, M., Absmanner, B., Weller, B., Barbosa, I. C., Willige, B. C., Fastner, A., et al. (2014). Auxin efflux by PIN-FORMED proteins is activated by two different protein kinases, D6 PROTEIN KINASE and PINOID. *Elife* 2014:e02860. doi: 10.7554/eLife.02860.035

Zourelidou, M., Muller, I., Willige, B. C., Nill, C., Jikumaru, Y., Li, H., et al. (2009). The polarly localized D6 PROTEIN KINASE is required for efficient auxin transport in *Arabidopsis thaliana*. *Development* 136, 627–636. doi: 10.1242/dev.028365

frontiers
in Plant Science

Divide and Be Conquered—Cell Cycle Reactivation in Arbuscular Mycorrhizal Symbiosis

Giulia Russo[1] and Andrea Genre[2]*

[1] Department of Agricultural, Forest, and Food Sciences, University of Turin, Turin, Italy, [2] Department of Life Science and Systems Biology, University of Turin, Turin, Italy

INTRODUCTION

Arbuscular mycorrhizas (AM) are widespread symbiotic associations between 78% of vascular plant species globally (Tedersoo et al., 2020) and soil borne Glomeromycotina fungi (Spatafora et al., 2016). Analogous associations with fungi appeared over 400 million years ago (Strullu-Derrien et al., 2014, 2018) and are thought to have played a major role in the transition of plants from aquatic to terrestrial environments (Wang et al., 2010). Indeed, extant AM fungi supply their host plants with water and mineral nutrients, resources that are as indispensable for them as they were for their earliest ancestors (Feijen et al., 2018).

AM development is a conserved process across extant host species with limited morphological variations in the symbiotic structures (Choi et al., 2018). Following an exchange of chemical signals (Zipfel and Oldroyd, 2017) root colonization starts with the formation of a hyphopodium on the root surface (Kobae et al., 2018). Fungal entry in epidermal cells is anticipated by the assembly of the prepenetration apparatus (PPA), a broad, nucleus-associated cytoplasmic bridge. Here, exocytic and endocytic processes (Genre et al., 2005, 2008, 2012; Russo et al., 2019b) contribute to build a novel cell compartment, the symbiotic interface, hosting hyphae within an invagination of the plant cell membrane and a layer of unstructured cell wall components (Balestrini et al., 1996; Parniske, 2008; Balestrini and Bonfante, 2014). Such symbiotic interfaces accommodate all intracellular hyphae as they develop toward the inner cortex, where their repeated branching originates arbuscules: the distinctive structures of this symbiosis, where mineral nutrients and water are transferred to the plant across the extensive periarbuscular interface (Luginbuehl and Oldroyd, 2017), in exchange for sugars (Roth and Paszkowski, 2017) and lipids (Keymer and Gutjahr, 2018).

The study of fungal accommodation has revealed that host cell rearrangement, calcium-mediated signals and major changes in gene expression extend to neighboring, uncolonized cells (Genre et al., 2008; Pumplin and Harrison, 2009; Gaude et al., 2012; Sieberer et al., 2012), indicating that signaling processes anticipate fungal development within the root tissues. In this context, we have recently shown that such prepenetration responses include cell cycle reactivation in cortical cells, with anticlinal cell divisions and recursive endoreduplication anticipating fungal colonization (Carotenuto et al., 2019a,b; Russo et al., 2019a,b). We here propose a model depicting the recruitment of cell cycle processes as a strategy for arbuscule accommodation, speculating on its conservation in other, more recent, biotrophic interactions.

CELL DIVISION AND ENDOREDUPLICATION ANTICIPATE ARBUSCULE ACCOMMODATION

Several studies had reported an increase in ploidy in mycorrhizal roots of different angiosperms (Berta et al., 2000; Fusconi et al., 2005; Bainard et al., 2011). Siciliano et al. (2007) reported that histone H2B1 gene was induced in root segments of *Medicago truncatula* on which hyphopodium formation had occurred, supporting the hypothesis that cell divisions determinants are co-opted by the plant cell in preparation of interface compartment construction. More recently, combined microscopy, flow cytometry and gene expression studies have revealed that the activation of cell division- related processes occurs since the early steps of AM development, often at a distance from the colonizing hyphae (Carotenuto et al., 2019a,b; Russo et al., 2019a,b). Firstly, the presence of sparse couples of "split cells" in the inner root cortex was consistently observed in both young and fully developed colonization units from diverse plants. Such split cells were half the length of the surrounding parenchymal cells, suggesting the occurrence of cell division after tissue differentiation. This was confirmed by the direct observation of dividing cells as early as 48 h post-hyphopodium formation in *Daucus carota* expressing a GFP fusion with TPLATE (Russo et al., 2019a), an adaptin-related protein that accumulates on the cell plate membrane and plasmalemma at the cortical division zone (Van Damme et al., 2006).

Furthermore, taking advantage of the correlation between flow cytometry data and detailed nuclear size measurements through confocal imaging, the precise localization of inner cortical cells with different levels of increased ploidy in the AM colonized areas was achieved (Carotenuto et al., 2019a). This revealed the diffuse occurrence of endoreduplication events— i.e., DNA duplication in the absence of cell division (Barow, 2006)—throughout AM development (Carotenuto et al., 2019b), as supported by the upregulation of several key endocycle and S-phase marker genes (Carotenuto et al., 2019a), such as negative regulators of G2-M-specific cyclins *MtAPC/C subunit 2* (Tarayre et al., 2004) and *MtCCS52A* (Cebolla et al., 1999), and markers of DNA replication during the S phase, such as two subunits of DNA Topoisomerase VI, *MtVAG1*, and *MtTOPO-VI B* (Bergerat et al., 1994, 1997), and the histone *MtHist-H4* (Lepetit et al., 1992).

In addition, uncolonized split cells often displayed lower ploidy than their neighboring undivided cells, suggesting that cell division and endoreduplication combine to generate the resulting mixed population of cells with diverse ploidy levels (Carotenuto et al., 2019b).

These observations outlined a previously unpredicted scenario of cell cycle reactivation in response to AM colonization. Attempting to explain the origin and role of these conserved and histologically localized responses, a few additional considerations should be discussed.

Firstly, cell divisions in the inner cortex have been observed when intraradical hyphae were limited to epidermal and outer cortical layers but not in later stages; by contrast, as demonstrated by combined flow cytometry and microscopy data, recursive endoreduplication cycles appear to be active for a longer period of time, with arbusculated and neighboring cells reaching levels of 128C ploidy, corresponding to up to 5 cycles of endoreduplication (Carotenuto et al., 2019a). In more detail, confocal imaging revealed that the increase in nuclear size— a hallmark of endoreduplication—surged at the front of fungal expansion and reached the highest peaks in the central area of infection units, suggesting the existence of a proportion between ploidy and the abundance (or age) of intraradical fungal structures. Importantly, Carotenuto et al. (2019b) also observed that the couples of split cortical cells derived from cell division often displayed different nuclear sizes, with larger nuclei in cells that were closer to the fungus or hosting an older arbuscule. This strongly suggests that cell division takes place before endoreduplication, or at least that endoreduplication can proceed after cell division.

Secondly, the observation of both cell division and ploidy increase at a distance from arbuscules or colonizing hyphae suggests the existence of a yet unidentified signaling process reactivating the cell cycle before fungal arrival.

In addition, the concentration of both ectopic cell divisions and endocycle events to the inner cortex envisages a remarkable correlation with the accommodation of arbuscules, which normally develop in the same cell layer. Cell proliferation, with its limited occurrence, appears to have a secondary role, if any, in the generation of additional space for arbuscule accommodation. By contrast, the sparse cell divisions observed in AM colonized areas might relate to the developmental fate of cortical cells. In the roots of most plants, in fact, cortical cell differentiation is determined with an endocycle that doubles their DNA content from 2C to 4C (Cebolla et al., 1999; Edgar et al., 2014) with a consequent size increase (Robinson et al., 2018). In line with that, *in situ* studies of cell ploidy in uncolonized roots of *M. truncatula* (Carotenuto et al., 2019a) revealed that most cortical cells had 4C nuclei, while a few of them displayed 8C and 16C ploidy levels. Even if experimentally challenging, it would be very interesting to investigate if there is a relationship between initial cell ploidy and the occurrence of ectopic cell division in early AM interaction.

Besides tissue differentiation, endoreduplication is also common in plant interactions with diverse microbes: replicating DNA produces multiple copies of each gene, intensifying cell responsiveness to microbial signals. Examples are numerous, from pathogens and parasites (de Almeida Engler and Gheysen, 2013; Chandran and Wildermuth, 2016; Wildermuth et al., 2017) to symbionts (Suzaki et al., 2014; Lace and Ott, 2018). Furthermore, endoreduplication-related cell enlargement is typically associated with the accommodation of several microbes, and specifically to arbuscules in AM (Balestrini and Bonfante, 2014; Heck et al., 2016).

The requirement of a specific rearrangement in the host cell organization for arbuscule accommodation is apparent from a simple observation of the structural and functional complexity of the periarbuscular interface (Luginbuehl and Oldroyd, 2017; Ivanov et al., 2019; Roth et al., 2019), compared to the tunnel-like interface hosting linear hyphae in outer root tissues. In fact, while epidermal and outer cortical PPAs are structured as

roughly linear cytoplasmic bridges across the vacuole, the PPAs that generate periarbuscular interfaces are much more complex and extensive, appearing as large accumulations of cytoplasm that extend from the hyphal penetration site and occupy most of the host cell central volume (Genre et al., 2008). Such a massive, centripetally-oriented exocytic event has striking ultrastructural and molecular similarities with the assembly of the cell plate on the cell equatorial plane at the end of mitosis, and indeed an evolutionary correlation between symbiotic interface biogenesis and cell plate deposition has been envisaged in both AM (Russo et al., 2019b) and N-fixing nodulation (Downie, 2014). In support of this hypothesis, *in vivo* imaging of GFP-TPLATE fusions revealed a strong accumulation of TPLATE at sites of PPA assembly and at sites of cell-to-cell hyphal passage, where the perifungal membrane fuses with the plasmalemma, in striking analogy with cell plate fusion with the cell membrane at the end of mitosis (Russo et al., 2019b).

If the recruitment of cell division processes to assemble the extensive periarbuscular interface now appears more convincing, developmental restraints could contribute to explain why sparse cell division and diffuse endoreduplication are limited to the cortex. Dong et al. (2021) have recently highlighted that a SHR-SCR module (known to regulate cortex/endodermis initial cell division in the root meristem) maintains its activity and is required for cell cycle reactivation in legume inner cortex during nodule organogenesis (Suzaki et al., 2014; Xiao et al., 2014). Even if analogous studies in rice (which does not form root nodules, but hosts AM fungi) did not confirm SHR-SCR expression in cortical cells, it is reasonable to speculate that analogous mechanisms involving meristematic transcription factors maintain a disposition to reactivate the cell cycle in inner cortical cells. This peculiarity has been related to the

evolution of root branching (Xiao et al., 2019), but appears to have later been co-opted in several plant interactions, from N-fixing symbioses (Dong et al., 2021) to nematode parasitism (de Almeida Engler and Gheysen, 2013), where both cell division and endoreduplication are required for microbe accommodation. While anyway such processes involve the formation of new organs (i.e. lateral roots, N-fixing nodules or nematode-hosting cysts), their occurrence in AM, where organogenesis is absent, appears puzzling; even more so if we consider that AM symbiosis appeared in land plants before the evolution of true roots (Strullu-Derrien et al., 2014, 2018).

CONCLUSIONS

By discussing the developmental and evolutionary context where cell cycle processes interweave with AM symbiosis, a scenario emerges (**Figure 1**) where the perception of AM fungal colonization in outer root tissues triggers a so-far unknown intraradical signaling process activating cell cycle-related processes ahead of the penetrating intraradical hyphae. Inner cortical cells may deploy two downstream responses: a few of them (possibly depending on their ploidy) complete mitosis, splitting in two smaller cells, as cell elongation is very limited in a mature tissue (Russo et al., 2019a,b); the remaining majority of inner cortical cells enter the endocycle, duplicating their DNA content up to several times, continuously stimulated by the approaching fungal symbiont—in fact endoreduplication also extends to those cells that had divided earlier (Carotenuto et al., 2019a,b). Such a model implies that host cells largely anticipate and direct fungal colonization, in line with previous
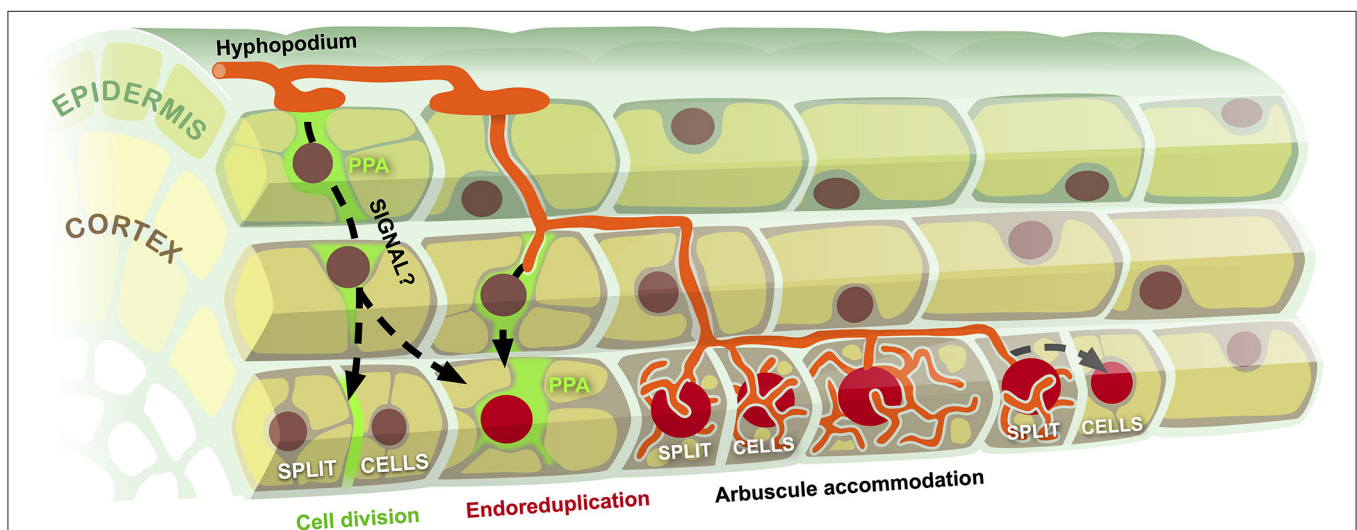
FIGURE 1 | Model of cell cycle reactivation during fungal accommodation in AM. Hyphal colonization of the root epidermis associated with prepenetration apparatus assembly (PPA) triggers a so far uncharacterized signaling process (black dashed arrows) targeting inner cortical cells. This causes the reactivation of cell cycle processes, leading to occasional cell divisions (split cells) and diffuse events of endoreduplication (large red nuclei). Progressive intraradical development of the symbiotic fungus reiterates the stimulation of cell cycle activation, leading to multiple rounds of endoreduplication in advance of arbuscule accommodation and to the expansion of the endoreduplication zone at the front of the developing colonization unit.

propositions that the plant holds substantial control over symbiosis development (Parniske, 2008).

We currently have no information on how the earliest land plants acquired the ability to host a symbiotic fungus inside their cells. One can speculate that initial surface interactions provided an advantageous exchange of nutrients, pressing toward more intimate contacts, such as the penetration of fungal hyphae between the plant cells and eventually inside their lumen. In this context, creating *de novo* a fully functional symbiotic interface—as in modern plants—appears unrealistic. By contrast, stimulating cell divisions in differentiated organs could have been a more amenable strategy to generate both crack openings in the surface tissues (an entry route that is conserved in many extant plant-microbe interactions; Ibáñez et al., 2017)

and irregular intercellular spaces in the inner ones, producing a protected niche for the fungus. The subsequent re-routing of cell plate formation toward the creation of a more efficient symbiotic interface appears achievable, especially in the light of the current findings, and the observation of split cells in some of the earliest fossils of AM hosts indicates that this is indeed an ancient response associated with fungal accommodation (Strullu-Derrien et al., 2018).

AUTHOR CONTRIBUTIONS

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

REFERENCES

Bainard, L. D., Bainard, J. D., Newmaster, S. G., and Klironomos, J. N. (2011). Mycorrhizal symbiosis stimulates endoreduplication in angiosperms. *Plant Cell Environ.* 34, 1577–1585. doi: 10.1111/j.1365-3040.2011.02354.x

Balestrini, R., and Bonfante, P. (2014). Cell wall remodeling in mycorrhizal symbiosis: a way towards biotrophism. *Front. Plant Sci.* 5:237. doi: 10.3389/fpls.2014.00237

Balestrini, R., Hahn, M. G., Faccio, A., Mendgen, K., and Bonfante, P. (1996). Differential localization of carbohydrate epitopes in plant cell walls in the presence and absence of arbuscular mycorrhizal fungi. *Plant Physiol.* 111, 203–213. doi: 10.1104/pp.111.1.203

Barow, M. (2006). Endopolyploidy in seed plants. *BioEssays.* 28, 271–281. doi: 10.1002/bies.20371

Bergerat, A., de Massy, B., Gadelle, D., Varoutas, P. C., Nicolas, A., and Forterre P. (1997). An atypical607 topoisomerase II from Archaea with implications for meiotic recombination. *Nature* 386, 414–417.

Bergerat, A., Gadelle, D., and Forterre, P. (1994). Purification of a DNA topoisomerase II from the hyperthermophilic archaeon *Sulfolobus shibatae*. A thermostable enzyme with both bacterial and eucaryal features. *J. Biol. Chem.*. 269, 27663–27669. doi: 10.1016/S0021-9258(18)47037-8

Berta, G., Fusconi, A., Sampo, S., Lingua, G., Perticone, S., and Repetto, O. (2000). Polyploidy in tomato roots as affected by arbuscular mycorrhizal colonization. *Plant Soil.* 226, 37–44. doi: 10.1007/978-94-017-2858-4_8

Carotenuto, G., Sciascia, I., Oddi, L., Volpe, V., and Genre, A. (2019b). Size matters: three methods for estimating nuclear size in mycorrhizal roots of *Medicago truncatula* by image analysis. *BMC Plant Biol.* 19:180. doi: 10.1186/s12870-019-1791-1

Carotenuto, G., Volpe, V., Russo, G., Politi, M., Sciascia, I., de Almeida-Engler, J., et al. (2019a). Local endoreduplication as a feature of intracellular fungal accommodation in arbuscular mycorrhizas. *New Phytol.* 223, 30–446. doi: 10.1111/nph.15763

Cebolla, A., Vinardell, J. M., Kiss, E., Oláh, B., Roudier, F., Kondorosi, A., et al. (1999). The mitotic inhibitor ccs52 is required for endoreduplication and ploidy-dependent cell enlargement in plants. *EMBO J.* 18, 4476–4484. doi: 10.1093/emboj/18.16.4476

Chandran, D., and Wildermuth, M. C. (2016). Modulation of host endocycle during plant-biotroph interactions. *Enzymes.* 40, 65–103. doi: 10.1016/bs.enz.2016.09.001

Choi, J., Summers, W., and Paszkowski, U. (2018). Mechanisms underlying establishment of arbuscular mycorrhizal symbioses. *Annu. Rev. Phytopathol.* 56, 135–160. doi: 10.1146/annurev-phyto-080516-035521

de Almeida Engler, J., and Gheysen, G. (2013). Nematode-induced endoreduplication in plant host cells: why and how? *Mol. Plant. Microbe. Interact.* 26, 17–24. doi: 10.1094/MPMI-05-12-0128-CR

Dong, W., Zhu, Y., Chang, H., Wang, C., Yang, J., Shi, J., et al. (2021). An SHR-SCR module specifies legume cortical cell fate to enable nodulation. *Nature.* 589, 586–590. doi: 10.1038/s41586-020-3016-z

Downie, J. A. (2014). Legume nodulation. *Curr. Biol.* 24, 184–190. doi: 10.1016/j.cub.2014.01.028

Edgar, B., Zielke, N., and Gutierrez, C. (2014). Endocycles: a recurrent evolutionary innovation for post-mitotic cell growth. *Nat. Rev. Mol. Cell. Biol.* 15, 197–210. doi: 10.1038/nrm3756

Feijen, F. A. A., Vos, R. A., Nuytinck, J., and Merckx, V. S. F. T. (2018). Evolutionary dynamics of mycorrhizal symbiosis in land plant diversification. *Sci. Rep.* 8:10698. doi: 10.1038/s41598-018-28920-x

Fusconi, A., Lingua, G., Trotta, A., and Berta, G. (2005). Effects of arbuscular mycorrhizal colonization and phosphorus application on nuclear ploidy in Allium porrum plants. *Mycorrhiza.* 15, 313–321. doi: 10.1007/s00572-004-0338-x

Gaude, N., Bortfeld, S., Duensing, N., Lohse, M., and Krajinski, F. (2012). Arbuscule containing and non-colonized cortical cells of mycorrhizal roots undergo extensive and specific reprogramming during arbuscular mycorrhizal development. *Plant J.* 69, 510–528. doi: 10.1111/j.1365-313X.2011.04810.x

Genre, A., Chabaud, M., Faccio, A., Barker, D. G., and Bonfante, P. (2008). Prepenetration apparatus assembly precedes and predicts the colonization patterns of arbuscular mycorrhizal fungi within the root cortex of both *Medicago truncatula* and *Daucus carota*. *Plant Cell.* 20, 1407–1420. doi: 10.1105/tpc.108.059014

Genre, A., Chabaud, M., Timmers, T., Bonfante, P., and Barker, D. G. (2005). Arbuscular mycorrhizal fungi elicit a novel intracellular apparatus in *Medicago truncatula* root epidermal cells before infection. *Plant Cell.* 17, 3489–3499. doi: 10.1105/tpc.105.035410

Genre, A., Ivanov, S., Fendrych, M., Faccio, A., Zársky, V., Bisseling, T., et al. (2012). Multiple exocytotic markers accumulate at the sites of perifungal membrane biogenesis in arbuscular mycorrhizas. *Plant Cell Physiol.* 53, 244–255. doi: 10.1093/pcp/pcr170

Heck, C., Kuhn, H., Heidt, S., Walter, S., Rieger, N., and Requena, N. (2016). Symbiotic fungi control plant root cortex development through the novel GRAS transcription factor MIG1. *Curr. Biol.* 26, 2770–2778. doi: 10.1016/j.cub.2016.07.059

Ibáñez, F., Wall, L., and Fabra, A. (2017). Starting points in plant-bacteria nitrogen-fixing symbioses: intercellular invasion of the roots. *J. Exp. Bot.* 68, 1905–1918. doi: 10.1093/jxb/erw387

Ivanov, S., Austin, J., Berg, R. H., and Harrison, M. J. (2019). Extensive membrane systems at the host-arbuscular mycorrhizal fungus interface. *Nat. Plants.* 5, 194–203. doi: 10.1038/s41477-019-0364-5

Keymer, A., and Gutjahr, C. (2018). Cross-kingdom lipid transfer in arbuscular mycorrhiza symbiosis and beyond. *Curr. Opin. Plant Biol.* 44, 137–144. doi: 10.1016/j.pbi.2018.04.005

Kobae, Y., Kameoka, H., Sugimura, Y., Saito, K., Ohtomo, R., Fujiwara, T., et al. (2018). Strigolactone biosynthesis genes of rice is required for the punctual entry of arbuscular mycorrhizal fungi into the roots. *Plant Cell Physiol.* 59, 544–553. doi: 10.1093/pcp/pcy001

Lace, B., and Ott, T. (2018). Commonalities and differences in controlling multipartite intracellular infections of legume roots by

symbiotic microbes. *Plant Cell Physiol.* 59, 666–677. doi: 10.1093/pcp/pcy043

Lepetit, M., Ehling, M., Chaubet, N., and Gigot, C. (1992). A plant histone gene promoter can direct both replication-dependent and -independent gene expression in transgenic plants. *Mol. Gen. Genet.* 231, 276–285. doi: 10.1007/BF00279801

Luginbuehl, L. H., and Oldroyd, G. E. D. (2017). Understanding the arbuscule at the heart of endomycorrhizal symbioses in plants. *Curr. Biol.* 27, R952–R963. doi: 10.1016/j.cub.2017.06.042

Parniske, M. (2008). Arbuscular mycorrhiza: the mother of plant root endosymbioses. *Nat. Rev. Microbiol.* 6, 763–775. doi: 10.1038/nrmicro1987

Pumplin, N., and Harrison, M. J. (2009). Live-cell imaging reveals periarbuscular membrane domains and organelle location in *Medicago truncatula* roots during arbuscular mycorrhizal symbiosis. *Plant Physiol.* 151, 809–819. doi: 10.1104/pp.109.141879

Robinson, D. O., Coate, J. E., Singh, A., Hong, L., Bush, M., Doyle, J. J., et al. (2018). Ploidy and size at multiple scales in the arabidopsis sepal. *Plant Cell.* 30, 2308–2329. doi: 10.1105/tpc.18.00344

Roth, R., Hillmer, S., Funaya, C., Chiapello, M., Schumacher, K., Lo Presti, L., et al. (2019). Arbuscular cell invasion coincides with extracellular vesicles and membrane tubules. *Nat. Plants.* 5, 204–211. doi: 10.1038/s41477-019-0365-4

Roth, R., and Paszkowski, U. (2017). Plant carbon nourishment of arbuscular mycorrhizal fungi. *Curr. Opin. Plant Biol.* 39, 50–56 doi: 10.1016/j.pbi.2017.05.008

Russo, G., Carotenuto, G., Fiorilli, V., Volpe, V., Chiapello, M., Van Damme, D., et al. (2019a). Ectopic activation of cortical cell division during the accommodation of arbuscular mycorrhizal fungi. *New Phytol.* 221, 1036–1048. doi: 10.1111/nph.15398

Russo, G., Carotenuto, G., Fiorilli, V., Volpe, V., Faccio, A., Bonfante, P., et al. (2019b). TPLATE recruitment reveals endocytic dynamics at sites of symbiotic interface assembly in arbuscular mycorrhizal interactions. *Front. Plant Sci.* 10:1628. doi: 10.3389/fpls.2019.01628

Siciliano, V., Genre, A., Balestrini, R., Cappellazzo, G., deWit, P. J., and Bonfante, P. (2007). Transcriptome analysis of arbuscular mycorrhizal roots during development of the prepenetration apparatus. *Plant Physiol.* 144, 1455–1466. doi: 10.1104/pp.107.097980

Sieberer, B. J., Chabaud, M., Fournier, J., Timmers, A. C., and Barker, D. G. (2012). A switch in Ca2+ spiking signature is concomitant with endosymbiotic microbe entry into cortical root cells of *Medicago truncatula. Plant J.* 69, 822–830. doi: 10.1111/j.1365-313X.2011.04834.x

Spatafora, J. W., Chang, Y., Benny, G. L., Lazarus, K., Smith, M. E., Berbee, M. L., et al. (2016). A phylum-level phylogenetic classification of zygomycete fungi based on genome-scale data. *Mycologia.* 108, 1028–1046. doi: 10.3852/16-042

Strullu-Derrien, C., Kenrick, P., Pressel, S., Duckett, J. G., Rioult, J. P., and Strullu, D. G. (2014). Fungal associations in Horneophyton ligneri from the Rhynie Chert (c. 407 million year old) closely resemble those in extant lower land plants: novel insights into ancestral plant-fungus symbioses. *New Phytol.* 203, 964–79. doi: 10.1111/nph.12805

Strullu-Derrien, C., Selosse, M. A., Kenrick, P., and Martin, F. M. (2018). The origin and evolution of mycorrhizal symbioses: from palaeomycology to phylogenomics. *New Phytol.* 220, 1012–1030. doi: 10.1111/nph.15076

Suzaki, T., Ito, M., Yoro, E., Sato, S., Hirakawa, H., Takeda, N., et al. (2014). Endoreduplication-mediated initiation of symbiotic organ development in *Lotus japonicus. Development.* 141, 2441–2445. doi: 10.1242/dev.107946

Tarayre, S., Vinardell, J. M., Cebolla, A., Kondorosi, A., and Kondorosi, E. (2004). Two classes of the cdh1-type activators of the anaphase-promoting complex in plants: novel functional domains and distinct regulation. *Plant Cell.* 16, 422–434. doi: 10.1105/tpc.018952

Tedersoo, L., Bahram, M., and Zobel, M. (2020). How mycorrhizal associations drive plant population and community biology. *Science.* 367, 867–876. doi: 10.1126/science.aba1223

Van Damme, D., Coutuer, S., De Rycke, R., Bouget, F. Y., Inzé, D., and Geelen, D. (2006). Somatic cytokinesis and pollen maturation in Arabidopsis depend on TPLATE, which has domains similar to coat proteins. *Plant Cell.* 18, 3502–3518. doi: 10.1105/tpc.106.040923

Wang, B., Yeun, L. H., Xue, J. Y., Liu, Y., Ané, J. M., and Qiu, Y. L. (2010). Presence of three mycorrhizal genes in the common ancestor of land plants suggests a key role of mycorrhizas in the colonization of land by plants. *New Phytol.* 186, 514–525. doi: 10.1111/j.1469-8137.2009.03137.x

Wildermuth, M. C., Steinwand, M. A., McRae, A. G., Jaenisch, J., and Chandran, D. (2017). Adapted biotroph manipulation of plant cell ploidy. *Annu. Rev. Phytopathol.* 55, 537–564. doi: 10.1146/annurev-phyto-080516-035458

Xiao, T. T., Schilderink, S., Moling, S., Deinum, E. E., Kondorosi, E., Franssen, H., et al. (2014). Fate map of *Medicago truncatula* root nodules. *Development.* 141, 3517–3528. doi: 10.1242/dev.110775

Xiao, T. T., van Velzen, R., Kulikova, O., Franken, C., and Bisseling, T. (2019). Lateral root formation involving cell division in both pericycle, cortex and endodermis is a common and ancestral trait in seed plants. *Development.* 146:dev182592. doi: 10.1242/dev.182592

Zipfel, C., and Oldroyd, G. E. (2017). Plant signalling in symbiosis and immunity. *Nature.* 543, 328–336. doi: 10.1038/nature22009

frontiers
in Plant Science

The Who or the How? Species vs. Ecosystem Function Priorities in Conservation Ecology

Sebastian Leuzinger[1*†] and Boris Rewald[2*†]

[1] School of Science, Auckland University of Technology, Auckland, New Zealand, [2] Department of Forest and Soil Sciences, University of Natural Resources and Life Sciences (BOKU), Vienna, Austria

Current conservation strategies are targeted at preserving species, without explicitly aiming at the maintenance of ecosystem functions. In a physically highly connected world, the unintentional relocation of terrestrial, marine, and microbial life is therefore unavoidable and has been an integral part of human evolution for thousands of years. Here, we challenge the default perception often shared among conservation ecologists that preserving native species at all costs and reducing the number of exotic species and their abundance is the only way to conservation and restoration success. While this strategy is valuable in cases where exotic species disrupt ecological function, there are examples where exotic species have similar functional traits to the threatened or extinct native species and can in fact help maintain the overall or target function of an ecosystem. In the race to cope with global environmental change, we argue that ecosystem function and ecosystem services need to be viewed not only through a taxonomic lens, but increasingly also through a functional, trait-based one.

Keywords: conservation ecology, ecosystem function and ecosystem services, exotic species, functional diversity, functional traits, invasion impact, invasive species, plant invasion

INTRODUCTION

The definition of biological species ultimately rests on human-made concepts, particularly in the realm of unicellular organisms (Hanage et al., 2005). Conversely, biological function (or traits) can more objectively be measured. For example, the rise of oxygen in the early atmosphere of the earth was caused by bacteria and had a massive and irreversible impact on all subsequent life. The quality and size of the impact, however, depended on the organisms' traits, i.e., it was entirely of *functional* nature, and did not hinge on what species and how many were involved – the *how* mattered, not the *who*. Traits are features of an individual organism that potentially affect the performance or fitness of the organism itself ("response traits"). However, a particular trait, or set of traits, of an organism also impacts its biotic and abiotic environment ("effect traits"; Díaz et al., 2013). Plant traits can be physical/morphological (e.g., leaf size and thickness), biochemical/physiological (secondary metabolite production, leaf stoichiometry, carbon assimilation pathway), or temporal/phenological (timing of leaf-out, leaf shedding; Violle et al., 2007). Great advances have been made in establishing

global, publicly available trait data (Kattge et al., 2011), although severe gaps in the availability of functionally "relevant" traits, and their intraspecific variability and plasticity under various environmental conditions, persist (Freschet et al., 2021). Importantly, a given trait (or function) is not necessarily associated with a particular species but can be similar in/performed by different taxa (Calow, 1987), although the likelihood of two species showing similarities in multiple traits naturally decreases.

Since the establishment of the section *Functional Plant Ecology* within Frontiers in Plant Science 10-years-ago (Körner, 2011), this journal has been able to highlight many trait-based studies, lastly, e.g., on how functional traits can be used to predict species assemblages (Li et al., 2021). Notwithstanding that our comprehension of trait–function relationships is still evolving, particularly below ground (Bergmann et al., 2020; van der Plas et al., 2020; Freschet et al., 2021), there is a growing consensus that a trait-based approach has a strong potential to help us understand (1) how functions are coordinated within organisms (Kurze et al., 2021), (2) how species perform under varying environmental conditions (Nikolova et al., 2020), and (3) how species affect ecosystem functioning including the services delivered to humans (Liu et al., 2021).

The numbers of non-native species in floras are steadily increasing in Europe (Lambdon et al., 2008), the United States (Pimentel et al., 2005), but also on much more easily to protect islands like New Zealand (Hulme, 2020). Non-native (exotic) plants in general and alien (invasive) species in particular are thus a pervasive global challenge (Millennium Ecosystem Assessment, 2005) – particularly affecting conservation of biodiversity and ecosystem functioning. While "invasiveness" is naturally often based on measures of population growth and spread in the new region (Pyšek et al., 2004), defining invasiveness also by the impact on the invaded ecosystems has been suggested (Davis and Thompson, 2001). While non-natives can have both positive and negative impacts on their host ecosystems, positive effects are rarely reported (Simberloff et al., 2013; Blackburn et al., 2014; Sladonja et al., 2015). In this perspective piece, we raise the delicate question of whether ecological function needs to be more carefully weighed off against the sheer conservation of native species assemblages. This may sometimes mean the acceptance of the role of exotic species in performing similar ecological function(s) to that of natives, whose protection often involves an extremely high cost (Fairburn et al., 2004; Moore et al., 2011). Ultimately, and particularly in the light of global environmental change, the maintenance of ecological function, and thus ecosystem services, are key and indisputably more valuable than sheer biodiversity metrics. We argue that, 10 years after a debate weighing off the problem of non-natives vs. the broader anthropogenic impact on our planet (Hulme et al., 2011; Thompson and Davis, 2011; van Kleunen et al., 2011), an increasing understanding of response and effect traits of both invasive and native species is urgently needed to support efficient decision making in conservation ecology. While we focus on plants, we also borrow from faunal examples, as in the present question, the same principles apply to all organisms.

ECOSYSTEM FUNCTIONING AS DEPENDENT ON SPECIES AND/OR FUNCTIONAL DIVERSITY

Ecosystem functions are the biotic and abiotic processes within an ecosystem. They are the foundation of ecosystem services (Costanza et al., 1997). Ecosystems are often managed or valued for several ecosystem functions – so-called ecosystem "multifunctionality" (Sanderson et al., 2004). However, sometimes particular functions are more important than others, e.g., in protection forests, mitigating or preventing the impact of rockfalls and landslides will be key (Moos et al., 2019), while, e.g., carbon sequestration will be of secondary importance. Under rapid environmental change, the key question is when and where such services rely on taxonomic vs. functional diversity.

The idea that species diversity *per se* could be an important determinant of ecosystem function (biodiversity-ecosystem functioning, BEF) has been debated for decades. For example, Isbell et al. (2011) show that if larger spatiotemporal scales are considered, functional redundancy is required *via* higher than expected species numbers. Similarly, Hector and Bagchi (2007) concluded earlier that because species often facilitate functions performed by others, studies focusing on individual processes in isolation will generally underestimate levels of biodiversity required to maintain multifunctional ecosystems. However, early studies addressing BEF were frequently criticized for not sufficiently separating complementarity (i.e., high-diversity plant communities can utilize resources more completely) from sampling (i.e., biased toward including highly productive or N-fixing species) effects (Eisenhauer et al., 2016). This resulted in experiments focusing less on taxonomic diversity but more on functional dissimilarity (Díaz and Cabido, 2001). As traits were shown to determine the contributions of species to ecosystem functions (Garnier et al., 2004; Funk et al., 2017), ecologists now often quantify trait variation within a species assemblage, generically referred to as "functional diversity." Functional diversity thus presupposes a mechanistic link between diversity and ecosystem function (Cadotte et al., 2011). Additional drivers such as intraspecific variation, species interactions under contemporary evolution, and interwoven abiotic factors may be needed to improve predictions of ecosystem functioning by models (Carroll et al., 2007; Valiente-Banuet et al., 2015; Funk et al., 2017; van der Plas et al., 2020). As the concept of functional diversity is thus by definition removed from the notion of individual taxonomic species, a specific ecological function can be achieved by the trait profile of taxonomically entirely different species – so-called "functional homologs" (Love, 2007). Ultimately, however, potential changes in community-weighted means and trait ranges (i.e., the "community trait profile"; change in trait profiles and trait "3" are exemplified in **Figure 1**) re-shape the functioning and resilience of the colonized ecosystem (Russell et al., 2014; Sodhi et al., 2019). Addressing realistic, real-world conservations tasks, it remains thus open to debate if the contribution of exotic species – acting as homologs for natives and sustaining target ecosystem services – should not be
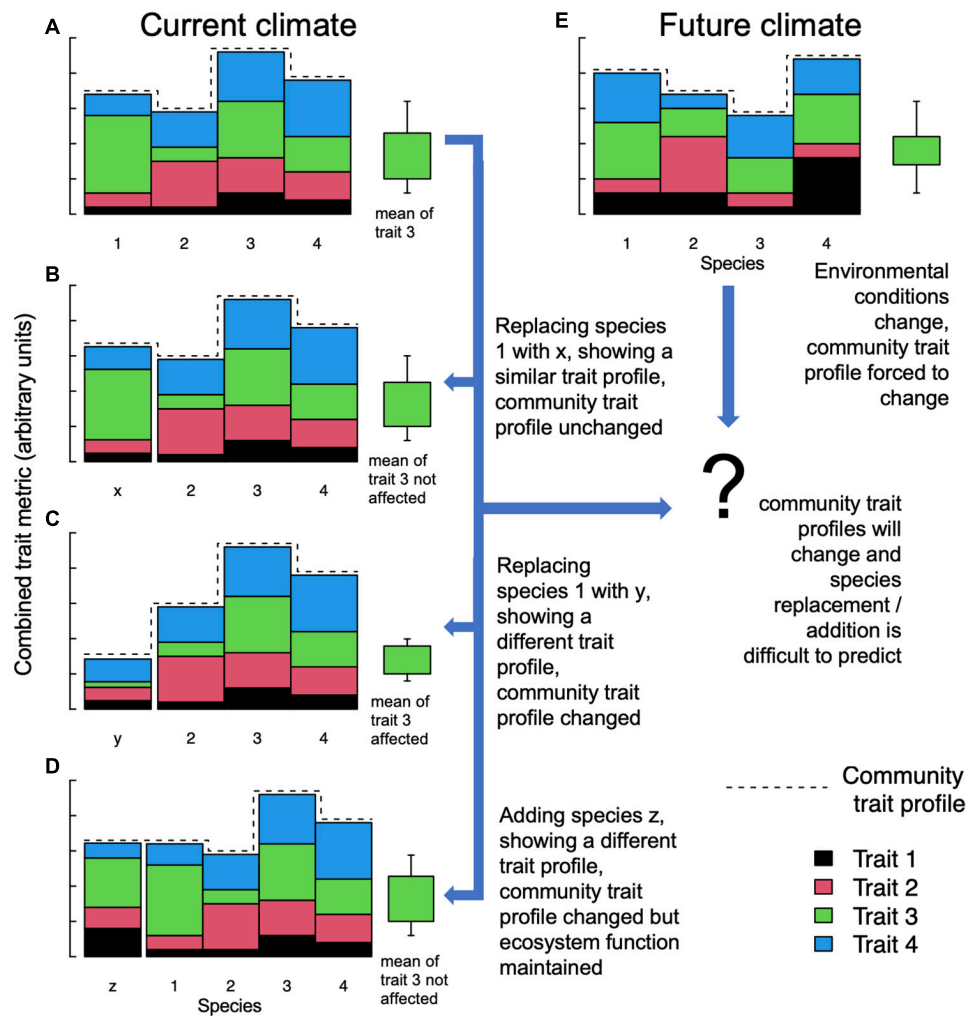
FIGURE 1 | Model ecosystem with four species and four functional traits in current and future climates. Traits are stacked and combined to represent a combined trait metric (no units). The dashed line symbolizes the community trait profile, i.e., the specific set of functional characteristics that have evolved in response to a given environment (Russell et al., 2014). The green boxplot exemplifies the community-weighed mean and range of trait "3" under different scenarios. **(A)** A pristine ecosystem holding four species. In example **(B)**, species 1 is replaced by alien species x, which shares a similar trait profile to 1, such that neither the mean of trait 3 nor the community trait profile is affected. In **(C)**, alien species y replaces species 1, but because its trait profile is substantially different, both the mean of trait 3 across all species and the community trait profile are affected, which might be compromising overall ecosystem functioning. In **(D)**, species z is added to the community without replacing a species and without affecting overall ecosystem function under the current climate but potentially adding redundancy under future environmental conditions. Under future climate **(E)**, the community trait profile and parameter values of specific traits may change even if species assemblages are initially preserved, and thus the impacts of alien species x, y, and z on ecosystem services are much more difficult to predict and can be either positive, neutral, or negative.

considered in conservation and restoration decisions rather than maintaining species assemblages *per se*.

CAN EXOTIC SPECIES MAINTAIN ECOSYSTEM FUNCTIONING, ECOSYSTEM SERVICES?

The movement and exchange of plants between people and regions have been one of the defining characteristics of the human species (Heywood, 2012). Apart from agricultural crops, the cultivation of non-native tree species for production (timber,

industrial wood, and biomass) or restoration purposes is probably one of the most widely accepted uses of exotic species to promote specific ecosystem functions (Dodet and Collet, 2012). This is despite some of the introduced non-native species in Europe, e.g., *Acacia* spp. and *Robinia pseudoacacia* turned out to be invasive or induce unexpected disservices (Souza-Alonso et al., 2017; Šibíková et al., 2019; Langmaier and Lapin, 2020). However, *R. pseudoacacia*, for example, is simultaneously regarded as valuable for the restoration of degraded croplands (Papaioannou et al., 2016). Similarly, exotic pine plantations in New Zealand can support a diverse native flora in their understorey (Forbes et al., 2019). However, as monospecific plantations typically provide

less non-production-related ecosystem services on all but least degraded sites (Bauhus et al., 2010), future planted forests may be designed, and management measures adapted, to integrate ineradicable alien species – maximizing multifunctionality (Liu et al., 2018; Messier et al., 2021). In specific, Baeten et al. (2019) reported that targeting high tree productivity does not necessarily trade-off against other ecosystem services. Thus, high productivity and multifunctionality can be combined with an informed management of tree species and assemblages that favors (target) ecosystem functions – combining native, locally dominant species with economically important exotics as well as locally present but ineradicable aliens alike. In urban forestry, where ecological function almost always outweighs the importance of species identity, the use of non-native species has become commonplace (Sjöman et al., 2016; Conway et al., 2019; Arrington, 2021).
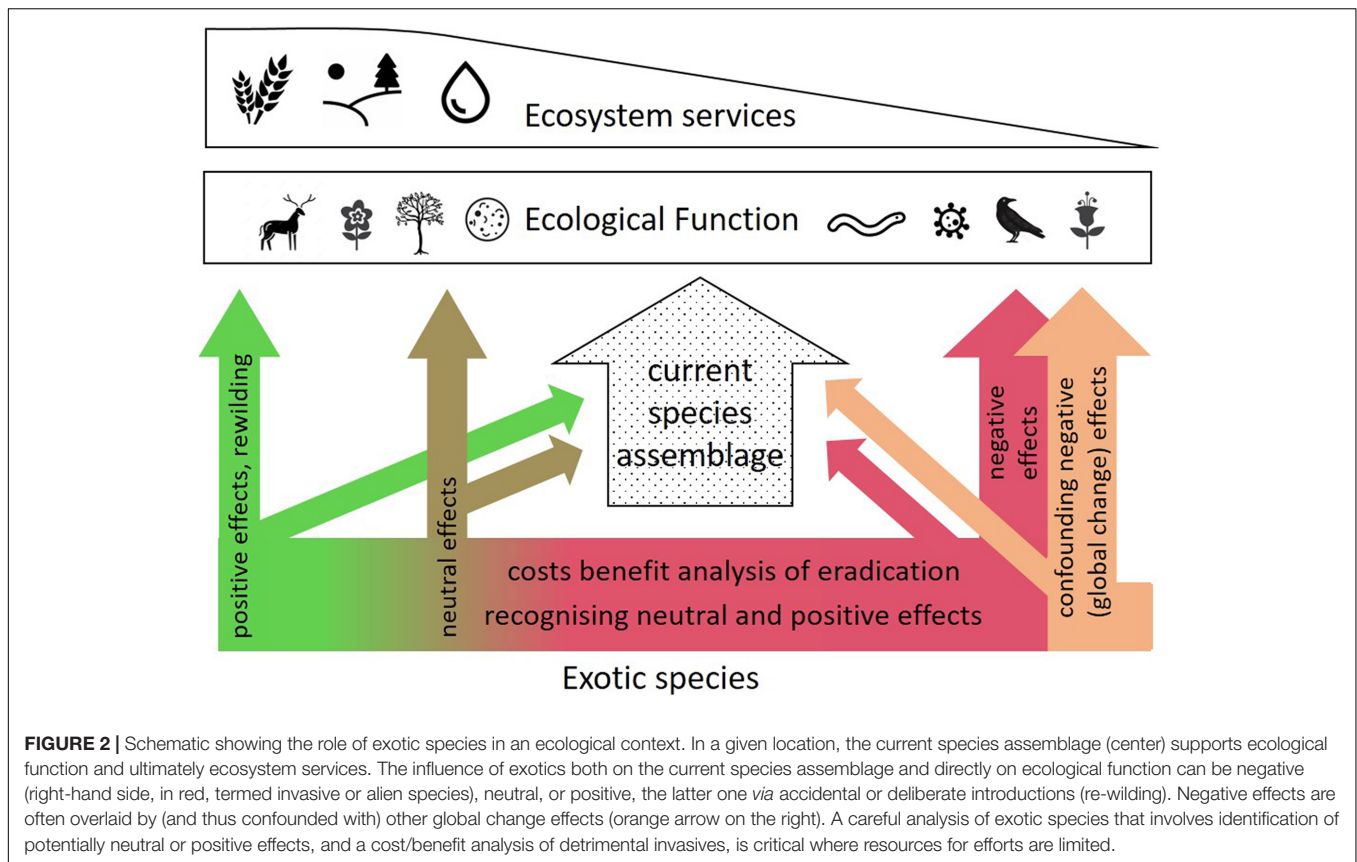
Lastly, "assisted colonization" or "re-wilding," almost exclusively used for animals and while heavily debated (Ricciardi and Simberloff, 2009; Schlaepfer et al., 2009), attempts to replace extinct or struggling natives with an alien homolog. This can work if the trait profile of the introduced alien is reasonably similar to the extinct species, and does not affect the overall community trait profile of an ecosystem (**Figure 1B**); this may of course change under altered environments (**Figure 1E**). One successful example is exotic tortoises that were released on Round Island, Mauritius to replace an extinct native tortoise. The functional trait profile of the introduced species seemed to match that of the extinct species sufficiently to restore overall ecosystem functioning (Griffiths et al., 2013). While "assisted colonization" often fails because of problems with the introduced species itself (see Fischer and Lindenmayer, 2000 for faunal examples), this threshold is removed in cases where aliens have already established, and their functional contribution to the ecosystem remains to be assessed. In the best case, such aliens complement the present species portfolio adding functional redundancy (Isbell et al., 2011; **Figure 1D**) or even serve as a replacement for lost natives as illustrated in the above example (**Figure 1B**).

More or less accidental or historic species introductions are ubiquitous and generally irreversible on a global scale, and if the community trait profile of the resulting ecosystem is sufficiently altered (**Figure 1C**) their impacts on ecosystems can be extremely detrimental (Lowe et al., 2000; Simberloff et al., 2013). In some cases, however, even though whole landscapes are transformed and species assemblages change, the overall function of the ecosystem remains remarkably unchanged. One example is the introduction of large succulents in the Mediterranean, which added a functional type rather than replacing one (**Figure 1D**; Vilà et al., 2003; Heywood, 2012). Often, if ecological function alone is considered, exotic species appear in a different light. The invasive tree species *Ailanthus altissima* (tree of heaven) increasingly occupies disturbed sites in Europe including forests in the Alps and is considered one of the worst invasive plants in Europe – not least due to its homogenization effects on species composition and impact on regeneration of (previously) dominating tree species (Sladonja et al., 2015; Langmaier and Lapin, 2020). *A. altissima* is thus the center of a large range of activities aiming to control the

invasion, but eradication is difficult, as it has a high regenerative capacity (Sladonja et al., 2015). While stand conversion into high forests has been proposed as a potential way of controlling the invasion of light-demanding *A. altissima* (Radtke et al., 2013), this conflicts with the superiority of coppice stands in providing rockfall protection (Jancke et al., 2009). Indeed, a key functional trait of mountain forests is the protective capacity against rockfall, avalanche, and erosion, and alien *A. altissima* intruded forests in Southern Switzerland seem to be providing this function (and ecosystem service) similar to other tree species in the region (Moos et al., 2019). One of those species showing a similar decay pattern, and thus a similar potential decrease in energy reduction capacity against rockfall, is *Castanea sativa* (European chestnut). Ironically, *C. sativa* was originally also introduced to the area (∼2k years ago; Conedera et al., 2004), and now enjoys considerable conservation efforts to protect it (Pereira-Lorenzo et al., 2020). It is an interesting phenomenon how with prolonged exposure, humans seem to legitimatize "naturalized" alien species, sometimes through the association of a historic connotation. From a purely functional perspective, however, a newly introduced species should be assessed using the same criteria as species we may perceive as less "alien" simply because they have been around for longer.

DISCUSSION: FUNCTIONAL DIVERSITY/TRAIT-GUIDED DECISION MAKING IN ECOSYSTEM MANAGEMENT AND CONSERVATION

According to the simple rule "never change a running system" and without an exhaustive understanding of ecosystems, it is often argued that all species should be preserved in their natural habitat because one cannot be certain exactly which species provides which ecological function (Ehrlich and Ehrlich, 1981). In a fully globalized economy, however, species introductions are ubiquitous and irreversible (Sladonja et al., 2015), and their management should carefully consider both detrimental and beneficial effects both on biodiversity *per se* and purely functional aspects of an ecosystem (**Figure 2**). Detrimental effects on natives are further often confounded with other global change drivers including climate-driven range shifts (e.g., Johnson et al., 2011; Wallingford et al., 2020). Clearly distinguishing between the two effects that often act in parallel, is often difficult (**Figure 2**). This was also recognized in very early studies from New Zealand. Allan (1936) points out that the detrimental effects on native ecosystems come from the direct human interventions, and much less *via* invasive species themselves. Despite extremely strict biosecurity regulations and ample pest eradication programs (Goldson et al., 2015), the island has more invasive plant species than any other island (Hulme, 2020). At the same time, protecting rare natives/eradicate invasive species involves a high cost (Fairburn et al., 2004; Moore et al., 2011). Conversely, some invasive species may partly play a positive role, e.g., the European legume *Ulex europaeus* (gorse), which acts as a nurse plant

FIGURE 2 | Schematic showing the role of exotic species in an ecological context. In a given location, the current species assemblage (center) supports ecological function and ultimately ecosystem services. The influence of exotics both on the current species assemblage and directly on ecological function can be negative (right-hand side, in red, termed invasive or alien species), neutral, or positive, the latter one *via* accidental or deliberate introductions (re-wilding). Negative effects are often overlaid by (and thus confounded with) other global change effects (orange arrow on the right). A careful analysis of exotic species that involves identification of potentially neutral or positive effects, and a cost/benefit analysis of detrimental invasives, is critical where resources for efforts are limited.

for native forest regeneration in many areas of New Zealand, although plant succession under *U. europaeus* follows initially a different trajectory from that occurring under the homolog native species (Norton, 2009). These examples highlight the delicate trade-off between pure species conservation and a more functional (traits) approach.

The examples in **Figure 1** illustrate how using trait-based frameworks might lead to better understanding and prediction of invasion impacts (see also Sodhi et al., 2019). We fully acknowledge that an incomplete understanding of hard-to-predict ecological interaction imposed by exotic species may pose a risk to multiple ecosystem functions. Examples of alien species disrupting local ecosystems disastrously are plentiful [e.g., Phillips et al. (2007), but see Lowe et al. (2000) for a complete list], and predicting such impacts is difficult (Dehlin et al., 2008). Efforts to avoid further unintentional introductions and dispersion must therefore always be supported, particularly where this can be achieved at a reasonable cost. However, the broad implementation of risk assessment strategies based on functional traits, facilitating the prediction of the capacity of a species to affect ecosystem functions, and to maintain or enhance these functions under future environments (Díaz et al., 2013; Cuthbert et al., 2019) may help inform a more nuanced ecosystem management approach. This is particularly true where neither exclusion nor eradication is realistic, or in cases where alien species either have a neutral or positive effect on BEF (left arrows in **Figure 2**). It is fully acknowledged that

our understanding of trait-function relationships, particularly including the consequences of variation and plasticity of trait sets under extreme climatic conditions and a rapidly changing environment, must evolve further to increase prediction accuracies (**Figure 1**, center right).

In brief, and colloquially expressed, it comes down to the question to what extent we can afford "open-air museums," and when it is more cost-effective to maintain ecosystem function, i.e., *how* (well) is the job done, not by *whom*. Ultimately, this may result in classification schemes for alien species beyond the current focus on the adverse impacts (e.g., Blackburn et al., 2014). Also, as Brodie et al. (2018) suggest, such schemes must look beyond the survival of individual species, but target key roles in species interactions and the maintenance of communities and ecosystems. In any case, there are valid arguments beyond ecological function [e.g., aesthetic, ethical, or cultural (Lindemann-Matthies et al., 2010; Aerts et al., 2016; Sacchelli et al., 2020)], which justify the combat of invasives and/or the protection of natives in their own right. In particular, societal and political processes may set the normative values guiding management decisions.

However, the partially positive impact of exotic and even invasive species on the functional diversity of species assemblages and target ecosystem functions, as exemplified above, seem currently not considered sufficiently in real-world ecological management decisions. We therefore strongly advocate for an evidence- and function-based decision-making process beyond

conserving species assemblages *per se* (see **Figure 2** for illustration). To evaluate to which extent the traits of specific colonizing species provide target ecosystem functions (and thus services) in real-world ecological systems compared to unaltered species assemblage requires empirical evidence. To achieve this, we suggest a research agenda at the interface of conservation, functional and ecosystem ecology, and an intensified societal/political discussion on conservation foci under an increasingly altered environment. Ultimately, the decision seems an economical one – if endless resources were available to conservation programs, nobody would ever argue to not preserve native and eradicate invasive species. However, given multiple global changes acting concurrently, and limited resources for conservation/restoration projects that have to service a large number of requirements (e.g., related to aesthetics, culture, carbon sequestration, and water quality), biodiversity – functional ecology trade-offs have to be considered carefully.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding authors.

AUTHOR CONTRIBUTIONS

SL and BR drafted the manuscript. SL provided the artwork. Both authors jointly revised and approved the manuscript.

ACKNOWLEDGMENTS

REFERENCES

Aerts, R., Van Overtveld, K., November, E., Wassie, A., Abiyu, A., Demissew, S., et al. (2016). Conservation of the Ethiopian church forests: threats, opportunities and implications for their management. *Sci. Total Environ.* 551, 404–414. doi: 10.1016/j.scitotenv.2016.02.034

Allan, H. H. (1936). Indigene versus alien in the New Zealand plant world. *Ecology* 17, 187–193. doi: 10.2307/1931458

Arrington, A. (2021). Urban foraging of five non-native plants in NYC: balancing ecosystem services and invasive species management. *Urban For. Urban Green.* 58:126896. doi: 10.1016/j.ufug.2020.126896

Baeten, L., Bruelheide, H., Van Der Plas, F., Kambach, S., Ratcliffe, S., Jucker, T., et al. (2019). Identifying the tree species compositions that maximize ecosystem functioning in European forests. *J. Appl. Ecol.* 56, 733–744. doi: 10.1111/1365-2664.13308

Bauhus, J., Van Der Meer, P., and Kanninen, M. (2010). *Ecosystem Goods and Services from Plantation Forests*. London: Earthscan.

Bergmann, J., Weigelt, A., Van Der Plas, F., Laughlin, D. C., Kuyper, T. W., Guerrero-Ramirez, N., et al. (2020). The fungal collaboration gradient dominates the root economics space in plants. *Sci. Adv.* 6:eaba3756. doi: 10.1126/sciadv.aba3756

Blackburn, T. M., Essl, F., Evans, T., Hulme, P. E., Jeschke, J. M., Kühn, I., et al. (2014). A unified classification of alien species based on the magnitude of their environmental impacts. *PLoS Biol.* 12:e1001850. doi: 10.1371/journal.pbio.1001850

Brodie, J. F., Redford, K. H., and Doak, D. F. (2018). Ecological function analysis: incorporating species roles into conservation. *Trends Ecol. Evol.* 33, 840–850. doi: 10.1016/j.tree.2018.08.013

Cadotte, M. W., Carscadden, K., and Mirotchnick, N. (2011). Beyond species: functional diversity and the maintenance of ecological processes and services. *J. Appl. Ecol.* 48, 1079–1087. doi: 10.1111/j.1365-2664.2011.02048.x

Calow, P. (1987). Towards a definition of Functional Ecology. *Funct. Ecol.* 1, 57–61. doi: 10.2307/2389358

Carroll, S. P., Hendry, A. P., Reznick, D. N., and Fox, C. W. (2007). Evolution on ecological time-scales. *Funct. Ecol.* 21, 387–393. doi: 10.1111/j.1365-2435.2007.01289.x

Conedera, M., Krebs, P., Tinner, W., Pradella, M., and Torriani, D. (2004). The cultivation of *Castanea sativa* (Mill.) in Europe, from its origin to its diffusion on a continental scale. *Veg. Hist. Archaeobot.* 13, 161–179. doi: 10.1007/s00334-004-0038-7

Conway, T. M., Almas, A. D., and Coore, D. (2019). Ecosystem services, ecological integrity, and native species planting: how to balance these ideas in urban forest management?. *Urban For. Urban Green.* 41, 1–5. doi: 10.1016/j.ufug.2019.03.006

Costanza, R., Darge, R., Degroot, R., Farber, S., Grasso, M., Hannon, B., et al. (1997). The value of the world's ecosystem services and natural capital. *Nature* 387, 253–260. doi: 10.1038/387253a0

Cuthbert, R. N., Dickey, J. W. E., Coughlan, N. E., Joyce, P. W. S., and Dick, J. T. A. (2019). The Functional Response Ratio (FRR): advancing comparative metrics for predicting the ecological impacts of invasive alien species. *Biol. Invasions* 21, 2543–2547. doi: 10.1007/s10530-019-02002-z

Davis, M. A., and Thompson, K. (2001). Invasion terminology: should ecologists define their terms differently than others? No, not if we want to be of any help! *Bull. Ecol. Soc. Am.* 82, 206–206.

Dehlin, H., Peltzer, D. A., Allison, V. J., Yeates, G. W., Nilsson, M.-C., and Wardle, D. A. (2008). Tree seedling performance and below-ground properties in stands of invasive and native tree species. *N. Z. J. Ecol.* 32, 67–79.

Díaz, S., and Cabido, M. (2001). Vive la différence: plant functional diversity matters to ecosystem processes. *Trends Ecol. Evol.* 16, 646–655. doi: 10.1016/S0169-5347(01)02283-2

Díaz, S., Purvis, A., Cornelissen, J. H. C., Mace, G. M., Donoghue, M. J., Ewers, R. M., et al. (2013). Functional traits, the phylogeny of function, and ecosystem service vulnerability. *Ecol. Evol.* 3, 2958–2975. doi: 10.1002/ece3.601

Dodet, M., and Collet, C. (2012). When should exotic forest plantation tree species be considered as an invasive threat and how should we treat them?. *Biol. Invasions* 14, 1765–1778. doi: 10.1007/s10530-012-0202-4

Ehrlich, P. R., and Ehrlich, A. H. (1981). *Extinction: The Causes and Consequences of the Disappearance of Species*. New York: Random House.

Eisenhauer, N., Barnes, A. D., Cesarz, S., Craven, D., Ferlian, O., Gottschall, F., et al. (2016). Biodiversity–ecosystem function experiments reveal the mechanisms underlying the consequences of biodiversity change in real world ecosystems. *J. Veg. Sci.* 27, 1061–1070. doi: 10.1111/jvs.12435

Fairburn, G. A., Hughey, K. F., and Cullen, R. (2004). Cost effectiveness of endangered species management: the kokako (*Callaeas cinerea*) in New Zealand. *N. Z. J. Ecol.* 28, 83–91.

Fischer, J., and Lindenmayer, D. B. (2000). An assessment of the published results of animal relocations. *Biol. Conserv.* 96, 1–11. doi: 10.1016/S0006-3207(00)00048-3

Forbes, A. S., Norton, D. A., and Carswell, F. E. (2019). Opportunities and limitations of exotic *Pinus radiata* as a facilitative nurse for New Zealand indigenous forest restoration. *N. Z. J. For. Sci.* 49, 1–21. doi: 10.33494/nzjfs492019x45x

Freschet, G. T., Roumet, C., Comas, L. H., Weemstra, M., Bengough, A. G., Rewald, B., et al. (2021). Root traits as drivers of plant and ecosystem functioning: current understanding, pitfalls and future research needs. *New Phytol.* 232, 1123–1158. doi: 10.1111/nph.17072

Funk, J. L., Larson, J. E., Ames, G. M., Butterfield, B. J., Cavender-Bares, J., Firn, J., et al. (2017). Revisiting the Holy Grail: using plant functional traits to

understand ecological processes. *Biol. Rev.* 92, 1156–1173. doi: 10.1111/brv. 12275

Garnier, E., Cortez, J., Billes, G., Navas, M. L., Roumet, C., Debussche, M., et al. (2004). Plant functional markers capture ecosystem properties during secondary succession. *Ecology* 85, 2630–2637. doi: 10.1890/03-0799

Goldson, S., Bourdôt, G., Brockerhoff, E., Byrom, A., Clout, M., Mcglone, M., et al. (2015). New Zealand pest management: current and future challenges. *J. R. Soc. N. Z.* 45, 31–58. doi: 10.1080/03036758.2014.1000343

Griffiths, C. J., Zuel, N., Jones, C. G., Ahamud, Z., and Harris, S. (2013). Assessing the potential to restore historic grazing ecosystems with tortoise ecological replacements. *Conserv. Biol.* 27, 690–700. doi: 10.1111/cobi.1 2087

Hanage, W. P., Fraser, C., and Spratt, B. G. (2005). Fuzzy species among recombinogenic bacteria. *BMC Biol.* 3:6. doi: 10.1186/1741-7007-3-6

Hector, A., and Bagchi, R. (2007). Biodiversity and ecosystem multifunctionality. *Nature* 448, 188–190. doi: 10.1038/nature05947

Heywood, V. H. (2012). The role of New World biodiversity in the transformation of Mediterranean landscapes and culture. *Bocconea* 24, 69–93.

Hulme, P. E. (2020). Plant invasions in New Zealand: global lessons in prevention, eradication and control. *Biol. Invasions* 22, 1539–1562. doi: 10.1007/s10530-020-02224-6

Hulme, P. E., Pyšek, P., and Duncan, R. P. (2011). Don't be fooled by a name: a reply to Thompson and Davis. *Trends Ecol. Evol.* 26:318. doi: 10.1016/j.tree.2011.03. 018

Isbell, F., Calcagno, V., Hector, A., Connolly, J., Harpole, W. S., Reich, P. B., et al. (2011). High plant diversity is needed to maintain ecosystem services. *Nature* 477, 199–202. doi: 10.1038/nature10282

Jancke, O., Dorren, L. K., Berger, F., Fuhr, M., and Köhl, M. (2009). Implications of coppice stand characteristics on the rockfall protection function. *For. Ecol. Manage.* 259, 124–131. doi: 10.1016/j.foreco.2009.10.003

Johnson, P. T., Mckenzie, V. J., Peterson, A. C., Kerby, J. L., Brown, J., Blaustein, A. R., et al. (2011). Regional decline of an iconic amphibian associated with elevation, land-use change, and invasive species. *Conserv. Biol.* 25, 556–566. doi: 10.1111/j.1523-1739.2010.01645.x

Kattge, J., Díaz, S., Lavorel, S., Prentice, C., Leadley, P., Bonisch, G., et al. (2011). TRY - a global database of plant traits. *Glob. Chang. Biol.* 17, 2905–2935. doi: 10.1111/j.1365-2486.2011.02451.x

Körner, C. (2011). The Grand Challenges in Functional Plant Ecology. *Front. Plant Sci.* 2:1. doi: 10.3389/fpls.2011.00001

Kurze, S., Engelbrecht, B. M. J., Bilton, M. C., Tielbörger, K., and Álvarez-Cansino, L. (2021). Rethinking the plant economics spectrum for annuals: a multi-species study. *Front. Plant Sci.* 12:640862. doi: 10.3389/fpls.2021.640862

Lambdon, P. W., Pysek, P., Basnou, C., Hejda, M., Arianoutsou, M., Essl, F., et al. (2008). Alien flora of Europe: species diversity, temporal trends, geographical patterns and research needs. *Preslia* 80, 101–149.

Langmaier, M., and Lapin, K. (2020). A systematic review of the impact of invasive alien plants on forest regeneration in European temperate forests. *Front. Plant Sci.* 11:1349. doi: 10.3389/fpls.2020.524969

Li, R., Zhu, S., Lian, J., Zhang, H., Liu, H., Ye, W., et al. (2021). Functional traits are good predictors of tree species abundance across 101 subtropical forest species in China. *Front. Plant Sci.* 12:541577. doi: 10.3389/fpls.2021.54 1577

Lindemann-Matthies, P., Junge, X., and Matthies, D. (2010). The influence of plant diversity on people's perception and aesthetic appreciation of grassland vegetation. *Biol. Conserv.* 143, 195–202. doi: 10.1016/j.biocon.2009.10.003

Liu, C., Li, Y., Yan, P., and He, N. (2021). How to Improve the predictions of plant functional traits on ecosystem functioning?. *Front. Plant Sci.* 12:622260. doi: 10.3389/fpls.2021.622260

Liu, C. L. C., Kuchma, O., and Krutovsky, K. V. (2018). Mixed-species versus monocultures in plantation forestry: development, benefits, ecosystem services and perspectives for the future. *Glob. Ecol. Conserv.* 15:e00419. doi: 10.1016/j. gecco.2018.e00419

Love, A. C. (2007). Functional homology and homology of function: biological concepts and philosophical consequences. *Biol. Philos.* 22, 691–708. doi: 10. 1007/s10539-007-9093-7

Lowe, S., Browne, M., Boudjelas, S., and De Poorter, M. (2000). *100 of the world's worst invasive alien species: a selection from the global invasive species database.* Auckland: Invasive Species Specialist Group.

Messier, C., Bauhus, J., Sousa-Silva, R., Auge, H., Baeten, L., Barsoum, N., et al. (2021). For the sake of resilience and multifunctionality, let's diversify planted forests!. *Conserv. Lett.* e12829. doi: 10.1111/conl.12829 [Epub ahead of print].

Millennium Ecosystem Assessment (2005). *Ecosystems And Human Well-Being.* Washington: Island Press.

Moore, J. L., Runge, M. C., Webber, B. L., and Wilson, J. R. (2011). Contain or eradicate? Optimizing the management goal for Australian acacia invasions in the face of uncertainty. *Divers. Distrib.* 17, 1047–1059.

Moos, C., Toe, D., Bourrier, F., Knüsel, S., Stoffel, M., and Dorren, L. (2019). Assessing the effect of invasive tree species on rockfall risk – The case of *Ailanthus altissima*. *Ecol. Eng.* 131, 63–72. doi: 10.1016/j.ecoleng.2019.03.001

Nikolova, P. S., Bauerle, T. L., Häberle, K.-H., Blaschke, H., Brunner, I., and Matyssek, R. (2020). Fine-root traits reveal contrasting ecological strategies in european beech and norway spruce during extreme drought. *Front. Plant Sci.* 11:1211. doi: 10.3389/fpls.2020.01211

Norton, D. A. (2009). Species invasions and the limits to restoration: learning from the New Zealand experience. *Science* 325, 569–571. doi: 10.1126/science. 1172978

Papaioannou, A., Chatzistathis, T., Papaioannou, E., and Papadopoulos, G. (2016). *Robinia pseudoacacia* as a valuable invasive species for the restoration of degraded croplands. *Catena* 137, 310–317. doi: 10.1016/j.catena.2015.09.019

Pereira-Lorenzo, S., Bischofberger, Y., Conedera, M., Piattini, P., Crovadore, J., Chablais, R., et al. (2020). Reservoir of the European chestnut diversity in Switzerland. *Biodivers. Conserv.* 29, 2217–2234. doi: 10.1007/s10531-020-01970-2

Phillips, B. L., Brown, G. P., Greenlees, M., Webb, J. K., and Shine, R. (2007). Rapid expansion of the cane toad (*Bufo marinus*) invasion front in tropical Australia. *Austral Ecol.* 32, 169–176. doi: 10.1111/j.1442-9993.2007.01664.x

Pimentel, D., Zuniga, R., and Morrison, D. (2005). Update on the environmental and economic costs associated with alien-invasive species in the United States. *Ecol. Econ.* 52, 273–288. doi: 10.1016/j.ecolecon.2004.10.002

Pyšek, P., Richardson, D. M., Rejmánek, M., Webster, G. L., Williamson, M., and Kirschner, J. (2004). Alien plants in checklists and floras: towards better communication between taxonomists and ecologists. *Taxon* 53, 131–143. doi: 10.2307/4135498

Radtke, A., Ambraß, S., Zerbe, S., Tonon, G., Fontana, V., and Ammer, C. (2013). Traditional coppice forest management drives the invasion of *Ailanthus altissima* and *Robinia pseudoacacia* into deciduous forests. *For. Ecol. Manage.* 291, 308–317. doi: 10.1016/j.foreco.2012.11.022

Ricciardi, A., and Simberloff, D. (2009). Assisted colonization is not a viable conservation strategy. *Trend Ecol. Evol.* 24, 248–253. doi: 10.1016/j.tree.2008. 12.006

Russell, M. B., Woodall, C. W., D'amato, A. W., Domke, G. M., and Saatchi, S. S. (2014). Beyond mean functional traits: influence of functional trait profiles on forest structure, production, and mortality across the eastern US. *For. Ecol. Manage.* 328, 1–9. doi: 10.1016/j.foreco.2014.05.014

Sacchelli, S., Grilli, G., Capecchi, I., Bambi, L., Barbierato, E., and Borghini, T. (2020). Neuroscience application for the analysis of cultural ecosystem services related to stress relief in forest. *Forests* 11:190. doi: 10.3390/f11020190

Sanderson, M., Skinner, R., Barker, D., Edwards, G., Tracy, B., and Wedin, D. (2004). Plant species diversity and management of temperate forage and grazing land ecosystems. *Crop Sci.* 44, 1132–1144. doi: 10.2135/cropsci2004.1132

Schlaepfer, M. A., Helenbrook, W. D., Searing, K. B., and Shoemaker, K. T. (2009). Assisted colonization: evaluating contrasting management actions (and values) in the face of uncertainty. *Trends Ecol. Evol.* 24, 471–472. doi: 10.1016/j.tree. 2009.05.008

Šibíková, M., Jarolímek, I., Hegedüšová, K., Májeková, J., Mikulová, K., Slabejová, D., et al. (2019). Effect of planting alien *Robinia pseudoacacia* trees on homogenization of Central European forest vegetation. *Sci. Total Environ.* 687, 1164–1175. doi: 10.1016/j.scitotenv.2019.06.043

Simberloff, D., Martin, J. L., Genovesi, P., Maris, V., Wardle, D. A., Aronson, J., et al. (2013). Impacts of biological invasions: what's what and the way forward. *Trends Ecol. Evol.* 28, 58–66. doi: 10.1016/j.tree.2012.07.013

Sjöman, H., Morgenroth, J., Sjöman, J. D., Sæbø, A., and Kowarik, I. (2016). Diversification of the urban forest—can we afford to exclude exotic tree species?. *Urban For. Urban Green.* 18, 237–241.

Sladonja, B., Sušek, M., and Guillermic, J. (2015). Review on invasive tree of heaven (*Ailanthus altissima* (Mill.) Swingle) conflicting values: assessment of

its ecosystem services and potential biological threat. *Environ. Manage.* 56, 1009–1034. doi: 10.1007/s00267-015-0546-5

Sodhi, D. S., Livingstone, S. W., Carboni, M., and Cadotte, M. W. (2019). Plant invasion alters trait composition and diversity across habitats. *Ecol. Evol.* 9, 6199–6210. doi: 10.1002/ece3.5130

Souza-Alonso, P., Rodríguez, J., González, L., and Lorenzo, P. (2017). Here to stay. Recent advances and perspectives about *Acacia* invasion in Mediterranean areas. *Ann. For. Sci.* 74:55. doi: 10.1007/s13595-017-0651-0

Thompson, K., and Davis, M. A. (2011). Why research on traits of invasive plants tells us very little. *Trends Ecol. Evol.* 26, 155–156. doi: 10.1016/j.tree.2011.01.007

Valiente-Banuet, A., Aizen, M. A., Alcántara, J. M., Arroyo, J., Cocucci, A., Galetti, M., et al. (2015). Beyond species loss: the extinction of ecological interactions in a changing world. *Funct. Ecol.* 29, 299–307. doi: 10.1111/1365-2435.12356

van der Plas, F., Schroder-Georgi, T., Weigelt, A., Barry, K., Meyer, S., Alzate, A., et al. (2020). Plant traits alone are poor predictors of ecosystem properties and long-term ecosystem functioning. *Nat. Ecol. Evol.* 4, 1602–1611. doi: 10.1038/s41559-020-01316-9

van Kleunen, M., Dawson, W., and Dostal, P. (2011). Research on invasive-plant traits tells us a lot. *Trends Ecol. Evol.* 26:317. doi: 10.1016/j.tree.2011.03.019

Vilà, M., Burriel, J. A., Pino, J., Chamizo, J., Llach, E., Porterias, M., et al. (2003). Association between *Opuntia* species invasion and changes in land-cover in the Mediterranean region. *Glob. Chang. Biol.* 9, 1234–1239. doi: 10.1046/j.1365-2486.2003.00652.x

Violle, C., Navas, M. L., Vile, D., Kazakou, E., Fortunel, C., Hummel, I., et al. (2007). Let the concept of trait be functional!. *Oikos* 116, 882–892. doi: 10.1111/j.2007.0030-1299.15559.x

Wallingford, P. D., Morelli, T. L., Allen, J. M., Beaury, E. M., Blumenthal, D. M., Bradley, B. A., et al. (2020). Adjusting the lens of invasion biology to focus on the impacts of climate-driven range shifts. *Nat. Clim. Chang.* 10, 398–405. doi: 10.1038/s41558-020-0768-2

Legume Breeding for the Agroecological Transition of Global Agri-Food Systems: A European Perspective

Diego Rubiales[1]*, Paolo Annicchiarico[2], Maria Carlota Vaz Patto[3] and Bernadette Julier[4]

[1]Institute for Sustainable Agriculture, CSIC, Córdoba, Spain, [2]Research Centre for Animal Production and Aquaculture, CREA, Lodi, Italy, [3]Instituto de Tecnologia Química e Biológica António Xavier, ITQB-NOVA, Oeiras, Portugal, [4]Institut National de Recherche Pour l'Agriculture, l'Alimentation et l'Environnement (INRAE), URP3F, Lusignan, France

Wider and more profitable legume crop cultivation is an indispensable step for the agroecological transition of global agri-food systems but represents a challenge especially in Europe. Plant breeding is pivotal in this context. Research areas of key interest are represented by innovative phenotypic and genome-based selection procedures for crop yield, tolerance to abiotic and biotic stresses enhanced by the changing climate, intercropping, and emerging crop quality traits. We see outmost priority in the exploration of genomic selection (GS) opportunities and limitations, to ease genetic gains and to limit the costs of multi-trait selection. Reducing the profitability gap of legumes relative to major cereals will not be possible in Europe without public funding devoted to crop improvement research, pre-breeding, and, in various circumstances, public breeding. While most of these activities may profit of significant public-private partnerships, all of them can provide substantial benefits to seed companies. A favorable institutional context may comprise some changes to variety registration tests and procedures.

Keywords: legume, breeding, agroecological transitions, agri-food systems, genomic selection

THE AGROECOLOGICAL CONTEXT AND THE EVOLUTION OF AGRI-FOOD SYSTEMS

Legume crops are characterized by their high-protein content and their ability to meet their own nitrogen demand by biological nitrogen fixation. Most major legume crops were domesticated at the very onset of agriculture and played a key historical role in agri-food systems as sources of protein complementing carbohydrates provided by cereals, tubers, or roots (Smýkal et al., 2015). Legumes offer specific functional attributes that deliver multiple benefits, including health and nutritional provisions, farmland biodiversity, and improved environmental sustainability (Iannetta et al., 2021). Even when N_2 fixation was not demonstrated till XIX century (Hellriegel and Wilfarth, 1888), the "reinvigorating" and "manuring" effect of legumes to the soil was already acknowledged by ancient Greek and Roman authors, who recommended their systematic use in crop rotations.

In spite of the known benefits of legume-supported production systems and diets, legume cultivation declined in modern agriculture, particularly in Europe. Grain and forage legumes

are grown in only about 1.5 and 1%, respectively, of the European arable area, compared with *circa* 15% in the world. The main reason for that is the spread of and the economic support to intensive cereal-based systems largely relying on the use of nitrogen fertilizers (Watson et al., 2017). Lower economic return from legume crops compared to cereals is frequently claimed by farmers, especially when neglecting the positive effect of a legume on subsequent or companion crops and the opportunity of domestic valorisation. Hence, legumes entered into a vicious circle at all steps of the production and the value chain, resulting in a technological lock-down: the lower production leading to lower use in the value chain, leading in turn to lower investment in agronomy, breeding, farm advisory, and processing, resulting in the increasing marginalization of legume cultivation and use (Magrini et al., 2016). This decline in legume cultivation paired with an increased feed protein demand for livestock and poultry production (Westhoek et al., 2011) has led to about 70% dependence from imported feed protein of Europe, relying essentially on imported soybean to supplement animal diets. This demand, added to the rising feed protein import by China (Gale et al., 2019), contributes to an unsustainable soybean production in South America, leading to wide-scale tropical deforestation (Bager et al., 2020). Conversely, the negative consequences on energy and resource use efficiency, greenhouse gas emissions, nitrogen biogeochemical fluxes, and agricultural biodiversity of not growing legumes locally (Foyer et al., 2016) have largely been overlooked.

The increasing awareness of the non-sustainability of current regional and global agri-food systems is leading to strategic plans aimed to expand legume cropping in the European Union (EC, 2018) and globally (Sivasankar et al., 2016). This is needed to respond to the expected sharp increase in the demand for high-protein food and feedstuff (Pilorgé and Muel, 2016) and the consequent insecurity and high predicted cost of plant protein supply in international market. Another major objective given to legumes is to introduce reactive nitrogen in agriculture. As a consequence, and also because of a high nitrogen demand to grow non-legume crops, agriculture is largely based on the use of nitrogen fertilizers, chemically produced in factories with huge consumption of fossil energy. Meeting this demand of protein production for food and feed and nitrogen input to agriculture is challenging and may require various changes, such as the extension of the cultivation of legumes and a dietary change toward less animal product-based diets (Westhoek et al., 2011; Searchinger et al., 2019; Willett et al., 2019).

The growing social awareness of environmental and health issues has returned legumes to prominence in food systems (McDermott and Wyatt, 2017; Cusworth et al., 2021). We are experiencing a higher demand not only of local vegetable protein for feed but also for food, which, particularly in EU, is associated with an increasing demand for organic and genetically modified free foods and a rise in the adoption of flexitarian, vegetarian, and vegan diets (EC, 2018). Legumes are in fact a key component of the Mediterranean diet, which is extending to other areas and is driving a culinary revival of local produce and home cooking. At the same time, legumes are entering rapidly into the processed food business (Cusworth et al., 2021). Legume-derived protein

is less expensive relative to meat, egg, and dairy proteins and is amenable to a wide variety of processing applications whose production sector has a two digits' annual growth (EC, 2018).

The positive impact of legume-rich grasslands on ruminant feeding and ecosystem services is increasingly acknowledged (Martin et al., 2020). Perennial legumes, compared with annual grain legumes, offer the additional advantage of maximizing the production of proteins per unit area by a factor of 2–4. The relative inefficiency of extensive livestock systems in terms of greenhouse gas emissions ought to be weighed against the highly positive effects of these systems on landscape preservation, animal and crop diversity, resource use recycling, and production of typical or organically produced food with high added value (Swagemakers et al., 2017), suggesting to rather safeguard and improve the efficiency of these systems by greater reliance on legume-rich perennial grasslands.

As a result, legume cultivation may speedily recover, and predictions are for its further increase. This should be paired with technical solutions to support this growth, making legumes competitive for land at produce prices. This can only be achieved through an integrative approach leading to the adjustment of cropping practices and the breeding of more adapted, attractive, and productive cultivars able to address producers and consumers' needs.

BREEDING FOR INCREASING CROP AND CROPPING SYSTEMS DIVERSITY

Increasing legume cropping requires a breeding investment on a large number of species (**Table 1**) adapted to the diverse agro-climatic conditions and product uses. A broad classification of major species can be made depending on their main use (food, feed, or cover crop), crop cycle (annual or perennial), and breeding system (allogamous or autogamous). Species grown in temperate regions mostly originated from the Fertile Crescent and first expanded in the Mediterranean region (with the exception of soybean, of Chinese origin, and common bean and one lupin species, of American origin). Most of them are relatively well adapted to northern latitudes, because of cold tolerance and/or cultivation as summer crops.

Breeding relies on genetic diversity, of which the maintenance in Genetic Resources Centers is crucial. At the European and world levels, wild and cultivated (landraces, old varieties) accessions of legume species are stored, multiplied, and shared (**Table 1**). Better knowledge on these accessions (phenotypic and genotypic data) is useful to make use of these genetic resources in breeding programs. The selection of varieties, while aiming to maximize breeding progress, tends to progressively reduce the genetic diversity used in agriculture. Breeding may also be challenged by insufficient diversity for specific emerging traits. This can be remedied by a continuing infusion of genetic diversity coming from old varieties, landraces, ecotypes, or wild populations. However, legume breeding programs are relatively small, often forcing breeders to fulfill short-term breeding goals by using elite germplasm rather than "exotic" germplasm, which may require lengthy pre-breeding (Dwivedi et al., 2016; Coyne et al., 2020; Pratap et al., 2021).

TABLE 1 | Major legume crops ranked by the number of cultivars registered in the EU as an indication of breeding efforts devoted to them.

Major crop species		No. of registered cultivars		No. of accessions in geneBanks		Genomic resources available (reference genome sequence or a recent review on the subject)
Scientific name	Common name	EU[1]	Globally[1]	European[2]	Globally[3]	
Phaseolus vulgaris	Common bean	3.728	5.956	49.662	136.167	Assefa et al., 2019
Pisum sativum	Pea	3.377	5.959	35.425	55.732	Kreplak et al., 2019; Pandey et al., 2021
Glycine max	Soybean	1.947	15.430	16.108	83.592	Chu et al., 2021
Medicago sativa	Lucerne	1.270	3.680	8.485	18.353	Chen et al., 2020
Vicia faba	Faba bean	830	1.398	13.229	26.044	Khazaei et al., 2021
Trifolium pratense	Red clover	739	1.485	10.507	11.548	De Vega et al., 2015
Trifolium repens	White clover	430	1.054	4.960	9.600	Griffiths et al., 2019
Vicia sativa	Common vetch	312	665	8.220	13.189	Zhu et al., 2019
Phaseolus coccineus	Runner bean	191	273	2.435	5.226	Xanthopoulou et al., 2019
Lupinus angustifolius	Narrow-leafed lupin	177	357	3.110	5.096	Kamphuis et al., 2021
Cicer arietinum	Chickpea	120	213	12.015	63.612	Roorkiwal et al., 2020
Trifolium incarnatum	Crimson clover	118	178	219	401	No info
Trifolium alexandrinum	Berseem clover	103	184	243	648	No info
Lotus corniculatus	Lotus	98	229	2.006	3.628	Mun et al., 2016
Lupinus albus	White lupin	96	174	4.043	5.490	Kamphuis et al., 2021
Onobrychis viciifolia	Sainfoin	55	87	690	1.179	Shen et al., 2019
Trifolium resupinatum	Persian clover	52	97	324	1.889	No info
Trifolium subterraneum	Subterranean clover	36	110	5.846	16.124	Hirakawa et al., 2016
Lupinus luteus	Yellow lupin	48	85	3.301	3.781	Iqbal et al., 2020
Lens culinaris	Lentil	33	250	9.113	30.846	Guerra-García et al., 2021
Arachis hypogaea	Groundnut	29	576	3.320	32.933	Bertioli et al., 2019
Hedysarum coronarium	Zulla	22	35	126	412	No info
Lathyrus cicera	Red pea	17	27	700	1.183	Santos et al., 2018
Trigonella foenum-graecum	Fenugreek	17	36	379	1.212	No info
Medicago lupulina	Black medic	11	19	594	1.489	No info
Ornithopus sativus	Bird's foot	10	31	230	708	No info
Vicia ervilia	Ervil	8	11	1.245	2.162	No info
Vicia narbonensis	Narbon bean	7	22	593	1.247	No info
Vigna unguiculata	Cowpea	6	81	4.144	40.384	Lonardi et al., 2019
Lathyrus sativus	Grass pea	6	40	3.671	7.663	Emmrich et al., 2020
Medicago polymorpha	Burr medic	6	23	1.012	9.144	Cui et al., 2021
Ornithopus compressus	Yellow bird's foot	4	15	906	3.297	No info
Medicago truncatula	Barrel medic	2	19	2.253	9.309	Pecrix et al., 2018
Vigna radiata	Mungbean	2	49	1.086	15.944	Ha et al., 2021

[1]*CPVO, 2021 (http://cpvoextranet.cpvo.europa.eu).*
[2]*EURISCO, 2021 (https://eurisco.ipk-gatersleben.de/).*
[3]*GENESYS, 2021 (https://www.genesys-pgr.org/).*

This emphasizes the importance of pre-breeding programs carried out by public institutions that exploit landraces and wild relatives and make the resulting germplasm globally available.

There is increasing interest by some farmers of the organic sector for greater cultivation of seeds of genetically heterogeneous material of inbred crops rather than pure line cultivars, because of the advantages in terms of crop resilience and yield stability that genetic diversity is expected to provide. This conflicts with the genetic uniformity required for cultivar registration. Recently, the EU has allowed to market "Organic Heterogeneous Materials," not fulfilling the cultivar requirements as regards uniformity under certain conditions (EU, 2018, 2021). These might include farmers' selection from populations or landraces, dynamic populations, and composite cross populations, devised to get progressively adapted to specific farm conditions under which they evolve, as a kind of modern farm ecotypes (Costanzo and Bickler, 2019). The strategy to grow heterogeneous material conflicts with the breeding theory that aims to produce the best genotype and suggests that micro-niches are exploited by different genotypes. The situations (stress,

spatial heterogeneity) in which a certain extent of diversity is favorable to yield and yield stability are an important area of future research. Multi-environment comparisons of line mixtures vs. their best component pure lines indicated the possibility to grow mixtures with higher yield stability and just a modest yield penalty for soybean (Carneiro et al., 2019), and the presence of an optimal level of genetic diversity that allows to increase the crop yield stability without a penalty on crop yield for the inbred forage subterranean clover (Pecetti et al., 2020).

BREEDING FOR EMERGING NEW TRAITS AND DIVERSIFIED TARGET USES AND ENVIRONMENTS

Breeding needs to identify and exploit genetic variation for relevant traits supported by innovative phenotyping tools and molecular markers-based procedures to develop "smart" cultivars

that yield more with low inputs. Key traits for grain or forage legume breeding were summarized in previous reviews (e.g., Annicchiarico et al., 2015a; Araújo et al., 2015; Duc et al., 2015; Rubiales et al., 2015; Vaz Patto et al., 2015). Hereafter, we focus on emerging traits as suggested by the changing climate and the needed agroecological transition.

The agroecological transition requires not only greater legume crop cultivation in rotations but also greater exploitation of legume-based intercrops, which, in modern agriculture, has been widespread only for some perennial legumes, especially white clover. The association of annual legumes with cereals, or that of perennial legumes with forage grasses, can exploit plant functional diversity to raise crop yields, yield stability, and/or crop quality, while simultaneously enhancing ecosystem services and reducing adverse environmental impacts (Martin-Guay et al., 2018). Breeding for intercropping may be challenging for legume breeders, requiring the identification of trait mean and variance that are responsible for the survival and the production of a target species grown with one or several companion species (Maamouri et al., 2017). Specific breeding for intercropping is supported by several studies on perennial legumes reviewed in Annicchiarico et al. (2019a), as well as by recent findings for an annual legume, such as pea (Annicchiarico et al., 2021).

Greater adaptation to low input conditions will be a leading priority in legume breeding, particularly for organic systems that are on the rise in EU with a mandate to reach 25% of the EU cropping area by 2030. Improved symbiosis and nitrogen use efficiency are therefore becoming compulsory (Sreeharsha et al., 2021). Phosphorous (P) use efficiency is also gaining importance as we are facing an alarming decline in the availability of natural sources of P for soil amendments. Efficiency in S, K, and Zn uptake can be a priority for certain soil types, especially where interactions with P- and N-use efficiency affect the final nutritional quality of the grain (Blair, 2013).

The global change and increasing instability of the climate pose additional challenges to breeders (Andrews and Hodge, 2010). It emphasizes a need for greater tolerance to major biotic (Rubiales et al., 2015) and abiotic stresses (Aráujo et al., 2015). Tolerance to drought will have even greater importance due to lower rainfall as well as lower available irrigation water in many regions (e.g., Polade et al., 2017). Tolerance to low winter temperatures of autumn-sown crops remains a key target in many regions despite the climate change, because occasional low temperatures on poorly hardened plants issued by a mild-winter period can be highly damaging (Araújo et al., 2015), but also because of the requested expansion of legume cultivation toward northern regions (Ergon et al., 2018) or anticipated sowing aimed to crop escape from summer drought. Heat waves at flowering and grain filling stage are an increasing threat, with heat tolerance becoming a priority not only in hot regions but also in spring sowings in temperate regions and even in winter sowings in Mediterranean Basin at low altitudes (Rubiales et al., 2021). Pest and diseases are becoming more and more critical in the predicted scenario of sharp decrease in pesticide uses during cultivation and storage. Also, global changes will affect the relative importance of pests and diseases impacting their geographic distribution (especially

northward) and frequency of outbreaks by affecting their overwintering survival and ability to develop more generations (Skendžić et al., 2021). For instance, pod borer insects (Sharma et al., 2020) and the parasitic weed broomrape (Rubiales, 2020) are expected to extend northward.

Climate change-related stresses will have a negative impact not only on yield but also on nutrient quality of grain legumes (Scheelbeek et al., 2018) with a clear nutritional quality ranking change for legume varieties under, for example, heat stress conditions (Mecha et al., 2021). Conversely, global warming should extend the period of production of perennial grasses, enabling an increase in annual forage yield in spring and autumn, although with risks on summer survival in some regions or years (Durand et al., 2010). In forage legumes, drought has a combined effect on protein content: The reduction in biomass is associated to protein content increase but drought may limit nitrogen fixation (Lemaire and Allirand, 1993).

The predicted increase of legume-based food consumption due to the rise of plant-based and flexitarian diets and the planned EU reduction of meat consumption will widen the economic opportunities for domestic cultivation of grain legumes, since food use provides greater added value than feed use. Until recently, food legume breeders' efforts have focused mainly on improving protein yield and reducing "undesirable" compounds contents (such as raffinose family oligosaccharides in lentils, phytates in pea, vicine-convicine in faba beans, and ODAP in grass pea), while largely neglecting sensory or processing important traits (Duc et al., 2015; Vaz Patto et al., 2015). The insufficient attention paid to the integrated approach needed for improving food legume quality resulted in a lack of innovation and low attractiveness of legume food products that, together with the emergence of novel food habits, resulted in a reduction in consumption (Vaz Patto et al., 2015). To reverse this trend, consumer's preference should be a major driver in food legume breeding. In the more sustainable (less energy needed during processing, with reduced wastes), minimal or mild processed legume-based foods (whole seed or flour consumption), besides appearance, the basic taste qualities (sweet, sour, bitter, salty, and umami), and certain texture properties are of most importance (Roland et al., 2017). Moreover, taste, aroma, and appearance are significantly associated with specific metabolites content in foods (Liem and Russell, 2019; Mecha et al., 2022) that might also influence their final nutritional and health benefits, which are aspects with increasing importance on consumers' choices. An example is the antioxidant phenolic compounds content in food legumes (Mecha et al., 2019). Sensory analysis cannot be attained unless pulses are cooked. In this way, besides the conventional cooking time determination, expedite sensory methodology combining the choice of product' attributes with their intensity scale classification are needed to overcome cost and time constraints of conventional approaches (Ares et al., 2014). When considering highly processed legume-based foods, involving isolated macromolecular fractions of the flours (isolates and concentrates), breeding objectives might focus on increasing the contents and isolation yields of the most valuable nutrient. Examples are protein, fiber, and starch concentrates or isolates (Vaz Patto et al., 2015) used on the

development of nutraceuticals, functional foods, ultra-processed foods made of recombined ingredients (such as meat and dairy products replacers), and nutritional supplements. Although not as sustainable as the minimal or mild processed legume-based food system, this is a rapidly growing market, attracting a new and mainstream audience (Cusworth et al., 2021). In any of the cases (minimal or highly processed legume-based food products), an increased food use will require collaborative developments between legumes breeders and the food industry, exploiting in the most efficient way the existing diversity in quality traits both within and between grain legume species.

For forage crops, useful new quality traits may include increased Omega-3 polyunsaturated fatty acids, vaccenic acid, and conjugated linoleic acid, with reduced levels of omega-6 fatty acids and palmitic acid in milk (Alothman et al., 2019). Considering the ruminants as consumers, breeding for increased content in condensed tannins is important to improve protein valorization in the digestive tract, reduce N release in the environment, and decrease the impact of parasitic nematodes (Mueller-Harvey et al., 2019).

Farmer's acceptability of cultivars cannot be considered a new trait by itself, but farmer-participatory selection is gaining increasing attention especially for organic farming. There is a paucity of formal assessments of its value for countries with developed agriculture, but its application to a large-scale pea breeding program in Italy produced greater yield gains than ordinary breeder's selection (Annicchiarico et al., 2019c).

Adaptation for use as a service (alias cover) crop is a further new trait of interest for some legume species in the European agriculture. Two main situations are encountered as: Either a crop (or a crop mixture) is grown in summer just after the last harvest until the new sowing (in autumn, end of winter, or spring) to catch residual nitrogen, control weeds and pests, avoid soil erosion, and improve the soil carbon content, or a crop is grown together with a cash-crop as a service-provider only. Annual legumes for these uses should possess rapid summer establishment and autumn growth (e.g., faba bean and crimson clover). Perennial legumes may offer a living mulch for grain crops over several years (e.g., white clover and prostrate-type lucerne). The breeding of these crops is challenged by greater interest of minor species or plant types and the use of test conditions and target traits that are completely different from those currently used as grain or forage legumes (e.g., evaluation of soil coverage, summer establishment, early vegetative growth, nitrogen content in the biomass, capacity to be destroyed by winter frost, and low competition with the companion grain crop). In addition, their seed cost ought to be very low.

NOVEL TECHNIQUES TO ENHANCE THE SELECTION EFFICIENCY AND EASE THE COMPLEXITY OF SELECTION

As outlined in the earlier sections, legume breeders have to cope with ever-increasing quantitative target traits and the need to reduce the profitability gap with major cereals by drastic yield improvements, in the presence of modest budgets. Field phenotyping remains a bottleneck for crop genetic improvement. Legume improvement can today be supported by the integration of modern genomics approaches, high-throughput phenomics, and simulation modeling (Araus et al., 2018; Varshney et al., 2018). The challenge for wider adoption by breeders is the cost per sample, which is today lower for genotyping than for field-based phenotyping and for high-through phenotyping platforms. The emergence of novel non-destructive root/aerial phenotyping methods can support selection for improved symbiosis as well as other agronomic traits. Affordable low-cost phenotyping tools are being developed, including unmanned aerial vehicles and sensors mounted on "phenomobiles" and can be particularly valuable to decrease the cost of field evaluations (Cazenave et al., 2019).

The use of markers in breeding programs got impulse from the development of high-throughput genotyping techniques, such as genotyping-by-sequencing (GBS), and large SNP array tools. Besides the QTL identified in restricted parental crosses that are hardly used in breeding programs, genome-wide association studies (GWAS) provided markers that explain trait variation in a chosen population. These QTL explain a portion of genetic variance, useful to track major alleles. Most agronomically important traits are polygenic and, as such, can greatly profit of genomic selection (GS), which enables small-effect loci to be incorporated into prediction equations. An interesting option would be to combine in a single model both QTL and all the markers covering the genome, through procedures that may also allow to define a subset of highly explanatory SNPs for inclusion in less expensive selection tools (Li et al., 2018).

A key question for legume breeders is whether GS may have greater selection efficiency than phenotypic selection for crop yield improvement. Pioneer studies highlighted greater predicted yield gain per unit time or unit cost for alfalfa (Annicchiarico et al., 2015b), soybean (Matei et al., 2018), and pea (Annicchiarico et al., 2019b). In legumes, GS displayed convenient predictive ability also for key grain quality (Stewart-Brown et al., 2019) or forage quality traits (Biazzi et al., 2017; Pégard et al., 2021) and emerging complex traits, such as drought tolerance (Li et al., 2019; Annicchiarico et al., 2020), performance in intercropping (Annicchiarico et al., 2021), and tolerance to some biotic stresses (Carpenter et al., 2018). However, research work is crucially needed to fully assess the potential of GS for different legume species and target traits, explore the transferability of its models to different breeding populations, and optimize its adoption within the breeding schemes. GS has high potential importance for breeding programs not only to enhance their selection efficiency but also to ease the cost and complexity of breeding for several target traits, once prediction equations are available. The number of evaluated genotypes for a fixed trait selection fraction increases exponentially as a function of the number of target traits, quickly reaching genotype numbers whose evaluation cost is beyond reach for

phenotypically evaluated traits. GS selection costs (which depends on the genotyping cost) are nearly zero from the second target trait onwards.

Variant detection (or allele mining) is also a way to identify genes involved in trait variation and alleles conferring a positive effect on a trait (Kumar et al., 2010). For autogamous species in which accessions are mostly pure lines, the detection of variants requires to investigate large panels of genetic resources. For allogamous species, variants, including lethal or sublethal ones at an evolutionary meaning, may be present at a heterozygous status. Such variants could be interesting in breeding, as exemplified for genes involved in lignin content in lucerne (Gréard et al., 2018). Prediction models can be useful also for cost-efficient mining of key traits in large germplasm collections (Jarquín et al., 2016). More generally, markers can bring more progress compared to phenotypic selection in heterozygous and more over tetraploid species, such as lucerne, lotus, and some clovers, because the recessive alleles conferring a positive effect can be selected (Julier et al., 2003).

INSTITUTIONAL AND RESEARCH POLICY ASPECTS

Wider and more profitable legume crop cultivation is an indispensable step for the agroecological transition of global agri-food systems but represents a challenge especially in Europe. Plant breeding is pivotal in this context. Variety breeding is reputed to be a fruitful business, enabling economic activity through seed marketing and genetic progress. However, when the surfaces are low, the return of the breeding activity is not sufficient to support strong breeding programs. Taking the number of registered legume cultivars (CPVO, 2021; **Table 1**) as an indication of the breeding efforts devoted to each species, it seems that the EU seed business is mostly active on common bean, pea, soybean, and faba bean among the grain legumes, and on lucerne, red clover and white clover among the forages. Even for the so-called major legume species (pea, faba bean, and lucerne), the breeding programs are less ambitious than on other non-legume crops, such as maize, sunflower, and oilseed rape.

Reducing the profitability gap of legumes relative to major cereals will not be possible in Europe without public funding devoted to crop improvement research and pre-breeding activities and, in various circumstances and especially for minor crops, public breeding. While most of these activities may profit of significant public-private partnerships, all of them can provide substantial benefits to seed companies (in the form of transferred breeding techniques, genetic resources, or varieties to be marketed). A favorable institutional context ought to comprise variety registration procedures and marketing regulations able to value innovative variety traits and to reverse, under specific circumstances, the trend toward the cultivation of genetically uniform varieties. In Europe, only France has a levy system on grain legume production (pea, faba bean, and lupin grown from certified or farmer'

seed) when collected by a cooperative and allocated to a fund that supports plant breeder's projects. This tax, known as "Cotisation Volontaire Obligatoire" (Mandatory Voluntary Contribution), is not applied (yet) to other grain legumes and does not apply to forage legumes, which are mostly not collected but used directly on farm to feed cattle. Similar systems apply in Canada and Australia, for instance, with returns to public breeding programs, mainly held by Universities, leading to continuous funder-led breeding improvements. Europe's policy makers should explore this further, facilitating public-private breeding interaction for the good of all. The lack of sufficient economic returns also explains the fact that public breeding programs still are the main contributors to legume crop improvement in most European countries. In any case, the support of public research institutions is crucial for the development of pre-breeding or breeding activities and innovative selection procedures (such as GS), as a form of research policy-supported public-private partnership.

At the moment, Genetically Modified Organisms (GMO) in Europe are submitted to complex regulation although are they are used for scientific research. Moreover, plants derived from genome editing enter into GMO category at the moment (Court of Justice of the European Union, 2018). Changes in current regulations and public perceptions are needed before these techniques can be widely adopted by breeders in Europe. Also variety registration procedures may require improvement in various respects, to verify and enforce the genetic progress performed by breeding programs. Important areas needing improvement include the assessment and adequate quantification of the importance of emerging traits related to crop quality, stress tolerance, or suitability for intercropping. A pan-European variety testing network, organized at the level of agrizones with a shared contribution of registration offices, could be a way to improve variety evaluation, increase the number of traits and conditions under study with a controlled budget (Gilliland et al., 2020). In addition, molecular marker-based procedures for variety distinctness, especially for species bred as synthetic varieties, such as lucerne, could help to solve the situation in which a genetic progress is proved but variety distinction, based on morphological traits, fails (Julier et al., 2018; Gilliland et al., 2020).

AUTHOR CONTRIBUTIONS

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

FUNDING

REFERENCES

Alothman, M., Hogan, S. A., Hennessy, D., Dillon, P., Kilcawley, K. N., O'Donovan, M., et al. (2019). The "grass-fed" milk story: understanding the impact of pasture feeding on the composition and quality of bovine milk. *Foods* 8:350. doi: 10.3390/foods8080350

Andrews, M., and Hodge, S. (2010). "Climate change, a challenge for cool season grain legume crop production," in *Climate Change and Management of Cool Season Grain Legume Crops*. eds. S. Yadav and R. Redden (Dordrecht: Springer).

Annicchiarico, P., Barrett, B., Brummer, E. C., Julier, B., and Marshall, A. H. (2015a). Achievements and challenges in improving temperate perennial forage legumes. *Crit. Rev. Plant Sci.* 34, 327–380. doi: 10.1080/07352689. 2014.898462

Annicchiarico, P., Collins, R. P., De Ron, A. M., Firmat, C., Litrico, I., and Hauggaard-Nielsen, H. (2019a). Do we need specific breeding for legume-based mixtures? *Adv. Agron.* 157, 141–215. doi: 10.1016/bs.agron.2019.04.001

Annicchiarico, P., Nazzicari, N., Laouar, M., Thami-Alami, I., Romani, M., and Pecetti, L. (2020). Development and proof-of-concept application of genome-enabled selection for pea grain yield under severe terminal drought. *Int. J. Mol. Sci.* 21:2414. doi: 10.3390/ijms21072414

Annicchiarico, P., Nazzicari, N., Li, X., Wei, Y., Pecetti, L., and Brummer, E. C. (2015b). Accuracy of genomic selection for alfalfa biomass yield in different reference populations. *BMC Genomics* 16:1020. doi: 10.1186/s12864-015-2212-y

Annicchiarico, P., Nazzicari, N., Notario, T., Martin, C. M., Romani, M., Ferrari, B., et al. (2021). Pea breeding for intercropping with cereals: variation for competitive ability and associated traits, and assessment of phenotypic and genomic selection strategies. *Front. Plant Sci.* 12:731949. doi: 10.3389/fpls.2021.731949

Annicchiarico, P., Nazzicari, N., Pecetti, L., Romani, M., and Russi, L. (2019b). Pea genomic selection for Italian environments. *BMC Genomics* 20:603. doi: 10.1186/s12864-019-5920-x

Annicchiarico, P., Russi, L., Romani, M., Pecetti, L., and Nazzicari, N. (2019c). Farmer-participatory vs. conventional market-oriented breeding of inbred crops using phenotypic and genome-enabled approaches: a pea case study. *Field Crop Res.* 232, 30–39. doi: 10.1016/j.fcr.2018.11.001

Araújo, S. S., Beebe, S., Crespi, M., Delbreil, B., González, E. M., Gruber, V., et al. (2015). Abiotic stress responses in legumes: strategies used to cope with environmental challenges. *Crit. Rev. Plant Sci.* 34, 237–280. doi: 10.1080/07352689.2014.898450

Araus, J. L., Kefauver, S. C., Zaman-Allah, M., Olsen, M. S., and Cairns, J. E. (2018). Translating high-throughput phenotyping into genetic gain. *Trends Plant Sci.* 23, 451–466. doi: 10.1016/j.tplants.2018.02.001

Ares, G., Bruzzone, F., Vidal, L., Cadena, R. S., Gimenez, A., Pineau, B., et al. (2014). Evaluation of a rating-based variant of check-all-that-apply questions: rate-all-that-apply (RATA). *Food Qual. Prefer.* 36, 87–95. doi: 10.1016/j. foodqual.2014.03.006

Assefa, T., Mahama, A. A., Brown, A. V., Cannon, E. K. S., Rubyogo, J. C., Rao, I. M., et al. (2019). A review of breeding objectives, genomic resources, and marker-assisted methods in common bean (*Phaseolus vulgaris* L.). *Mol. Breed.* 39:20. doi: 10.1007/s11032-018-0920-0

Bager, S., Persson, M., and Reis, T. (2020). Reducing commodity-driven tropical deforestation: political feasibility and 'theories of change' for EU policy options. *SSRN Electron. J.* doi: 10.2139/ssrn.3624073

Bertioli, D. J., Jenkins, J., Clevenger, J., Dudchenko, O., Gao, G., Seijo, G., et al. (2019). The genome sequence of segmental allotetraploid peanut *Arachis hypogaea*. *Nat. Genet.* 51, 877–884. doi: 10.1038/s41588-019-0405-z

Biazzi, E., Nazzicari, N., Pecetti, L., Brummer, E. C., Palmonari, A., Tava, A., et al. (2017). Genome-wide association mapping and genomic selection for alfalfa (*Medicago sativa*) forage quality traits. *PLoS One* 12:e0169234. doi: 10.1371/journal.pone.0169234

Blair, M. W. (2013). "Breeding approaches to increasing nutrient-use efficiency: examples from common bean," in *Improving Water and Nutrient-Use Efficiency in Food Production Systems*. ed. Z. Rengel (Wiley Online Library), 161–175.

Carpenter, M. A., Goulden, D. S., Woods, C. J., Thomson, S. J., Kenel, F., Frew, T. J., et al. (2018). Genomic selection for ascochyta blight resistance in pea. *Front. Plant Sci.* 9:1878. doi: 10.3389/fpls.2018.01878

Carneiro, A. K., Bruzi, A. T., Pereira, J. L., and Zambiazzi, E. V. (2019). Stability analysis of pure lines and a multiline of soybean in different locations. *Crop Breed. Appl. Biotechnol.* 19, 395–401. doi: 10.1590/1984-70332019v1 9n4a56

Cazenave, A. B., Shah, K., Trammell, T., Komp, M., Hoffman, J., Motes, C. M., et al. (2019). High-throughput approaches for phenotyping alfalfa germplasm under abiotic stress in the field. *Plant Phenome J.* 2:190005. doi: 10.2135/ tppj2019.03.0005

Chen, H., Zeng, Y., Yang, Y., Huang, L., Tang, B., Zhang, H., et al. (2020). Allele-aware chromosome-level genome assembly and efficient transgene-free genome editing for the autotetraploid cultivated alfalfa. *Nat. Commun.* 11:2494. doi: 10.1038/s41467-020-16338-x

Chu, J. S. C., Peng, B., Tang, K., Yi, X., Zhou, H., Wang, H., et al. (2021). Eight soybean reference genome resources from varying latitudes and agronomic traits. *Sci. Data* 8:164. doi: 10.1038/s41597-021-00947-2

Costanzo, A., and Bickler, C. (2019). Proposal for a Toolbox for Identification and Description of Organic Heterogeneous Material. Liveseed D2.8. Available at: https://www.liveseed.eu/wp-content/uploads/2020/01/LIVESEED_D2.8_ heterogeneous_material_toolbox.pdf (Accessed October 25, 2021).

Court of Justice of the European Union (2018). Organisms Obtained by Mutagenesis are GMOs and are, in Principle, Subject to the Obligations Laid Down by the GMO Directive. PRESS RELEASE No 111/18, Luxembourg, 25 July 2018, Judgment in Case C-528/16. Available at: https://curia.europa. eu/jcms/upload/docs/application/pdf/2018-07/cp180111en.pdf (Accessed October 25, 2021).

Coyne, C. J., Kumar, S., Von Wettberg, E. J. B., Marques, E., Berger, J. D., Redden, R. J., et al. (2020). Potential and limits of exploitation of crop wild relatives for pea, lentil, and chickpea improvement. *Legume Sci.* 2:e36. doi: 10.1002/leg3.36

CPVO (2021). Community Plant Variety Office. Available at: http://cpvoextranet. cpvo.europa.eu (Accessed August 16, 2021).

Cui, J., Lu, Z., Wang, T., Chen, G., Mostafa, S., Ren, H., et al. (2021). The genome of *Medicago polymorpha* provides insights into its edibility and nutritional value as a vegetable and forage legume. *Hortic. Res.* 8:47. doi: 10.1038/s41438-021-00483-5

Cusworth, G., Garnett, T., and Lorimer, J. (2021). Legume dreams: the contested futures of sustainable plant-based food systems in Europe. *Glob. Environ. Chang.* 69:102321. doi: 10.1016/j.gloenvcha.2021.102321

De Vega, J. J., Ayling, S., Hegarty, M., Kudrna, D., Goicoechea, J. L., Ergon, Å., et al. (2015). Red clover (*Trifolium pratense* L.) draft genome provides a platform for trait improvement. *Sci. Rep.* 5:17394. doi: 10.1038/ srep17394

Duc, G., Agrama, H., Bao, S., Berger, J., Bourion, V., De Ron, A. M., et al. (2015). Breeding annual grain legumes for sustainable agriculture: new methods to approach more complex traits and target new cultivar ideotypes. *Crit. Rev. Plant Sci.* 34, 381–411. doi: 10.1080/07352689.2014.898469

Durand, J. L., Bernard, F., Lardy, R., and Graux, A. I. (2010). "Climate change and grassland: the main impacts," in *The Green Book of the CLIMATOR Project 2007–2010: Climate Change, Agriculture and Forests in France: Simulations of the Impacts on the Main Species, Chapter C4 The Crops, Grasslands*. eds. N. Brisson and F. Levrault (Angers: ADEME Editions), 181–190.

Dwivedi, S. L., Ceccarelli, S., Blair, M. W., Upadhyaya, H. D., Are, A. K., and Ortiz, R. (2016). Landrace germplasm for improving yield and abiotic stress adaptation. *Trends Plant Sci.* 21, 31–42. doi: 10.1016/j.tplants.2015. 10.012

EC (2018). Report from the Commission to the Council and the European Parliament on the Development of Plant Proteins in the European Union. Available at: https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CEL EX:52018DC0757&from=EN (Accessed October 25, 2021).

Emmrich, P., Sarkar, A., Njaci, I., Kaithakottil, G. G., Ellis, N., Moore, C., et al. (2020). A draft genome of grass pea (*Lathyrus sativus*), a resilient diploid legume. bioRxiv [Preprint]. doi: 10.1101/2020.04.24.058164

Ergon, Å., Seddaiu, G., Korhonen, P., Virkajärvi, P., Bellocchi, G., Jørgensen, M., et al. (2018). How can forage production in Nordic and Mediterranean Europe adapt to the challenges and opportunities arising from climate change? *Eur. J. Agron.* 92, 97–106. doi: 10.1016/j.eja.2017.09.016

EU (2018). Regulation (EU) 2018/848 of the European Parliament and of the Council of 30 May 2018 on Organic Production and Labelling of Organic

Products and Repealing Council Regulation (EC) No 834/2007. Official Journal of the European Union. Available at: https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32018R0848&from=EN (Accessed October 25, 2021).

EU (2021). Commission Delegated Regulation (EU) of 7.5.2021 SUPPLEMENTING REGULATION (EU) 2018/848 of the European Parliament and of the Council as Regards the Production and Marketing of Plant Reproductive Material of Organic Heterogeneous Material of Particular Genera or Species. Available at: https://op.europa.eu/fr/publication-detail/-/publication/e66cb78f-af24-11eb-9767-01aa75ed71a1/language-en (Accessed October 25, 2021).

EURISCO (2021). European Search Catalogue for Plant Genetic Resources. Available at: https://eurisco.ipk-gatersleben.de/ (Accessed August 16, 2021).

Foyer, C. H., Lam, H. M., Nguyen, H. T., Siddique, K. H. M., Varshney, R. K., Colmer, T. D., et al. (2016). Neglecting legumes has compromised human health and sustainable food production. *Nat. Plants* 2:16112. doi: 10.1038/nplants.2016.112

Gale, F., Valdes, C., and Ash, M. (2019). Interdependence of China, United States, and Brazil in Soybean Trade. USDA, Economic Research Service, 48.

GENESYS (2021). Available at: https://www.genesys-pgr.org/ (Accessed August 16, 2021).

Gilliland, T. J., Annicchiarico, P., Julier, B., and Ghesquière, M. (2020). A proposal for enhanced EU herbage VCU and DUS testing procedures. *Grass Forage Sci.* 75, 227–241. doi: 10.1111/gfs.12492

Gréard, C., Barre, P., Flajoulot, S., Santoni, S., and Julier, B. (2018). Sequence diversity of five Medicago sativa genes involved in agronomic traits to set up allele mining in breeding. *Mol. Breed.* 38:141. doi: 10.1007/s11032-018-0898-7

Griffiths, A. G., Moraga, R., Tausen, M., Gupta, V., Bilton, T. P., Campbell, M. A., et al. (2019). Breaking free: the genomics of allopolyploidy-facilitated niche expansion in white clover. *Plant Cell* 31, 1466–1487. doi: 10.1105/tpc.18.00606

Guerra-García, A., Gioia, T., von Wettberg, E., Logozzo, G., Papa, R., Bitocchi, E., et al. (2021). Intelligent characterization of lentil genetic resources: evolutionary history, genetic diversity of germplasm, and the need for well-represented collections. *Curr. Protoc.* 1:e134. doi: 10.1002/cpz1.134

Ha, J., Satyawan, D., Jeong, H., Lee, E., Cho, K. H., Kim, M. Y., et al. (2021). A near-complete genome sequence of mungbean (*Vigna radiata* L.) provides key insights into the modern breeding program. *Plant Genome* 2021:e20121. doi: 10.1002/tpg2.20121

Hellriegel, H., and Wilfarth, H. (1888). Untersuchungen über die Stickstoffnahrung der Gramineon und Leguminosen. Beil Ztscher Ver Dt ZuckInd. Available at: https://archive.org/details/untersuchungen00hell (Accessed October 25, 2021).

Hirakawa, H., Kaur, P., Shirasawa, K., Nichols, P., Nagano, S., Appels, R., et al. (2016). Draft genome sequence of subterranean clover, a reference for genus *Trifolium*. *Sci. Rep.* 6:30358. doi: 10.1038/srep30358

Iannetta, P. P. M., Hawes, C., Begg, G. S., Maaß, H., Ntatsi, G., Savvas, D., et al. (2021). A multifunctional solution for wicked problems: value-chain wide facilitation of legumes cultivated at bioregional scales is necessary to address the climate-biodiversity-nutrition nexus. *Front. Sustain. Food Syst.* 5:692137. doi: 10.3389/fsufs.2021.692137

Iqbal, M. M., Erskine, W., Berger, J. D., Udall, J. A., and Nelson, M. N. (2020). "Genomics of yellow lupin (*Lupinus luteus* L.)," in *The Lupin Genome. Compendium of Plant Genomes*. eds. K. Singh, L. Kamphuis and M. Nelson (Cham: Springer), 151–159.

Jarquín, D., Specht, J., and Lorenz, A. (2016). Prospects of genomic prediction in the USDA soybean germplasm collection: historical data creates robust models for enhancing selection of accessions. *G3 (Bethesda)* 6, 2329–2341. doi: 10.1534/g3.116.031443

Julier, B., Barre, P., Hébert, Y., Huguet, T., and Huyghe, C. (2003). Methodology of alfalfa breeding: a review of recent achievements. *Czech J. Genet. Plant Breed.* 39, 71–83.

Julier, B., Barre, P., Lambroni, P., Delaunay, S., Thomasset, M., Lafaillette, F., et al. (2018). Use of GBS markers to distinguish among lucerne varieties, with comparison to morphological traits. *Mol. Breed.* 38:133. doi: 10.1007/s11032-018-0891-1

Kamphuis, L. G., Garg, G., Foley, R., and Singh, K. B. (2021). Genomic resources for lupins are coming of age. *Legume Sci.* 3, 1–15. doi: 10.1002/leg3.77

Khazaei, H., O'Sullivan, D. M., Stoddard, F. L., Adhikari, K., Paull, J., Schulman, A. H., et al. (2021). Recent advances in faba bean genetic and genomic tools for crop improvement. *Legume Sci.* 3, 1–13. doi: 10.1002/leg3.75

Kreplak, J., Madoui, M. A., Cápal, P., Novák, P., Labadie, K., Aubert, G., et al. (2019). A reference genome for pea provides insight into legume genome evolution. *Nat. Genet.* 51, 1411–1422. doi: 10.1038/s41588-019-0480-1

Kumar, G. R., Sakthivel, K., Sundaram, R. M., Neeraja, C. N., Balachandran, S. M., Rani, N. S., et al. (2010). Allele mining in crops: prospects and potentials. *Biotechnol. Adv.* 28, 451–461. doi: 10.1016/j.biotechadv.2010.02.007

Lemaire, G., and Allirand, J. M. (1993). Relation entre croissance et qualité de la luzerne: interaction génotypemode d'exploitation. *Fourrages* 134, 183–198.

Li, B., Zhang, N., Wang, Y. G., George, A. W., Reverter, A., and Li, Y. (2018). Genomic prediction of breeding values using a subset of snps identified by three machine learning methods. *Front. Genet.* 9:237. doi: 10.3389/fgene.2018.00237

Li, Y., Ruperao, P., Batley, J., Edwards, D., Khan, T., Colmer, T. D., et al. (2019). Investigating drought tolerance in chickpea using genome-wide association mapping and genomic selection based on whole-genome resequencing. *Front. Plant Sci.* 19:3389. doi: 10.3389/fpls.2018.00190

Liem, D. G., and Russell, C. G. (2019). The influence of taste liking on the consumption of nutrient rich and nutrient poor foods. *Front. Nutr.* 6:174. doi: 10.3389/fnut.2019.00174

Lonardi, S., Muñoz-Amatriaín, M., Liang, Q., Shu, S., Wanamaker, S. I., Lo, S., et al. (2019). The genome of cowpea (*Vigna unguiculata* [L.] Walp.). *Plant J.* 98, 767–782. doi: 10.1111/tpj.14349

Maamouri, A., Louarn, G., Béguier, V., and Julier, B. (2017). Performance of lucerne genotypes for biomass production and nitrogen content differs in monoculture and in mixture with grasses and is partly predicted from traits recorded on isolated plants. *Crop Pasture Sci.* 68, 942–951. doi: 10.1071/CP17052

Magrini, M. B., Anton, M., Choleza, C., Corre-Hellou, G., Duc, G., Jeuffroy, M. H., et al. (2016). Why are grain-legumes rarely present in cropping systems despite their environmental and nutritional benefits? Analyzing lock-in in the French agrifood system. *Ecol. Econ.* 126, 152–162. doi: 10.1016/j.ecolecon.2016.03.024

Martin, G., Durand, J. L., Duru, M., Gastal, F., Julier, B., Litrico, I., et al. (2020). Role of ley pastures in tomorrow's cropping systems. A review. *Agron. Sustain. Dev.* 40:17. doi: 10.1007/s13593-020-00620-9

Martin-Guay, M. O., Paquette, A., Dupras, J., and Rivest, D. (2018). The new green revolution: sustainable intensification of agriculture by intercropping. *Sci. Total Environ.* 615, 767–772. doi: 10.1016/j.scitotenv.2017.10.024

Matei, G., Woyann, L. G., Milioli, A. S., Bem Oliveira, I., Zdziarski, A. D., Zanella, R., et al. (2018). Genomic selection in soybean: accuracy and time gain in relation to phenotypic selection. *Mol. Breed.* 38:117. doi: 10.1007/s11032-018-0872-4

McDermott, J., and Wyatt, A. J. (2017). The role of pulses in sustainable and healthy food systems. *Ann. N. Y. Acad. Sci.* 1392, 30–42. doi: 10.1111/nyas.13319

Mecha, E., Erny, G. L., Guerreiro, A. C. L., Feliciano, R. P., Barbosa, I., Bento da Silva, A., et al. (2022). Metabolomics profile responses to changing environments in a common bean (*Phaseolus vulgaris* L.) germplasm collection. *Food Chem.* 370:131003. doi: 10.1016/j.foodchem.2021.131003

Mecha, E., Figueira, M. E., Vaz Patto, M. C., and Bronze, M. R. (2019). "Two sides of the same coin: the impact of grain legumes on human health—common bean (*Phaseolus vulgaris* L.) as a case study," in *Legume Seed Nutraceutical Research*. eds. J. Jiménez-López and C. Clemente (InTechOpen).

Mecha, E., Natalello, S., Carbas, B., Bento da Silva, A., Leitão, S. T., Brites, C., et al. (2021). Disclosing the nutritional quality diversity of Portuguese common beans—the missing link for their effective use on protein quality breeding programmes. *Agronomy* 11:221. doi: 10.3390/agronomy11020221

Mueller-Harvey, I., Bee, G., Dohme-Meier, F., Hoste, H., Karonen, M., Kolliker, R., et al. (2019). Benefits of condensed tannins in forage legumes fed to ruminants: importance of structure, concentration, and diet composition. *Crop Sci.* 59, 861–885. doi: 10.2135/cropsci2017.06.0369

Mun, T., Bachmann, A., Gupta, V., Stougaard, J., and Andersen, S. U. (2016). Lotus base: an integrated information portal for the model legume *Lotus japonicus*. *Sci. Rep.* 6:39447. doi: 10.1038/srep39447

Pandey, A. K., Rubiales, D., Wang, Y., Fang, P., Sun, T., Liu, N., et al. (2021). Omics resources and omics-enabled approaches for achieving high productivity and improved quality in pea (*Pisum sativum* L.). *Theor. Appl. Genet.* 134, 755–776. doi: 10.1007/s00122-020-03751-5

Pecetti, L., Carroni, A. M., and Annicchiarico, P. (2020). Performance and adaptability of subterranean clover pure lines and line mixtures of different complexity across contrasting Mediterranean environments. *Field Crop Res.* 256:107907. doi: 10.1016/j.fcr.2020.107907

Pecrix, Y., Staton, S. E., Sallet, E., Lelandais-Brière, C., Moreau, S., Carrère, S., et al. (2018). Whole-genome landscape of *Medicago truncatula* symbiotic genes. *Nat. Plants* 4, 1017–1025. doi: 10.1038/s41477-018-0286-7

Pégard, M., Leuenberger, J., Julier, B., and Barre, P. (2021). Genomic prediction of lucerne forage yield and quality. Eucarpia Congress, Fodder Plants and Amenity Grasses Section, 3–5 September 2021, (in press).

Pilorgé, E., and Muel, F. (2016). What vegetable oils and proteins for 2030? Would the protein fraction be the future of oil and protein crops? *OCL* 23:D402. doi: 10.1051/ocl/2016030

Polade, S. D., Gershunov, A., Cayan, D. R., Dettinger, M. D., and Pierce, D. W. (2017). Precipitation in a warming world: assessing projected hydro-climate changes in California and other Mediterranean climate regions. *Sci. Rep.* 7:10783. doi: 10.1038/s41598-017-11285-y

Pratap, A., Das, A., Kumar, S., and Gupta, S. (2021). Current perspectives on introgression breeding in food legumes. *Front. Plant Sci.* 11:589189. doi: 10.3389/fpls.2020.589189

Roland, W. S. U., Pouvreau, L., Curran, J., van de Velde, F., and de Kok, P. M. T. (2017). Flavor aspects of pulse ingredients. *Cereal Chem. J.* 94, 58–65. doi: 10.1094/CCHEM-06-16-0161-FI

Roorkiwal, M., Bharadwaj, C., Barmukh, R., Girish, P. D., Thudi, M., Gaur, P. M., et al. (2020). Integrating genomics for chickpea improvement: achievements and opportunities. *Theor. Appl. Genet.* 133, 1703–1720. doi: 10.1007/s00122-020-03584-2

Rubiales, D. (2020). Broomrape threat to agriculture. *Outlooks Pest Manag.* 31, 141–145. doi: 10.1564/v31_jun_12

Rubiales, D., Fondevilla, S., Chen, W., Gentzbittel, L., Higgins, T. J. V., Castillejo, M. A., et al. (2015). Achievements and challenges in legume breeding for pest and disease resistance. *Crit. Rev. Plant Sci.* 34, 195–236. doi: 10.1080/07352689.2014.898445

Rubiales, D., Moral, A., and Flores, F. (2021). Heat waves and broomrape are the major constraints for lentil cultivation in southern Spain. *Agronomy* 11:1871. doi: 10.3390/agronomy11091871

Santos, C., Almeida, N. F., Alves, M. L., Horres, R., Krezdorn, N., Leitao, S. T., et al. (2018). First genetic linkage map of *Lathyrus cicera* based on RNA sequencing-derived markers: key tool for genetic mapping of disease resistance. *Hortic. Res.* 5:45. doi: 10.1038/s41438-018-0047-9

Scheelbeek, P. F. D., Birda, F. A., Tuomistob, H. L., Greena, R., Harrisa, F. B., Joya, E. J. M., et al. (2018). Effect of environmental changes on vegetable and legume yields and nutritional quality. *Proc. Natl. Acad. Sci. U. S. A.* 115, 6804–6809. doi: 10.1073/pnas.1800442115

Searchinger, T., Waite, R., Hanson, C., Ranganathan, J., Dumas, P., Matthews, E., et al. (2019). Creating a Sustainable Food Future: A Menu of Solutions to Feed Nearly 10 Billion People by 2050. Final Report. Washington, DC: World Resources Institute.

Sharma, H. C., Srivastava, C. P., Durairaj, C., and Gowda, C. L. L. (2020). "Pest management in grain legumes and climate change," in *Climate Change and Management of Cool Season Grain Legume Crops.* eds. S. S. Yadav and R. Redden (Springer), 115–139.

Shen, S., Chai, X., Zhou, Q., Luo, D., Wang, Y., and Liu, Z. (2019). Development of polymorphic EST-SSR markers and characterization of the autotetraploid genome of sainfoin (*Onobrychis viciifolia*). *PeerJ* 7:e6542. doi: 10.7717/peerj.6542

Sivasankar, S., Ellis, N., Buruchara, R., Henry, C., Rubiales, D., Singh, J., et al. (2016). 10-year Research Strategy for Pulse Crops. Available at: https://pulses.org/future-of-food/10-year-research-strategy (Accessed October 25, 2021).

Skendžić, S., Zovko, M., Živković, I. P., Lešić, V., and Lemić, D. (2021). The impact of climate change on agricultural insect pests. *Insects* 12:440. doi: 10.3390/insects12050440

Smýkal, P., Coyne, C. J., Ambrose, M. J., Maxted, N., Schaefer, H., Blair, M. W., et al. (2015). Legume crops phylogeny and genetic diversity for science and breeding. *Crit. Rev. Plant Sci.* 34, 43–104. doi: 10.1080/07352689.2014.897904

Sreeharsha, R. V., Mudalkar, S., Unnikrishnan, D. K., Venkata Mohan, S., and Reddy, A. R. (2021). "Improving nitrogen use efficiency of legumes under changing climate through omics technologies," in *Omics Technologies for Sustainable Agriculture and Global Food Security. Vol. 1.* eds. A. Kumar, R. Kumar, P. Shukla and M. K. Pandey (Singapore: Springer).

Stewart-Brown, B. B., Song, Q., Vaughn, J. N., and Li, Z. (2019). Genomic selection for yield and seed composition traits within an applied soybean breeding program. *G3* 9, 2253–2265. doi: 10.1534/g3.118.200917

Swagemakers, P., Garcia, M. D. D., Onofa Torres, A., Oostindie, H., and Groot, J. C. J. (2017). A values-based approach to exploring synergies between livestock farming and landscape conservation in Galicia (Spain). *Sustainability* 9:1987. doi: 10.3390/su9111987

Varshney, R. H., Thudi, M., Pandey, M. K., Tardieu, F., Ojiewo, C., Vadez, V., et al. (2018). Accelerating genetic gains in legumes for the development of prosperous smallholder agriculture: integrating genomics, phenotyping, systems modelling and agronomy. *J. Exp. Bot.* 69, 3293–3312. doi: 10.1093/jxb/ery088

Vaz Patto, M. C., Amarowicz, R., Aryee, A., Boye, J., Chung, H. J., Martín-Cabrejas, M. A., et al. (2015). Achievements and challenges in improving the nutritional quality of food legumes. *Crit. Rev. Plant Sci.* 34, 105–143. doi: 10.1080/07352689.2014.897907

Watson, C. A., Reckling, M., Preissel, S., Bachinger, J., Bergkvist, G., Kuhlman, T., et al. (2017). Grain legume production and use in European agricultural systems. *Adv. Agron.* 144, 235–303. doi: 10.1016/bs.agron.2017.03.003

Westhoek, H. J., Rood, G. A., van den Berg, M., Janse, J. H., Nijdam, D. S., Reudink, M. A., et al. (2011). The protein puzzle: the consumption and production of meat, dairy and fish in the European Union. *Europ. J. Nutr. Food Saf.* 1, 123–144.

Willett, W., Rockström, J., Loken, B., Springmann, M., Lang, T., Vermeulen, S., et al. (2019). Food in the anthropocene: the EAT–lancet commission on healthy diets from sustainable food systems. *Lancet* 393, 447–492. doi: 10.1016/S0140-6736(18)31788-4

Xanthopoulou, A., Kissoudis, C., Mellidou, I., Manioudaki, M., Bosmali, I., Tsakonitis, V., et al. (2019). Expanding *Phaseolus coccineus* genomic resources: de novo transcriptome assembly and analysis of landraces 'gigantes' and 'elephantes' reveals rich functional variation. *Biochem. Genet.* 57, 747–766. doi: 10.1007/s10528-019-09920-6

Zhu, Y., Liu, Q., Xu, W., Zhang, J., Wang, X., Nie, G., et al. (2019). De novo assembly and discovery of genes that involved in drought tolerance in the common vetch. *Int. J. Mol. Sci.* 20:328. doi: 10.3390/ijms20020328

Check for updates

Grand Challenges in Sustainable and Intelligent Phytoprotection

Kai Huang[1,2] and Lei Shu[2,3*]

[1] National Engineering and Technology Center for Information Agriculture, Nanjing, China, [2] Nanjing Agricultural University, Nanjing, China, [3] School of Engineering, University of Lincoln, Lincoln, United Kingdom

Plant enhances the capacity of the ecosystem and agricultural systems, enabling human beings to survive and multiply on the earth. Since the human being appeared, plants and their production have played a pivotal role in the development of human civilization, which is mainly reflected in four aspects, including ecological value, edible and medicinal value, ornamental value, and cultural value. In the growth process of both aquatic and terrestrial plants, we need to consider plant protection from the four following levels. 1) at the level of maintaining ecological diversity: we need to explore soil improvement and protection, ecological and supply-demand balance of precise fertilization, meteorological prediction and warning and prediction of plant pest and disease epidemic patterns, etc.; 2) at the level of agricultural production: scientific breeding and seed selection, plant phenotyping, food safety and traceability, intelligent diagnosis and control of plant diseases and pests, intelligent weeding, automatic harvesting, quality grading and division, water and fertilizer integration, quality monitoring, growth monitoring, plant nutrition maintenance, ecology, soil, fertilizer and crop resources and nutrient balance, etc.; 3) at the level of plant high standardized farmland construction: explore smart orchard, smart tea garden, smart field, smart facility farmland, etc., explore recycling agriculture, Photovoltaic Agriculture (Huang et al., 2020), green and organic agriculture; 4) at the level of sustainable development: explore low carbon models, light-weight computing, small sample models, low-cost and high-reliability equipment, open database for sharing and crowdfunding, etc. In the field of plant protection, we should not merely consider one of the above levels, but consider all, that is, from the perspective of ecosystem, landscape, and economics, which is also a grand challenge for the development of plant protection.

As a new interdisciplinary subject, Smart Phytoprotection emerges under the background of the continuous development of science and technology and the in-depth transfer from computer science to plant science. Traditional plant protection discipline mainly focuses on biological sciences which refer to other disciplines including chemistry, biology, and ecology. Then researches have been toward to reduce pesticide pollution, protect the environment, and realize ecological sustainable development.

At present, the fast development toward various information and communication technologies, e.g., Internet of Things (Chen et al., 2020), Satellite Remote Sensing (Zhang et al., 2021), Aerial Image Processing (Su et al., 2021), Big Data (Wolfert et al., 2017), Cloud Computing (Gai et al., 2020), Artificial Intelligence (Jiang et al., 2020), 5G technology (Tang et al., 2021), Blockchain (Zhou et al., 2020; Liu et al., 2021), Quantum communication (Gisin and Thew, 2007), and Robotics (Karoly et al., 2020), has provided new opportunities for agriculture applications (Friha et al., 2021), further promoting the integration of both computer science and plant science and leading to realizing "Smart Phytoprotection" with novel research ideas and solutions involved in plant protection and development, which refers to green, smart plant protection, and evolving technology. However, various challenges from different fields need to be addressed to push the boundaries of developments in Smart Phytoprotection. Specifically, due to the lack of

in-depth integration between sensors, information and communication technology (ICT), and plant protection technology, some grand challenges are becoming increasingly arduous, resulting in several key research themes.

1. RESEARCH FIELD OF SUSTAINABLE AND INTELLIGENT PEST IDENTIFICATION AND CONTROL

Plant diseases and pests pose a great threat to agriculture production, which can directly or indirectly lead to a drop of crop yield and even crop failure. It is critical to monitoring the state of plant diseases and pests efficiently and accurately, and to preventing and controlling them in time. In the agricultural field, if the identification of plant diseases, pests and weeds is carried out manually, we will face the following four main challenges:

1) There are fewer experienced farmers, and it is still difficult for new farmers to screen disease species in the early stage of the disease, or even during the outbreak;
2) Wrong or delaying diagnosis during the grow control period is the fundamental cause of large yield reduction even extinction;
3) Existing methods of pest, diseases and weed control mostly focus on chemical control, which causes large areas of soil and water pollution and is not conducive to sustainable and low-carbon development, not to mention carbon neutrality in the future. More researches should focus on physical and biological control methods;
4) Plant growers face hazards in the field and suffer from occupational diseases (e.g., lumbar muscle strain, skin disease, arthritis) caused by long-term labor work.

Similar challenges also exist while judging whether an insect is a pest or a beneficial insect, and judging what kind of a weed it is. At present, in order to effectively cope with the grand challenges, we suggest the research on the following five aspects:

1) Based on Internet of Things, Image Processing, and Knowledge Graph technology (Liu et al., 2016), we can carry out researches on intelligent diagnosis and control methods for pests, diseases and weeds of various crops. Establish intelligent diagnosis system from single disease to multi-disease and from single crop to multi-crop;
2) Based on Internet of Things, Artificial Intelligence, Satellite Remote Sensing, Aerial Image Processing, Big Data, and other technologies, we can launch the researches on intelligent prevention and control of crop, forest, and grass plant diseases, such as locust monitoring based on remote sensing;
3) Based on Internet of Things, Radar Detection Technology (Hu et al., 2016), and Drone Technology, migratory pests are monitored, prevented and controlled intelligently. For example, the use of Solar Insecticidal Lamps Internet of Things (Li et al., 2019) and radar monitoring network for insect migration;
4) Based on process-based models and statistical models, we can launch the researches on assessing the best

management practices to control pests and crop diseases, and forecasting the impact of global climate change on future pest management;
5) Based on Internet of Things and Artificial Intelligence, better resources (e.g., water, pesticides) management tools and decision support system could be supplied to planters to ensure the implementation of the necessary changes (e.g., smart farming technologies).

2. RESEARCH FIELD OF GREEN INTELLIGENT BIOLOGICAL CONTROL TECHNOLOGY

In the process of biological control, it is necessary to consider the impact of various factors, e.g., environmental conditions, host plants, host pests, application methods, product batches, on natural enemies of pests and pathogenic microorganisms. It is of great importance to confirm the effect of biological control through large-area monitoring. However, the traditional manual method is time-consuming and laborious to know the whole control effect accurately, which is not beneficial for the development of biological control technology, and for the establishment of evaluation standards and systems about biological control effect.

In order to effectively cope with the challenges of green intelligent biological control, we suggest the researches on the following two aspects:

1) Based on Internet of Things, Artificial Intelligence, Satellite Remote Sensing, Aerial Image Processing, Big Data, Knowledge Graph, and other technologies, we can carry out the researches on the epidemic law, and comprehensive prevention and control for plant diseases and pests, weeds, on the treatment of degraded grassland, and on the integrated soil and water management. For example, the pest monitoring network can help cultivators anticipate problems in advance and take the initiative to solve them (Wohleb et al., 2021);
2) Based on Internet of Things, Aerial Image Processing, Remote Sensing Satellites, Big Data, and other technologies, large-scale alien species invasion can be identified, monitored, prevented, and controlled, for example, the prevention and control of buffelgrass spreading in the United States (Elkind et al., 2019).

3. RESEARCH FIELD OF GREEN INTELLIGENT ECOLOGICAL CONTROL TECHNOLOGY

By intelligently adjusting the planting environment parameters, such as water, light, temperature, CO_2, fertilizer, etc., we can create a suitable growing environment for crops, further improving the disease resistance of crops. Moreover, it is helpful to select high-quality pest-resistant varieties, improve crop planting structure, protect beneficial organisms, and control the occurrence rate of plant diseases and pests through regulating biodiversity. Therefore, it is of great significance to carry out researches on green intelligent ecological regulation

technology. How to realize such green intelligent ecological control technology is a grand challenge in this research field.

At present, in order to effectively cope with the above challenges of green intelligent ecological control, we suggest the researches on the following six aspects:

1) Based on the Internet of Things, Artificial Intelligence, Satellite Remote Sensing, Aerial Image Processing, Big Data, Drones, etc., we can carry out researches on intelligent water and fertilizer irrigation integration;
2) Based on the Internet of Things, Artificial Intelligence, Intelligent Equipment, Big Data, etc., we can carry out researches on coordinating the power generation of Photovoltaic Agriculture and crop growth, intelligent control of environmental temperature, humidity, light and ventilation of facility agriculture, etc., meanwhile, focus on intelligent greenhouse of the Internet of Things;
3) Based on the Internet of Things, Artificial Intelligence, Photovoltaic Agriculture, etc., we can carry out researches on combining with light-transmitting film, multi-spectrum, automatic adjustment of light duration and CO_2 concentration, etc., and also combining with plant nutrition, concentrate on precision organic planting;
4) Based on Internet of Things, Crowd Sensing, Aerial Image Processing, Big Data, we could carry out researches on protecting beneficial insects. For instance, prevention and control of large-scale bees' death, beekeepers' perception on environmental changes, and even pollution in bees' activity areas;
5) Based on Internet of Things, Artificial Intelligence, Satellite Remote Sensing, Aerial Image Processing, Big Data, we could carry out researches on biodiversity control for plant diseases, weeds, and pests, and on the pest prediction models by monitoring, investigation and research of pests. Then, make regulatory decisions to give full play to the natural regulatory advantages of biodiversity;
6) Based on advanced sensing, Machine Learning, and Big Data, we could use eco-evolutionary models to learn from both epidemic and genome data for deployment of disease-resistant cultivars and fungicides in space and time to both control plant diseases and impede their adaptation.

4. RESEARCH FIELD OF SCIENTIFIC BREEDING AND QUALITY CONTROL TECHNOLOGY

Food safety is one of the most important evaluation indicators in plant protection. Due to the wide variety of varieties, it is a lack of intelligent variety recommendation to select high quality pest and disease resistant varieties. In terms of quality control technology for the whole planting process, it requires the active guarantee of each link to make the crops in food safety to be visualized and traced. Thus, it is necessary to conduct researches on the application technology of agricultural product traceability based

on the Internet of Things, Artificial Intelligence, Big Data, and other technologies.

At present, in order to effectively cope with the grand challenges, we can launch the researches on the following three aspects:

1) Based on the Internet of Things, Artificial Intelligence, Satellite Remote Sensing, Aerial Image Processing, Big data, and Blockchain, we can carry out researches on variety quality resource evaluation and variety selection and breeding recommendation;
2) Based on the Internet of Things, Image Processing, Satellite Remote Sensing, Big Data, and Intelligent Equipment, we can carry out researches on crop quality grading and division techniques;
3) Based on the Internet of Things, Artificial Intelligence, GPS, Big Data, and Blockchain, we can carry out researches on agricultural product traceability and tracking control.

5. RESEARCH FIELD OF GREEN SMART AND SCIENTIFIC PESTICIDE APPLICATION TECHNOLOGY

In the scientific pesticide application technology, researchers have continuously developed high-efficiency, low-toxicity, low-residue, and environment-friendly pesticides for plant diseases and pests (Ferentinos, 2018). How to improve the pesticide application level from the perspective of both technology and equipment, and to achieve intelligent, accurate, low-dosage and high-efficiency pesticide application effect, are two grand challenges for further popularization and application of the above pesticides. Thus, it is necessary to carry out scientific pesticide application technology based on Internet of Things, Artificial Intelligence, Big Data, and other technologies, e.g., intelligent spraying pesticides by unmanned aerial vehicles (Chen et al., 2021) and selective spraying with ground vehicles.

6. RESEARCH FIELD OF INTELLIGENT WEATHER DISASTER PREVENTION TECHNOLOGY

Through analysis of current meteorological conditions, we can better adjust crops planting and management patterns. By predicting and controlling meteorological disasters, we can largely protect crops from damage and reduce crops losses. Therefore, it is of great significance to research intelligent and green technologies for preventing meteorological disasters. How to realize such technology is a grand challenge in this field. At present, in order to effectively deal with the above challenges, we can conduct researches from the following four aspects:

1) Based on the Internet of Things, Artificial Intelligence, Satellite Remote Sensing, Aerial Image Processing, Meteorological Sensors, and Blockchain, we can carry out crops' meteorological disaster analysis and early warning model research;

FIGURE 1 | The era of unmanned smart plant protection.
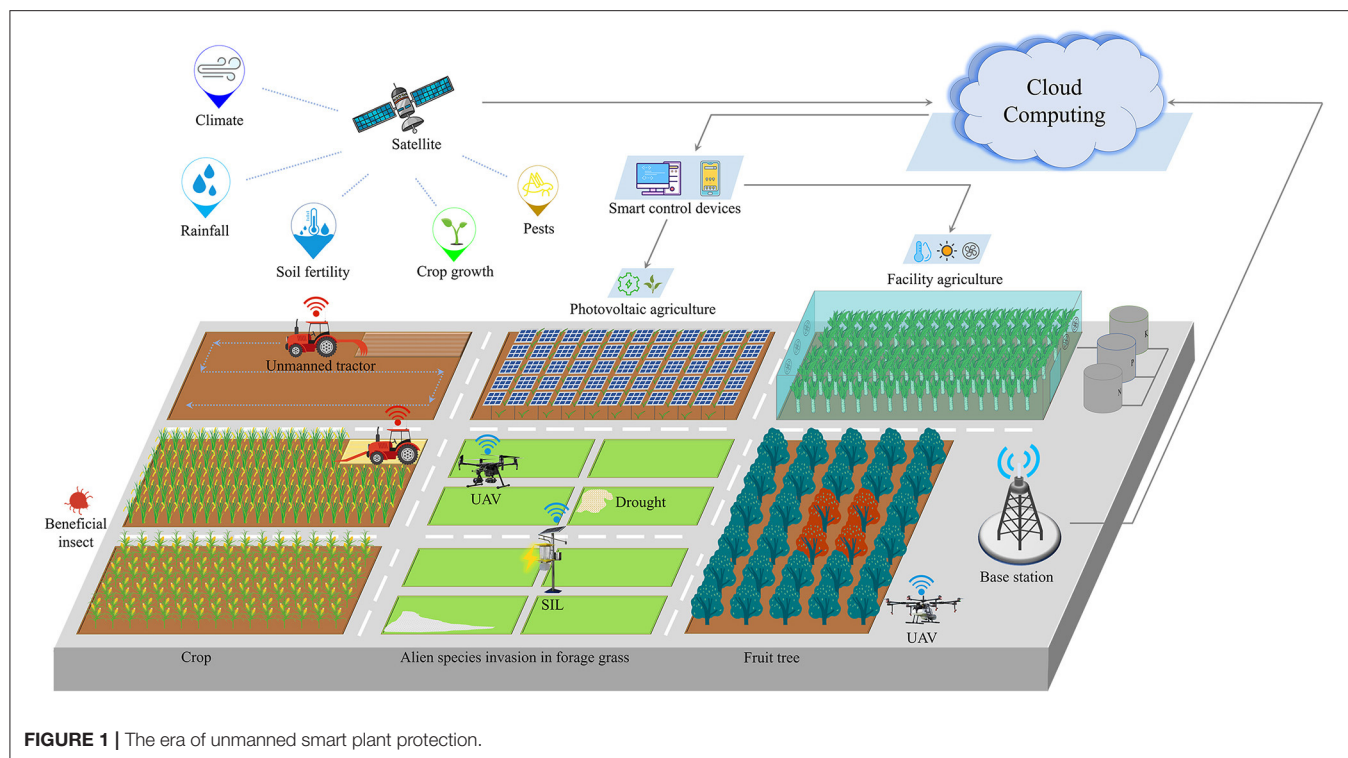
2) Based on the Internet of Things, Vision technology, and Machine Learning technologies, we can conduct researches on weather inspection robots. For instance, prevent and control frost disasters in tea gardens and meteorological pollution in tea gardens through moving inspections;

3) Based on the emerging LoRaWAN and other technologies, we can expand the wireless communication range of weather stations so that the agriculture and forestry environment can obtain the newest weather data in time and make weather disaster prevention work;

4) Based on Big Data Analysis, Statistics, Operations Research, and Mathematical Modeling, we can realize the estimation of the early warning and optimal response mechanism for meteorological disasters.

In addition, while emerging cutting-edge agricultural production patterns appear, it is worth to carry out studies on new prevention and control, and protection technologies. With the continuous development of ICT, it is critical to develop and apply intelligent, compound plant protection equipment which is suitable for various agricultural production scenarios. In the future, plant protection will become sustainable, intelligent, and toward the era of unmanned smart plant protection, as depicted in **Figure 1**:

1) Based on Satellite Remote Sensing and Aerial Image Processing, we can carry out researches on combining with the Solar Insecticidal Lamps Internet of Things to monitor plant diseases and pests, analyzing the prevalence of plant diseases

and pests, judging the effectiveness of control, and collecting climate data for weather disaster prevention;

2) Based on the Internet of Things, Artificial Intelligence Technology, Big Data, Scheduling Optimization, and UAV, we can carry out researches on accurately spraying pesticides on diseased fruit trees, intelligently reducing the amount of pesticide application and improving the effect of pesticide spraying. And the consumers can trace the pesticide application on fruit through the blockchain;

3) Based on the Internet of Things, Global Navigation Satellite System (GNSS), Intelligent Equipment, and UAV, we can carry out researches on guiding the unmanned tractor to work on the designated path and guiding the UAV to collect plant growth information on the preset path;

4) Based on Satellite Remote Sensing and Aerial Image Processing, we can carry out researches on evaluating forest and pasture germplasm resources, selecting breed, protecting beneficial insect, and monitoring the farmland, e.g., drought of farmland and the alien species invasion in forage grass.

In the era of unmanned smart plant protection, a high level of plant science is technologically achievable with the favor of computer science.

AUTHOR CONTRIBUTIONS

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

FUNDING

ACKNOWLEDGMENTS

REFERENCES

Chen, C.-J., Huang, Y.-Y., Li, Y.-S., Chen, Y.-C., Chang, C.-Y., and Huang, Y.-M. (2021). Identification of fruit tree pests with deep learning on embedded drone to achieve accurate pesticide spraying. *IEEE Access* 9, 21986–21997. doi: 10.1109/ACCESS.2021.3056082

Chen, W.-L., Lin, Y.-B., Ng, F.-L., Liu, C.-Y., and Lin, Y.-W. (2020). Ricetalk: Rice blast detection using internet of things and artificial intelligence technologies. *IEEE Internet Things J.* 7, 1001–1010. doi: 10.1109/JIOT.2019.2947624

Elkind, K., Sankey, T. T., Munson, S. M., and Aslan, C. E. (2019). Invasive bufflegrass detection using high-resolution satellite and uav imagery on google earth engine. *Remote Sens. Ecol. Conserv.* 5, 318–331. doi: 10.1002/rse2.116

Ferentinos, K. P. (2018). Deep learning models for plant disease detection and diagnosis. *Comput. Electron. Agric.* 145, 311–318. doi: 10.1016/j.compag.2018.01.009

Friha, O., Ferrag, M. A., Shu, L., Maglaras, L., and Wang, X. (2021). Internet of things for the future of smart agriculture: a comprehensive survey of emerging technologies. *IEEE/CAA J. Automat. Sin.* 8, 718–752. doi: 10.1109/JAS.2021.1003925

Gai, K., Guo, J., Zhu, L., and Yu, S. (2020). Blockchain meets cloud computing: a survey. *IEEE Commun. Surv. Tutorials* 22, 2009–2030. doi: 10.1109/COMST.2020.2989392

Gisin, N., and Thew, R. (2007). Quantum communication. *Nat. Photonics*. 1, 165–171. doi: 10.1038/nphoton.2007.22

Hu, G., Lim, K. S., Horvitz, N., Clark, S. J., Reynolds, D. R., Sapir, N., et al. (2016). Mass seasonal bioflows of high-flying insect migrants. *Science* 354, 1584–1587. doi: 10.1126/science.aah4379

Huang, K., Shu, L., Li, K., Yang, F., Han, G., Wang, X., et al. (2020). Photovoltaic agricultural internet of things towards realizing the next generation of smart farming. *IEEE Access* 8, 76300–76312. doi: 10.1109/ACCESS.2020.2988663

Jiang, H., Zhang, C., Qiao, Y., Zhang, Z., Zhang, W., and Song, C. (2020). Cnn feature based graph convolutional network for weed and crop recognition in smart farming. *Comput. Electron. Agric.* 174:105450. doi: 10.1016/j.compag.2020.105450

Karoly, A. I., Galambos, P., Kuti, J., and Rudas, I. J. (2020). Deep learning in robotics: Survey on model structures and training strategies. *IEEE Trans. Syst. Man Cybern. Syst.* 51, 266–279. doi: 10.1109/TSMC.2020.3018325

Li, K., Shu, L., Huang, K., Sun, Y., Yang, F., Zhang, Y., et al. (2019). Research and prospect of solar insecticidal lamps internet of things. *Smart Agric.* 1, 13–28.

Liu, H., Cao, Y., Chen, Y., Jing, F., and Fang, W. (2021). Crop germplasm resources registration blockchain model. *J. Plant Genet. Resour.* 22, 28–37.

Liu, Q., Li, Y., Duan, H., Liu, Y., and Qin, Z. (2016). Knowledge graph construction techniques. *J. Comput. Res. Dev.* 53, 582–600.

Su, J., Yi, D., Su, B., Mi, Z., Liu, C., Hu, X., et al. (2021). Aerial visual perception in smart farming: Field study of wheat yellow rust monitoring. *IEEE Trans. Ind. Inform.* 17, 2242–2249. doi: 10.1109/TII.2020.2979237

Tang, Y., Dananjayan, S., Hou, C., Guo, Q., Luo, S., and He, Y. (2021). A survey on the 5g network and its impact on agriculture: challenges and opportunities. *Comput. Electron. Agric.* 180:105895. doi: 10.1016/j.compag.2020.105895

Wohleb, C. H., Waters, T. D., and Crowder, D. W. (2021). Decision support for potato growers using a pest monitoring network. *Am. J. Potato Res.* 98, 5–11. doi: 10.1007/s12230-020-09813-0

Wolfert, S., Ge, L., Verdouw, C., and Bogaardt, M.-J. (2017). Big data in smart farming - a review. *Agric. Syst.* 153, 69–80. doi: 10.1016/j.agsy.2017.01.023

Zhang, N., Yang, G., Zhao, C., Zhang, J., Yang, X., Pan, Y., et al. (2021). Progress and prospects of hyperspectral remote sensing technology for crop diseases and pests. *Natl. Remote Sens. Bull.* 25, 403–422.

Zhou, Q., Huang, H., Zheng, Z., and Bian, J. (2020). Solutions to scalability of blockchain: a survey. *IEEE Access* 8, 16440–16455. doi: 10.1109/ACCESS.2020.2967218

Check for updates

A Grand Challenge in Development and Evodevo: Quantifying the Role of Development in Evolution

Aaron R. Leichty* and Neelima Roy Sinha

Department of Plant Biology, University of California, Davis, Davis, CA, United States

The identification and characterization of genes affecting development and their integration into regulatory networks has been the dominant endeavor over the past 30 years of plant development. The field of evolutionary developmental biology has been greatly aided by these findings, promoting a vast array of comparative work examining how these developmental pathways function in diverse species. However, evodevo as a discipline is not just concerned with how development evolves, but also how development contributes to the process of evolution. Historically, this avenue of study has taken on two major forms—either a focus on how developmental systems constrain or channel evolution in certain directions and not others (Alberch, 1982; Maynard Smith et al., 1985) or how development can promote or lead during evolution, with a particular focus on developmental plasticity and the role of the environment in the production of phenotypic variation (West-Eberhard, 2003). In our view, a grand challenge going forward is for the broader inclusion of these latter questions into the comparative framework that has already been so successfully applied across major morphological transitions of the plant phylogeny. Central to this inclusion will be the focus on well-characterized genetic regulatory networks (GRN) and the evaluation of the phenotypic variation they are capable of producing using ecologically and phylogenetically relevant genetic and environmental manipulations. Together, this focus on the range of phenotypic variation that can be generated and the fitness consequences of such variation will help in quantifying the role of development in generating evolutionary change.

The fact that an organism's developmental systems are incapable of generating phenotypic variation equally in all directions, and therefore impose directionality on the trajectory of evolution, is by now well-accepted (Jablonski, 2020; Salazar-Cuidad, 2021). In plants, work on this concept of developmental constraint (and the inter-related concepts of developmental bias and developmental drive) have been extensive—ranging from studies on the evolution of floral organs (Wessinger and Hileman, 2016) to the role that it may play in structuring defenses to herbivores and pathogens during a plant's life cycle (Boege and Marquis, 2005)—and parallels work done in animals. In some instances, such as flower color, these constraints on trait evolution and their relation to the underlying GRN have been extensively documented (e.g., Rausher, 2008; Smith, 2011; Larter et al., 2018; Ng et al., 2018). However, in many cases of morphological evolution, there remains a great deal of opportunity for defining the gaps in phenotypic potential produced by a particular GRN and to what degree these gaps have coincided with evolutionary trajectories in a phylogenetic context.

Contrary to seeing development as a limiting force in evolutionary change, in recent years research programs have begun to explore how environmentally induced phenotypic variation might actually promote evolution (West-Eberhard, 2003). In particular, this work often centers on the possibility that phenotypic plasticity precedes genetic changes during adaptation—a process now referred to as plasticity-first or plasticity-led evolution (Levis and Pfennig, 2016, 2020). The evidence for this mode of evolution remains small, yet examples in both animals and plants are beginning to accumulate (Bock et al., 2018; Corl et al., 2018). Additionally, the lack of evidence is at

least in part a consequence of most instances of putative mutation-first evolution having not been examined for the role that environment may have played in the initial phase of an adaptive event (Wund et al., 2008; Muschick et al., 2011). Further, despite the large number of environmentally regulated phenomena in plants (Olsen, 2019), work examining such developmental events has lagged behind work in animals (Levis and Pfennig, 2020) creating a large potential for new insights into this aspect of developmental evolution.

While the solution to this under-representation must undoubtedly involve increased focus from within the plant community, we believe the way forward is not to directly focus on whether development constrains or promotes evolutionary change (it certainly does both), but to characterize the range and type of phenotypes produced (Salazar-Cuidad, 2006, 2021) by specific GRN through evaluations of ecologically relevant environmental conditions and genetic perturbations within a phylogenetic context. Taking the leaf as an example, multiple GRN responsible for the final form of a leaf have now been described (Chitwood and Sinha, 2016; Conklin et al., 2019) and many of these networks have been characterized across broad phylogenetic scales. For example, it has been shown that the repeated evolution of complex leaves from simple leaves has been mediated by recruitment of KNOXI proteins into networks controlling leaf morphogenesis (Bharathan et al., 2002; Hay and Tsiantis, 2010). Interestingly, more recent work has demonstrated that this same gene regulatory network is responsible for the environmentally induced shifts in leaf morphology of the amphibious plant *Rorippa aquatica*. When this species is grown in a terrestrial environment, simple leaves develop, whereas aquatic conditions produce highly compounded leaves (Fassett, 1930). Further, it was recently found that this difference in leaf morphogenesis is mediated by shifts in KNOXI abundance induced by changes in the light and temperature at which the plants were grown (Nakayama et al., 2014).

In turn, this focus on the GRN underlying leaf complexity, how they are deployed at macroevolutionary scales, and how they are modulated by environmental inputs, opens the door to questions about their role in facilitating the evolutionary trajectories of different lineages. For example, one hypothesis of plasticity-led evolution is that lineages approximating the ancestral species should exhibit developmental plasticity in the trait of interest (Levis and Pfennig, 2016). In the case of *R. aquatica*, this would mean that growing other *Rorippa* species under different light and temperature regimens would be expected to produce phenotypic variation consistent with the alternative phenotypes found in R. *aquatica*. Further, the genetic regulation of these responses will be expected to have undergone refinement in *R. aquatica*, as a result of changes that enhance the association between environmental conditions and the developed phenotype (Levis and Pfennig, 2016). Going forward, we believe that assessing the phenotypic variation of well-characterized GRN under ecologically and phylogenetically relevant conditions will aid in a better understanding of the role development plays in generating evolutionary changes.

Additionally, although constraint has been a major area of focus in plant evodevo we believe that new methods for analysis

of phenotypic variation have the potential for informing how GRN features bias the range of phenotypes seen in lineages. In many instances it is helpful to develop a set of phenotypic expectations against which hypothesis of constraint can be tested (i.e., are there phenotypes missing or over represented in a particular group?). One method for generating such hypotheses is the construction of morphospaces, whereby either mathematical models of development or observational data of trait variation can be plotted to infer the potential variation for a set of traits (e.g., Stebbins, 1951; Raup and Michelson, 1965). Within this theoretical or observational space one can hypothesize why certain trait values are rare or abundant. This method has been employed extensively for studies of animal development and is beginning to see more widespread adoption in plants (Chartier et al., 2014; Li et al., 2018), in part aided by methods for the analysis of shape (Chitwood et al., 2016).

Again using leaves as an example, Chitwood and Otoni examined the morphology of leaves across the heteroblastic transition of 40 *Passiflora* species, finding that the earliest leaves produced by each species were more similar across species than leaves produced later in development (Chitwood and Otoni, 2017a,b). When this finding is integrated with what is known about the GRN underlying age-dependent changes in morphology (also known as heteroblasty), a set of testable hypotheses emerge. In *Arabidopsis thaliana*, and many other species, the miR156-SPL pathway is primarily responsible for the heteroblastic transition in leaf morphology (Wu and Poethig, 2006; Wang et al., 2011). Early in development, levels of miR156 are high, but temporal silencing of *MIR156* genes releases *SPL* transcripts from target cleavage or translational repression (Xu et al., 2016). In *A. thaliana* and *Cardamine hirsuta*, heteroblasty is mediated by the competition between SPL and TCP proteins for interaction with CUC proteins which are in turn responsible for production of serrations or leaflets (Blein et al., 2008; Rubio-Somoza et al., 2014). Assuming that this is a general mechanism for increasing complexity during heteroblastic transitions, it would make sense then that *Passiflora* species (and plants more generally) are constrained in their morphological variation early in development when *SPL* genes are repressed by high levels of miR156. More generally the *SPL* gene family may be a node in the GRN that remains relatively unconstrained, thereby allowing for phenotypic divergence between species at later stages of development. Put another way, are the GRN regulating juvenile and adult leaves different in their potential for producing phenotypic variation and does this explain why juvenile morphologies are often conserved within lineages (e.g., **Figures 1A,B**)? Attempts to experimentally accelerate or alter early leaf morphogenesis would allow for ecological and developmental tests of these possibilities.

In part, the logic of such hypothesis testing rests on the premise that if developmental systems can be manipulated (either environmentally or genetically) to produce phenotypic variation in a new context, then at the very least, a strict developmental constraint can be ruled out. Of course, if such phenotypic variation is experimentally demonstrated, this does not rule out selective constraints that disfavor the appearance of such phenotypic variation. Therefore, these novel phenotypic
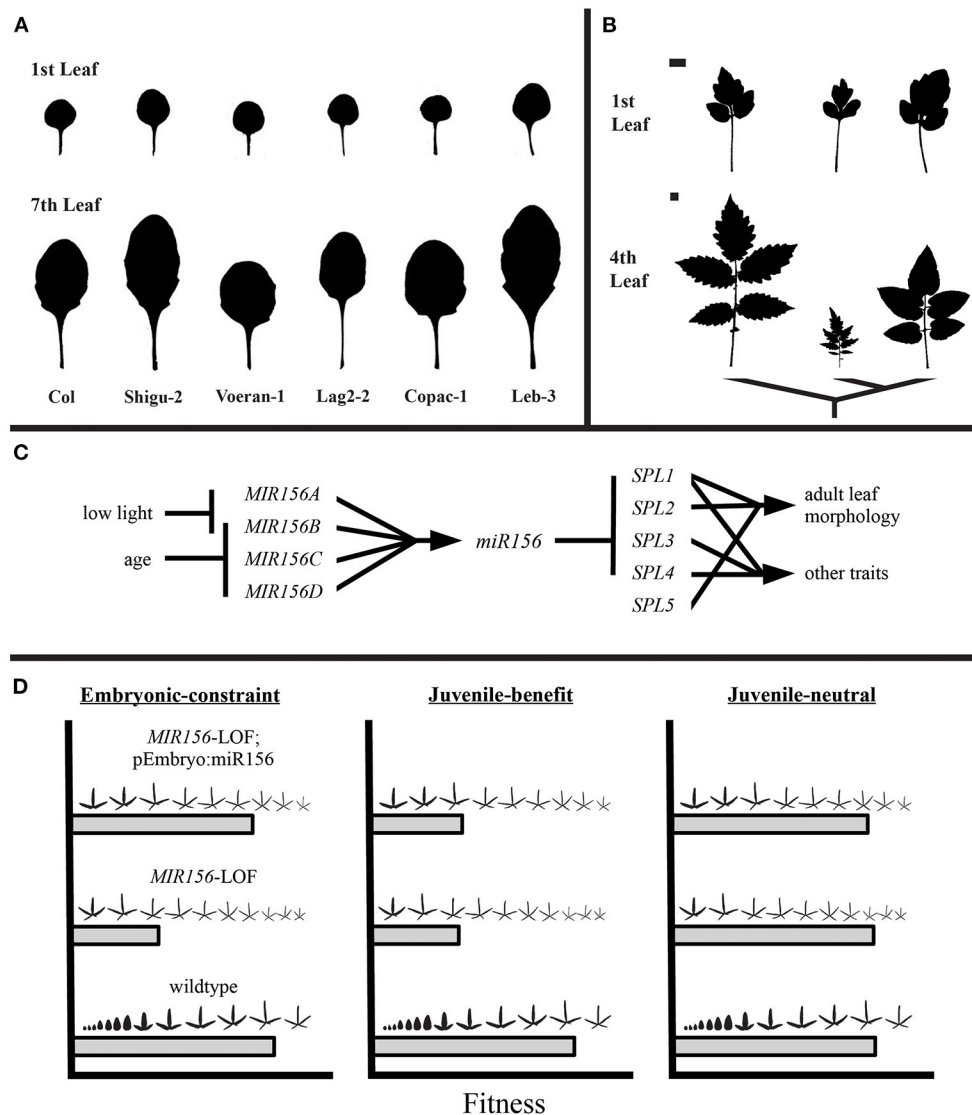
FIGURE 1 | Is there developmental constraint on early leaf morphology? In many clades, the morphology of leaves produced early in the plant life cycle are conserved between species [(A,B), top rows within a panel] relative to leaves produced later [(A,B), bottom rows within a panel]. As the GRN for many aspects of leaf morphogenesis and vegetative transitions are now known, it is theoretically possible to test how development and evolutionary forces such as selection may explain these macroevolutionary patterns. (A) Leaves are from *Arabidopsis thaliana* accessions. Adapted from He (2017). (B) Leaves from three tomato species. Adapted from Chitwood et al. (2012). Scale bars represent 1 cm and apply for leaves within each row. (C) The miR156-SPL pathway in a hypothetical species. (D) Predicted outcomes for experimental manipulation of juvenile leaf production and tests of fitness consequences in a hypothetical species. If the need for high levels of miR156 in the embryo ensures that SPL-mediated morphologies cannot be produced early in development, then the fitness of *MIR156* loss-of-function mutants (*MIR156*-LOF) should be rescued by embryo-specific expression of miR156 (left panel). Alternatively, if the phylogenetic conservation of juvenile leaves is due to a common adaptive function across species, then *MIR156*-LOF mutants would be expected to exhibit reduced fitness (middle panel). Conversely, selection on juvenile leaf morphology may be weak or absent, thereby creating minimal patterns of divergence between species (right panel). Leaf heteroblastic series adapted from Chitwood and Otoni (2017a).

variants must be scrutinized to pinpoint their underlying tradeoffs. In practice—taking again the example of conserved juvenile leaf morphology across many lineages (**Figures 1A,B**)—it has been demonstrated that miR156 is necessary for the production of leaves with juvenile morphology. Consequently, the question then becomes what is the performance of plants where the earliest leaves have been manipulated to

have an adult morphology? This can be achieved by loss-of-function mutations to key *MIR156* genes (He et al., 2018) which accelerates the production of the adult morphology (**Figures 1C,D**, *MIR156*-LOF). These precocious mutants could then be evaluated for various components of fitness to better understand their phylogenetic scarcity. For example, in *A. thaliana SPL* genes interfere with proper embryo

development (Nodine and Bartel, 2010). Therefore, shifting SPL-mediated phenotypes to earlier stages of development will be constrained by the need for high levels of miR156 in the embryo. Theoretically the embryo defects could be rescued by embryo-specific expression of miR156, allowing for direct quantification of these selective constraints (**Figure 1D**, *MIR156-LOF; pEmbryo:miR156*). Additionally, alternative explanations for conserved juvenile morphology are that selection favors a common morphology early in development due to conserved functional requirements (e.g., Lawrence et al., 2020) or that selection for divergent phenotypes is weak (**Figure 1D**, "juvenile-benefit" and "juvenile-neutral," respectively). Expanding such an analysis across multiple species would then quantify the primary drivers for stasis of juvenile morphology. It should be noted that with all these examples, shifts in morphology are confounded with other biochemical and physiological traits regulated by these same GRN. However, in many cases as more is learned about the molecular genetic mechanisms of a pathway, it may become possible to decouple the multiple traits that it coordinates. For example, once the targets of a particular SPL transcription factor are known, *cis*-elements could be targeted that alter leaf morphogenesis while leaving other biochemical targets intact.

It is exactly these sorts of tests that are required to more fully assess how development limits and leads in the evolution of lineages. Work on flower color has undoubtedly led the way in this regard (Sobel and Streisfeld, 2013), but expanding the study of GRN to include ecologically relevant environmental inputs in combination with knowledge of evolutionary trajectories (derived from phylogenetics and morphometrics) will bring new insights into many new and old model systems. Further, by experimentally manipulating GRN to produce relevant phenotypic variation, it will be possible to test the potential role that selection and development play in determining patterns of trait evolution between species. This work will undoubtedly be aided by new methods for exploring the morphospace of lineages, and by continued development of ecologically relevant model systems.

AUTHOR CONTRIBUTIONS

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

FUNDING

REFERENCES

Alberch, P. (1982). "Developmental constraints in evolutionary processes" in *Evolution and Development*, ed J. T. Bonner (New Yoek, NY: Springer), 313–332. doi: 10.1007/978-3-642-45532-2_15

Bharathan, G., Goliber, T. E., Moore, C., Kessler, S., Pham, T., and Sinha, N. R. (2002). Homologies in leaf form inferred from KNOXI gene expression during development. *Science* 296, 1858–1860. doi: 10.1126/science.1070343

Blein, T., Pulido, A., Vialette-Guiraud, A., Nikovics, K., Morin, H., Hay, A., et al. (2008). A conserved molecular framework for compound leaf development. *Science* 322, 1835–1839. doi: 10.1126/science.1166168

Bock, D. G., Kantar, M. B., Caseys, C., Matthey-Doret, R., and Rieseberg, L. H. (2018). Evolution of invasiveness by genetic accommodation. *Nat. Ecol. Evol.* 2, 991–999. doi: 10.1038/s41559-018-0553-z

Boege, K., and Marquis, R. J. (2005). Facing herbivory as you grow up: the ontogeny of resistance in plants. *Trends Ecol. Evol.* 20, 441–448. doi: 10.1016/j.tree.2005.05.001

Chartier, M., Jabbour, F., Gerber, S., Mitteroecker, P., Sauquet, H., von Balthazar, M., et al. (2014). The floral morphospace – a modern comparative approach to study angiosperm evolution. *New Phytol.* 204, 841–853. doi: 10.1111/nph.12969

Chitwood, D. H., Headland, R. L., Kumar, R., Peng, J., Maloof, J. N., and Sinha, N. R. (2012). The developmental trajectory of leaflet morphology in wild tomato species. *Plant Physiol.* 158, 1230–1240. doi: 10.1104/pp.111.192518

Chitwood, D. H., Klein, L., O'Hanlon, R., Chacko, S., Greg, M., Kitchen, C., et al. (2016). Latent developmental and evolutionary shapes embedded within the grapevine leaf. *New Phytol.* 210, 343–355. doi: 10.1111/nph.13754

Chitwood, D. H., and Otoni, W. (2017a). Divergent leaf shapes among Passiflora species arise from a shared juvenile morphology. *Plant Direct.* 1, e00028. doi: 10.1101/067520

Chitwood, D. H., and Otoni, W. (2017b). Morphometric analysis of Passiflora leaves: the relationship between landmarks of the vasculature and elliptical Fourier descriptors of the blade. *Gigascience* 6, 1–13. doi: 10.1093/gigascience/giw008

Chitwood, D. H., and Sinha, N. R. (2016). Evolutionary and environmental forces sculpting leaf development. *Curr. Biol.* 26, R297–R306. doi: 10.1016/j.cub.2016.02.033

Conklin, P., Strable, J., Li, S., and Scanlon, M. J. (2019). On the mechanisms of development in monocot and eudicot leaves. *New Phytol.* 221, 706–724. doi: 10.1111/nph.15371

Corl, A., Bi, K., Luke, C., Challa, A. S., Stern, A. J., Sinervo, B., et al. (2018). The genetic basis of adaptation following plastic changes in coloration in a novel environment. *Curr. Biol.* 28, 2970–2977. doi: 10.1016/j.cub.2018.06.075

Fassett, N. C. (1930). *A Manual of Aquatic Plants*. Madison, WI: The University of Wisconsin Press.

Hay, A., and Tsiantis, M. (2010). KNOX genes: versatile regulators of plant development and diversity. *Development* 137, 3153–3165. doi: 10.1242/dev.030049

He, J. (2017). *Developmental Functions of miR156 and miR157 in Arabidopsis*. Publicly Accessible Penn Dissertations. 2338. Availabel online at: https://repository.upenn.edu/edissertations/2338.= (accessed August 31, 2021).

He, J., Xu, M., Willmann, M. R., McCormick, K., Hu, T., Yang, L., et al. (2018). Threshold-dependent repression of SPL gene expression by miR156/miR157 controls vegetative phase change in *Arabidopsis thaliana*. *PLoS Genet.* 14, e1007337. doi: 10.1371/journal.pgen.1007337

Jablonski, D. (2020). Developmental bias, macroevolution, and the fossil record. *Evol. Dev.* 22, 103–125. doi: 10.1111/ede.12313

Larter, M., Dunbar-Wallis, A., Berardi, A., and Smith, S. D. (2018). Convergent evolution at the pathway level: predictable regulatory changes during flower color transitions. *Mol. Biol. Evol.* 35, 2159–2169. doi: 10.1093/molbev/msy117

Lawrence, E. H., Springer, C. J., Helliker, B. R., and Poethig, R. S. (2020). MicroRNA156-mediated changes in leaf composition lead to altered photosynthetic traits during vegetative phase change. *New Phytol.* 231, 1008–1022. doi: 10.1111/nph.17007

Levis, N., and Pfennig, D. (2016). Evaluating 'plasticity first' evolution in nature: key criteria and empirical approaches. *Trends Ecol. Evol.* 31, P563–574. doi: 10.1016/j.tree.2016.03.012

Levis, N., and Pfennig, D. (2020). Plasticity-led evolution: a survey of developmental mechanisms and empirical tests. *Evol. Dev.* 22, 71–87. doi: 10.1111/ede.12309

Li, M., Hong, A., Angelovici, R., Bagaza, C., Batushansky, A., Clark, L., et al. (2018). Topological data analysis as a morphometric method: using persistent homology to demarcate a leaf morphospace. *Front. Plant Sci.* 9, 553. doi: 10.3389/fpls.2018.00553

Maynard Smith, J., Burian, R., Kauffman, S., Alberch, P., Campbell, J., Goodwin, B., et al. (1985). Developmental constraints and evolution. *Q. Rev. Biol.* 6, 265–287. doi: 10.1086/414425

Muschick, M., Barluenga, M., Salzburger, W., and Meyer, A. (2011). Adaptive phenotypic plasticity in the Midas cichlid fish pharyngeal jaw and its relevance in adaptive radiation. *BMC Evol. Biol.* 11, 116. doi: 10.1186/1471-2148-11-116

Nakayama, H., Nakayama, N., Seiki, S., Kojima, M., Sakakibara, H., Sinha, N., et al. (2014). Regulation of the KNOX-GA gene module induces heterophyllic alteration in North American Lake Cress. *Plant Cell* 26, 4733–4748. doi: 10.1105/tpc.114.130229

Ng, J., Freitas, L. B., and Smith, S. D. (2018). Stepwise evolution of floral pigmentation predicted by biochemical pathway structure. *Evolution* 72, 2792–2802. doi: 10.1111/evo.13589

Nodine, M. D., and Bartel, D. P. (2010). MicroRNAs prevent precocious gene expression and enable pattern formation during plant embryogenesis. *Genes Dev.* 24, 2678–2692. doi: 10.1101/gad.1986710

Olsen, M. E. (2019). Plant evolutionary ecology in the age of the extended evolutionary synthesis. *Integr. Comp. Biol.* 59, 493–502. doi: 10.1093/icb/icz042

Raup, D. M., and Michelson, A. (1965). Theoretical morphology of the coiled shell. *Science* 147, 1294–1295. doi: 10.1126/science.147.3663.1294

Rausher, M. D. (2008). Evolutionary transitions in floral color. *Int. J. Plant Sci.* 169, 7–21. doi: 10.1086/523358

Rubio-Somoza, I., Zhou, C. M., Confraria, A., Martinho, C., von Born, P., Baena-Gonzalez, E., et al. (2014). Temporal control of leaf complexity by miRNA-regulated licensing of protein complexes. *Curr. Biol.* 24, 2714–2719. doi: 10.1016/j.cub.2014.09.058

Salazar-Cuidad, I. (2006). Developmental constraints vs. variational properties: how pattern formation can help to understand evolution and development. *J. Exp. Zool.* 306, 107–125. doi: 10.1002/jez.b.21078

Salazar-Cuidad, I. (2021). Why call it developmental bias when it is just development. *Biol. Direct* 16, 3. doi: 10.1186/s13062-020-00289-w

Smith, S. D. (2011). Gene loss and parallel evolution contribute to species difference in flower color. *Mol. Biol. Evol.* 28, 2799–2810. doi: 10.1093/molbev/msr109

Sobel, J. M., and Streisfeld, M. A. (2013). Flower color as a model system for studies of plant evo-devo. *Front. Plant Sci.* 4, 321. doi: 10.3389/fpls.2013.00321

Stebbins, G. L. (1951). Natural selection and the differentiation of angiosperm families. *Evolution* 5, 299–324. doi: 10.1111/j.1558-5646.1951.tb02789.x

Wang, J., Park, M., Wang, L., Koo, Y., Chen, X., Weigel, D., et al. (2011). MiRNA control of vegetative phase change in trees. *PLoS Genet.* 7, e1002012. doi: 10.1371/journal.pgen.1002012

Wessinger, C. A., and Hileman, L. C. (2016). Accessibility, constraint, and repetition in adaptive floral evolution. *Dev. Biol.* 419, 175–183. doi: 10.1016/j.ydbio.2016.05.003

West-Eberhard, M. J. (2003). *Developmental Plasticity and Evolution*. New York, NY: Oxford University Press. doi: 10.1093/oso/9780195122343.003.0008

Wu, G., and Poethig, R. S. (2006). Temporal regulation of shoot development in *Arabidopsis thaliana* by miR156 and its target SPL3. *Development* 133, 3539–3547. doi: 10.1242/dev.02521

Wund, M. A., Baker, J. A., Clancy, B., Golub, J. L., and Fosterk, S. A. (2008). A test of the "flexible stem" model of evolution: ancestral plasticity, genetic accommodation, and morphological divergence in the threespine stickleback radiation. *Amer. Natural.* 172, 449–462. doi: 10.1086/590966

Xu, M., Hu, T., Smith, M. R., and Poethig, R. S. (2016). Epigenetic regulation of vegetative phase change in Arabidopsis. *Plant Cell* 28, 28–41. doi: 10.1105/tpc.15.00854

Widening the Perspectives for Legume Consumption: The Case of Bioactive Non-nutrients

*Rafaela Geraldo, Carla S. Santos, Elisabete Pinto and Marta W. Vasconcelos**

CBQF - Centro de Biotecnologia e Química Fina – Laboratório Associado, Escola Superior de Biotecnologia, Universidade Católica Portuguesa, Porto, Portugal

Legume grains have provided essential nutrients in human diets for centuries, being excellent sources of proteins, carbohydrates, fatty acids, and fibers. They also contain several non-nutrients that historically have been connotated as toxic but that in recent years have been shown to have interesting bioactive properties. The discussion on the role of bioactive non-nutrients is becoming more important due to increasing science-based evidence on their potential antioxidant, hypoglycemic, hypolipidemic, and anticarcinogenic properties. At a time when legume-based products consumption is being strongly promoted by national governments and health authorities, there is a need to clearly define the recommended levels of such non-nutrients in human diets. However, there is insufficient data determining the ideal amount of non-nutrients in legume grains, which will exert the most positive health benefits. This is aligned with insufficient studies that clearly demonstrate if the positive health effects are due to the presence of specific non-nutrients or a result of a dietary balance. In fact, rather than looking directly at the individual food components, most nutritional epidemiology studies relate disease risk with the food and dietary patterns. The purpose of this perspective paper is to explore different types of non-nutrients present in legume grains, discuss the current evidence on their health benefits, and provide awareness for the need for more studies to define a recommended amount of each compound to identify the best approaches, either to enhance or reduce their levels.

Keywords: anti-nutrients, bioactive, legume grains, health, sustainability

INTRODUCTION

The intensification of agriculture and the unbalanced consumption of animal protein has called for increased consumption of alternative sources of protein, such as legumes. However, legume production and consumption levels are at a historic low in many parts of the world, including in many European countries (Cusworth et al., 2021). Food and feed-wise, legumes are often subdivided into three subgroups: fresh legumes (e.g., beans and peas), oilseed legumes (like peanuts and soybeans), and pulses (dried and edible seeds of legume plants, such as chickpeas, dried peas, and dried beans; Mullins and Arjmandi, 2021). Legume production may help reduce greenhouse gas emissions, improve soil carbon sequestration, and overall reduce fossil energy inputs in farming systems (Mus et al., 2016; Stagnari et al., 2017). Oftentimes legumes grow well in poor soils and

with unfavorable weather conditions and may be used as cover crops, which contribute to a reduction in soil erosion. These benefits, combined with the fact that they form symbiotic relations with nitrogen-fixing bacteria, make them excellent rotational crops (Maphosa and Jideani, 2017; Liu et al., 2018).

Legumes are generally low in fat, cholesterol-free and excellent sources of protein/amino acids, providing a large share of human dietary protein requirement (Smýkal et al., 2015), fatty acids, fibers, carbohydrates, vitamins, and minerals (Ganesan and Xu, 2017; Mirali et al., 2017; Bazghaleh et al., 2018; Balázs et al., 2021; Iannetta et al., 2021), like calcium (Ca), chromium (Cr), copper (Cu), iron (Fe), magnesium (Mg), phosphorus (P), potassium (K), selenium (Se), and zinc (Zn; Kouris-Blazos and Belski, 2016).

The consumption of legumes, as a part of a balanced diet, can bring human health benefits, including a reduced risk of cardiovascular disease (CVD; Marventano et al., 2017) and related CVD issues, like obesity, high blood pressure, type-2 diabetes, dyslipidemia, and stroke (Polak et al., 2015; Becerra-Tomás et al., 2019; Ferreira et al., 2021; Mullins and Arjmandi, 2021). Its reduction is possible due to the low glycemic index of legumes (avoid peaks in blood glucose), their high fiber content, and the presence of the non-nutrients (phytosterols, saponins, and lectins, among others; Duranti, 2006). Besides, legumes also improve the microbial diversity of gut, colon health, oxidative stress, inflammatory status, and even help to reduce cancer (Santos et al., 2017; Mirmiran et al., 2018; Mullins and Arjmandi, 2021; Ferreira et al., 2022).

Nonetheless, legumes have historically been associated with the presence of specific classes of anti-nutrients (or bioactive non-nutrients) which, if processed inappropriately, may have secondary effects, such as toxicity or legume-related food allergies (e.g., peanut and soybean). The negative connotation began several years ago, and one of the earliest pieces of evidence comes from the story of the Greek philosopher and mathematician, Pythagoras, who forbade his disciples to consume the Greek fava beans because it made many people sick with the so-called "favism" (Meletis, 2012). It is claimed that Pythagoras died at the hands of the enemy because he decided not to escape through a fava bean field (Meletis, 2012). Currently, it is known that favism is a form of hemolytic anemia and jaundice caused by a genetically inherited deficiency in the enzyme glucose-6-phosphate dehydrogenase (G6PD; Luzzatto and Arese, 2018). The cause of favism in such individuals is due to the presence of two fava bean anti-nutrients, the pyrimidine glycosides vicine and convicine (Luzzatto and Arese, 2018; Khazaei et al., 2019). These compounds are thermostable, but their concentration can be greatly reduced by seed soaking, frying, boiling, microwave irradiation, roasting, or fermenting (Pulkkinen et al., 2019). For non-nutrients that are clearly unsafe, breeding could help in the reduction/elimination of undesired non-nutrients levels (Khazaei et al., 2019; Robinson et al., 2019). In fact, low vicine and convicine fava bean cultivars are now available and researchers are also investigating ways to completely eliminate them (Khazaei et al., 2019). While the reduction of vicine and convicine levels has been successfully achieved, with a consensus that this reduction would be important for a broader consumption of fava beans,

we cannot say the same for all anti-nutritional compounds. For some, health benefits may be promoted, and increasing their levels could be considered. Nonetheless, a thorough discussion is needed to decide when (and if) these compounds should be bred "in" or "out" or kept "as is."

Even though legumes provide several health and environmental advantages there is a persistent barrier to their increased consumption related to the presence of bioactive non-nutrients. For example, legumes are highly associated with causes of unwanted flatulence, due to the presence of oligosaccharides (raffinose, stachyose, and verbascose; Abdel-Gawad, 1993; Han and Baik, 2006). In times where legumes have been put forward as an important protein source and as a vehicle to provide well-balanced nutrition, while safeguarding the environment, there is a need to clarify the real concerns (or lack thereof) of these compounds. Does the presence of non-nutrients bring positive or negative impacts, and how to balance the two? The present perspective takes a close look at this question and discusses some of the angles that need to be considered when discussing future research needs.

NON-NUTRIENTS

The non-nutrients can be broadly divided into two major categories: the proteinaceous group and the non-proteinaceous group. The former includes lectins, agglutinins, bioactive peptides, and protease inhibitors, and the second group includes alkaloids, phytic acid, tannins, and saponins (Sánchez-Chino et al., 2015). The accumulation in edible seeds is a natural process, triggered by plant defense mechanisms against insects, parasites, fungi, and herbivorous animals (Sánchez-Chino et al., 2015). They can also act as a nutritional pool to maintain plant growth under unfavorable conditions (Sánchez-Chino et al., 2015). Although some non-nutrients are mostly found in certain types of legumes, such as vicine and convicine in fava bean (Khamassi et al., 2013), not all of them are legume-exclusive; phytic acid is also present in cereals, oil seeds, nuts, and plants (Gupta et al., 2015); oxalates in spinach, Swiss chard, rhubarb, and potatoes; tannins in tea, cocoa, grapes, and wine (Petroski and Minich, 2020).

It is important to note that legumes that share similar nutritional profiles may have significant variations in the relative abundances of individual nutrients (Mirali et al., 2017). This variability extends not only to protein and other macronutrients but also for bioactive compounds (**Table 1**), and this should be considered when evaluating the right amount of each in a dietary serving. Among the proteinaceous non-nutrients, the glycoproteins lectins or hemagglutinins have the capacity of reversibly attaching carbohydrates on cells, like red blood cells, resulting in erythrocyte agglutination (Petroski and Minich, 2020; Samtiya et al., 2020). Lectins, present especially in common beans (*Phaseolus vulgaris*) and peas (*Pisum sativum*; **Table 1**), have a negative role in nutrient absorption (by binding intestinal epithelial cells), and in the integrity of the mucosa, causing intestinal hyperplasia and high permeability (**Figure 1**; Petroski and Minich, 2020; Samtiya et al., 2020), which allow bacteria contact with the bloodstream (Samtiya et al., 2020).

TABLE 1 | Summary of main legume species and concentrations of the non-nutrient's lectins, oxalates, total phenolics, phytates, saponins, and tannins (in yield range), and of trypsin and alpha-amylase inhibitors (in activity units).

Non-nutrient	Legume species	Yield range (mg/100 g seeds)	References
Lectins	*Cicer arietinum* L.	95	Gautam et al., 2018
	Glycine max	360	Barca et al., 1991
	Lens culinaris	48	El-Araby et al., 2020
	Phaseolus vulgaris	13–1,100	Lam and Ng, 2010
		174	Shang et al., 2016
	Pisum sativum	148–160	El-Araby et al., 2020
	Vicia faba	50	El-Araby et al., 2020
Oxalates	*Arachis hypogaea*	41	Guo et al., 2021
	Cicer arietinum	192–199	Shi et al., 2018
	Glycine max	370	
	Lens culinaris	168–289	
	Phaseolus vulgaris	99–117	
	Pisum sativum	244–280	
	Macrotyloma uniflorum	88–123	Vashishth et al., 2021
	Vicia faba	241–291	Shi et al., 2018
Total phenolics	*Canavalia* spp.	640–1,818	Sridhar and Sahadevan, 2006
	Glycine max	1.77–2.48	Król-Grzymała and Amarowicz, 2020
	Lens culinaris	12	Piecyk et al., 2012
	Lupinus angustifolius	94.66	Karnpanit et al., 2016
	Mucuna pruriens	0.565	Siddhuraju and Becker, 2005
	Phaseolus vulgaris	35.5–45.6	Barreto et al., 2021
		105.8	
			Piecyk et al., 2012
	Pisum sativum	11.6	Piecyk et al., 2012
	Vicia faba		
	Vigna unguiculata	1,210	Kalpanadevi and Mohan, 2013
Phytates	*Cicer arietinum* L.	1,133–1,400	Shi et al., 2018
	Glycine max	2,291	
	Lens culinaris	856–1710	
	Lupinus angustifolius	0.80	Karnpanit et al., 2016
	Macrotyloma uniflorum	42–45	Vashishth et al., 2021
	Mucuna pruriens	950	Siddhuraju and Becker, 2005
	Phaseolus vulgaris	310	Shang et al., 2016
		1,580	Carbas et al., 2020
		1,564–1,882	
		1,760–2,080	Shi et al., 2018
			Barreto et al., 2021
	Pisum sativum	855–993	Shi et al., 2018
	Vicia faba	1,965	Shi et al., 2018
		112–1,281	Mayer Labba et al., 2021
	Vigna unguiculata	360–510	Avanza et al., 2013

(Continued)

TABLE 1 | Continued

Non-nutrient	Legume species	Yield range (mg/100 g seeds)	References
Saponins	*Cajanus cajan*	2,164	Duhan et al., 2001
	Canavalia spp.	571–1,005	Sridhar and Sahadevan, 2006
	Medicago sativa	800–1,650	Hadidi et al., 2020
	Mucuna pruriens	1,210	Siddhuraju and Becker, 2005
	Phaseolus vulgaris	940–1,180	Emire and Rakshit, 2007
		373	
			Shang et al., 2016
	Vigna radiata	2,848	Kataria et al., 1988
	Vigna umbellata	2,175–2,450	Kaur and Kapoor, 1992
Tannins	*Canavalia* spp.	230–900	Sridhar and Sahadevan, 2006
	Lupinus angustifolius	46.41	Karnpanit et al., 2016
	Macrotyloma uniflorum	90–92	Vashishth et al., 2021
	Mucuna pruriens	300	Siddhuraju and Becker, 2005
	Phaseolus vulgaris	170–1,770	Carbas et al., 2020
	Vicia faba	1,370	Sharma and Sehgal, 1992
	Vigna unguiculata	380	Kalpanadevi and Mohan, 2013
		110–820	Avanza et al., 2013
	Activity units (U/mg)		
Trypsin inhibitors	*Arachis hypogea*	5.60	Embaby, 2010
	Cajanus cajan	4.75	Sangronis and Machado, 2007
	Cicer arietinum	12.60–14.51	Muzquiz et al., 2012
		14.22–16.24	Shi et al., 2017
	Lens culinaris	3–8	Guillamón et al., 2008
		7.40	
		4.98–6.29	Świeca and Baraniak, 2014
			Shi et al., 2017
	Phaseolus vulgaris	17–51	Guillamón et al., 2008
		15.18–20.83	
			Shi et al., 2017
	Pisum sativum	5.75–12.55	Muzquiz et al., 2012
		3.16–4.92	Shi et al., 2017
	Vicia faba	5–10	Guillamón et al., 2008
		5.96–6.10	
		4.47	Shi et al., 2017
			Alonso et al., 2000
	Vigna unguiculata	7.52	Rivas-Vega et al., 2006
Alfa-amylase inhibitors	*Cajanus cajan*	0.07	Choi et al., 2019
	Cicer arietinum	0.09	Choi et al., 2019
		0.02–0.08	Mulimani et al., 1994
	Phaseolus vulgaris	0.786–1.37	Shi et al., 2017
		0.25	Alonso et al., 2000
	Vicia faba	0.02	Alonso et al., 2000
	Vigna angularis	0.12	Choi et al., 2019
	Vigna radiata	0.14	
	Vigna unguiculata	0.18	
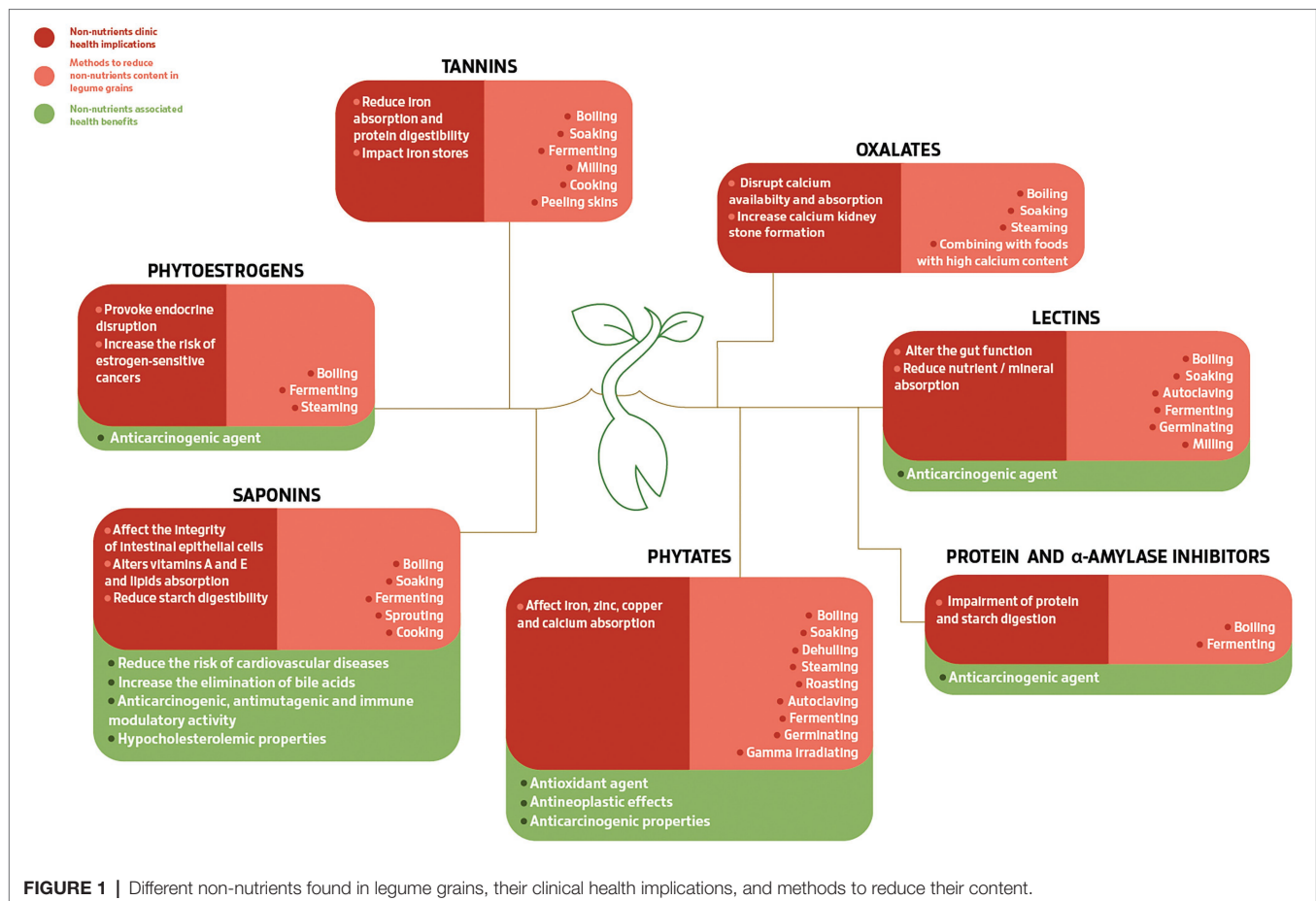
FIGURE 1 | Different non-nutrients found in legume grains, their clinical health implications, and methods to reduce their content.

Despite lectins are resistant to enzymes in the gastrointestinal tract, they can be reduced/removed by boiling, soaking, autoclaving, fermenting, germinating, and milling (**Figure 1**; Petroski and Minich, 2020). For example, boiling white and red kidney beans can eliminate lectin content (Nciri et al., 2015). However, lectins may have clinical benefits, for example, some studies show that they can recognize different glycan production of cancer cells and therefore can be potentially used in cancer treatments (**Figure 1**; Panda et al., 2014; Gautam et al., 2018, 2020; Bhutia et al., 2019; Mullins and Arjmandi, 2021). Besides, they positively activate the immune system, modifying the expression of interleukins and some protein kinases, and have been demonstrated as possible antiviral and antimicrobial agents (**Figure 1**; Lagarda-Diaz et al., 2017; Mullins and Arjmandi, 2021). For instance, in the treatment of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), responsible for the currently COVID-19 pandemic, lectins can bind complex-type-N-glycans on viral glycoproteins, like coronaviruses spike and prevent the production of viral proteins and the cytopathic effect in host cells (Liu et al., 2020).

Protein and α-amylase inhibitors may present higher activity units in common beans and chickpeas (*Cicer arietinum*; **Table 1**) and are natural plant inhibitors that interfere with mineral bioavailability, nutrient absorption, and protein and starch

digestibility (**Figure 1**; Sánchez-Chino et al., 2015; Samtiya et al., 2020). Although studies are limited and not recent and this subject remains controversial, it has been broadly reviewed that some of the inhibitors, like Bowman-Birk, may present anticarcinogenic effects (**Figure 1**; Muzquiz et al., 2012; Sánchez-Chino et al., 2015; Srikanth and Chen, 2016; Kårlund et al., 2021). Boiling and fermenting may reduce their amount (**Figure 1**; Maphosa and Jideani, 2017), and there are already studies aimed to reduce these compounds, through natural or induced biodiversity screening (Sparvoli et al., 2016).

Oxalates are often labeled as deleterious non-nutrients and are frequently present in soybean, fava bean, and peas (**Figure 1**), but also in non-legumes (Mitchell et al., 2019; Petroski and Minich, 2020). They are usually associated with a reduction in mineral bioavailability and absorption (through chelating minerals) and with favoring kidney stones formation (Shi et al., 2018; Petroski and Minich, 2020). Oxalates are usually excreted in urine (Shi et al., 2018), and its excretion can be promoted *via* proper hydration, Ca consumption (Ca binds to oxalates during digestion), and vitamin C balance (which may influence the oxalate endogenous production; Mitchell et al., 2019). Boiling, soaking, steaming, and combining with high Ca-rich foods help to reduce oxalate content (**Figure 1**; Petroski and Minich, 2020). For example, soaking seeds of different legumes species reduced

the oxalate content by 17–52% and the reduction even increased after cooking, 31–66% (Shi et al., 2018). Nevertheless, it is necessary to have into account that legumes are not the only oxalate source; cooked and raw spinach is considered the major supplier since ingestion of 50–100 g of spinach (normal portion) provides around 500–1,000 mg of oxalate (Mitchell et al., 2019); also in cocoa powder, oxalates content was found to be 619 mg/100 g; in sweet potatoes 496 mg/100 g and in okra 317 mg/100 g (Siener et al., 2020).

Phytate or phytic acid, a non-proteinaceous non-nutrient (Raes et al., 2014), frequently present in soybeans, fava beans, and common beans (**Table 1**), can chelate Fe, Zn, and Cu, and can negatively affect their absorption in the gastrointestinal tract (**Figure 1**; Samtiya et al., 2020). People that consume a large amount of legume grains as a part of their diet can have lower levels of Fe. In extreme cases, this can cause anemia (Shi et al., 2018), if the recommended daily doses are exceeded or it is not maintained a balanced diet. The adequate provision of vitamin C in the diet is a good option to counteract these negative effects since it keeps Fe available for absorption (Bohn et al., 2008; Petroski and Minich, 2020). Nevertheless, phytates have important health benefits, such as anticarcinogenic properties and antioxidant activity (**Figure 1**), chelating toxic metals, palladium and cadmium, or excess Fe, thus preventing harmful Fenton reactions (Shi et al., 2018; Petroski and Minich, 2020). Several techniques can reduce their content, for example, boiling, autoclaving, among others (**Figure 1**; Maphosa and Jideani, 2017) and, in the last decades, several mutants with low phytic acid have been developed, like in common beans (Campion et al., 2009; Sparvoli et al., 2016; Cominelli et al., 2018), to improve the nutritional quality of this seed crop (Cominelli et al., 2020).

Phenolic compounds, present in *Canavalia* spp. and cowpea (*Vigna unguiculata*; **Table 1**), can have anti-inflammatory and antioxidant properties, improve gut health (Filosa et al., 2018), lead to the inhibition of glucose regulation enzymes α-amylase and amyloglucosidase (Sánchez-Chino et al., 2015), and reduce the risk of CVD, type 2 diabetes, metabolic syndrome, ischemic stroke, and atherosclerotic vascular disease (Petroski and Minich, 2020). Nevertheless, not all polyphenolic compounds have health benefits, for example, tannins. Found mostly in the outer layers of grains and seed coats and, in higher concentration, in fava beans (**Table 1**), but also in cocoa beans, tea, wine, and fruits, they have the capacity of interfering with Fe absorption and storage, contributing to Fe deficiency anemia (**Figure 1**; Raes et al., 2014; Petroski and Minich, 2020). They can also form protein complexes, reducing protein digestibility and inactivating digestive enzymes (**Figure 1**; Samtiya et al., 2020). Methods like boiling, soaking, fermenting, milling, cooking, and de-coating allow the reduction of their content in legume seeds (**Figure 1**; Petroski and Minich, 2020; Samtiya et al., 2020). In the case of fava bean, genetic improvement has been applied to obtain zero-tannin cultivars (Gutierrez et al., 2008). Phytoestrogens, present especially in soy products (tofu, tempeh, and soymilk), have a similar structure to the female primary sex hormone, 17-β-estradiol, and also have some health concerns; they may be involved in endocrine disruption and increase the risk of estrogen-sensitive cancers (**Figure 1**; Petroski and Minich, 2020). However, there are some references to anticarcinogenic effects

(**Figure 1**; Sánchez-Chino et al., 2015; Petroski and Minich, 2020). These compounds can be reduced through boiling, fermenting, and steaming (**Figure 1**; Petroski and Minich, 2020).

Saponins in plant foods can interact with erythrocytes increasing the risk of hemolysis, inhibit digestive enzyme activities causing indigestibility disorders, and reduce vitamin absorption (**Figure 1**; Samtiya et al., 2020). However, saponins can also reduce the risk of CVD, cancer, blood cholesterol, and blood glucose; increase bile acids excretion, cell proliferation regulation, and have anti-inflammatory and immune-stimulatory activities (**Figure 1**; Sánchez-Chino et al., 2015; Singh et al., 2017). Once again, several standard processing methods are effective at reducing their amount (**Figure 1**; Maphosa and Jideani, 2017; Samtiya et al., 2020), for example, soaking navy beans reduced the level of saponins by 6.3% and soaking and cooking by 42.3% (Shi et al., 2009).

Since the consumption of non-nutrients has contrasting health effects, the possibility of reducing or increasing their content in different legumes has been considered (Gutierrez et al., 2008; Cominelli et al., 2018; Khazaei et al., 2019). The vast majority can be reduced or even eliminated by traditional food preparation procedures (**Figure 1**), and proper processing methods can reduce their amount and increase the protein digestibility and biological value of legumes (Samtiya et al., 2020). These methods are well documented in the literature according to the perspective that these compounds need to be eliminated (Samtiya et al., 2020) but the discovery that these can have beneficial effects has opened a new path of study. Some can indeed be present after food preparation procedures, and their health implications need to be further explored. However, benefits or deleterious effects are related to intake amount (Conti et al., 2021), which is absent in the literature, emphasizing the need to develop guidelines for recommended intake. Nevertheless, legumes are currently being used in alternative ways (e.g., flours), where they may not be subjected to these kinds of processing methods, therefore new breeding approaches are required. Hence, further studies on specific levels for these compounds that may bring positive health outcomes without jeopardizing human and animal health are necessary.

Furthermore, climatic changes can have an impact on the composition of these compounds, and thus, the future breeding programs and selection of high or low-bioactive legumes must be adapted (Hummel et al., 2018; Herrera et al., 2019). For example, mild hydric stress in common bean culture increased the non-nutrient content (phenolic compounds and saponins; Herrera et al., 2019).

To better evaluate the real need of reducing non-nutrients levels in plant foods or showing the benefits of such compounds, specific nutritional epidemiology studies are needed, but they are quite limited. It is imperative to have studies looking for associations between foods or even dietary patterns and diseases risk, rather than looking directly at the nutrients and components of individual foods (Hu, 2002). For example, several research studies show an inverse relationship between consumption of different legumes and CVD risk (Macarulla et al., 2001; Jukema et al., 2005; Winham and Hutchins, 2007; Abeysekara et al., 2012; Zhu et al., 2012; Ferreira et al., 2021). This benefit could be partially justified by these bioactive compounds in combination with others, in synergistic relationships (Hu, 2002; Bhupathiraju and Tucker, 2011).

Furthermore, it should be considered that although some non-nutrients are more abundant in specific legumes, their intake dosage, within a diversified diet, can balance the beneficial and adverse effects. This could ensure their recognition as non-nutrient or pro-nutrient (Muzquiz et al., 2012; Popova and Mihaylova, 2019). For example, phytic acid represents a non-nutrient factor in the context of a poor diet, that lacks in minerals and vitamins, or in unfavored segments of the population (such as elders and infants), while it can have health properties in a rich diet, typical of the industrialized countries (Nissar et al., 2017). Saponins may also have opposite effects, that is, when consumed in low amounts may contribute with the previously mentioned benefits, but when ingested in high amounts may have deleterious effects (Kumar and Pandey, 2020). Nonetheless, more studies are needed to determine the recommended amount of these compounds to avoid these harmful effects.

FUTURE RESEARCH NEEDS

Legume consumption provides health and environmental gains. However, the presence of non-nutrients continues to affect their consumption, and the goal of increasing the levels of these is a complex subject. The purpose of this perspective is not to give breeding directions for these non-nutrients but to raise awareness of this topic and underline the need for further studies and knowledge on specific amounts of these compounds that may bring health benefits without compromising general health and determine the need to either increase or decrease them. These may be a challenge since these compounds are not ingested isolated but in meals containing further compounds that can have synergic relationships. Besides, the human clinical trials that investigate the non-nutrients effects are quite limited and the alternative epidemiological/observational studies used are difficult to implement due to different variables. There are also great discrepancies in legume consumption habits, linked to cultural aspects, dietary habits, processing methods, and

socioeconomics, among others, that need to be integrated into a multidisciplinary approach for proper guidance of future research efforts. Therefore, in the future, more research is needed to make a proper position and clarify these knowledge gaps, including a technical perspective from breeders, public health specialists, sociologists, policymakers that takes into consideration all these aspects.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

MV defined the concept. RG, MV, CS, and EP offered contributions to the design and writing of the manuscript, as well as to the analysis and interpretation of data for the work, and revised the manuscript critically. All authors contributed to the article and approved the submitted version.

FUNDING

ACKNOWLEDGMENTS

REFERENCES

Abdel-Gawad, A. S. (1993). Effect of domestic processing on oligosaccharide content of some dry legume seeds. *Food Chem.* 46, 25–31. doi: 10.1016/0308-8146(93)90070-V

Abeysekara, S., Chilibeck, P. D., Vatanparast, H., and Zello, G. A. (2012). A pulse-based diet is effective for reducing total and LDL-cholesterol in older adults. *Br. J. Nutr.* 108, S103–S110. doi: 10.1017/S0007114512000748

Alonso, R., Aguirre, A., and Marzo, F. (2000). Effects of extrusion and traditional processing methods on antinutrients and in vitro digestibility of protein and starch in faba and kidney beans. *Food Chem.* 68, 159–165. doi: 10.1016/S0308-8146(99)00169-7

Avanza, M., Acevedo, B., Chaves, M., and Añón, M. (2013). Nutritional and anti-nutritional components of four cowpea varieties under thermal treatments: principal component analysis. *LWT Food Sci. Technol.* 51, 148–157. doi: 10.1016/j.lwt.2012.09.010

Balázs, B., Kelemen, E., Centofanti, T., Vasconcelos, M. W., and Iannetta, P. P. M. (2021). Integrated policy analysis to identify transformation paths to more-sustainable legume-based food and feed value-chains in Europe. *Agroecol. Sustain. Food Syst.* 45, 1–23. doi: 10.1080/21683565.2021.1884165

Barca, A. M. C., Vázquez-Moreno, L., and Robles-Burgueño, M. R. (1991). Active soybean lectin in foods: isolation and quantitation. *Food Chem.* 39, 321–327. doi: 10.1016/0308-8146(91)90149-I

Barreto, N. M. B., Pimenta, N. G., Braz, B. F., Freire, A. S., Santelli, R. E., Oliveira, A. C., et al. (2021). Organic black beans (*Phaseolus vulgaris* L.) from Rio de Janeiro state, Brazil, present more phenolic compounds and better nutritional profile than nonorganic. *Foods* 10:900. doi: 10.3390/foods10040900

Bazghaleh, N., Prashar, P., Purves, R. W., and Vandenberg, A. (2018). Polyphenolic composition of lentil roots in response to infection by *Aphanomyces euteiches*. *Front. Plant Sci.* 9:1131. doi: 10.3389/fpls.2018.01131

Becerra-Tomás, N., Papandreou, C., and Salas-Salvadó, J. (2019). Legume consumption and cardiometabolic health. *Adv. Nutr.* 10, S437–S450. doi: 10.1093/advances/nmz003

Bhupathiraju, S. N., and Tucker, K. L. (2011). Coronary heart disease prevention: nutrients, foods, and dietary patterns. *Clin. Chim. Acta* 412, 1493–1514. doi: 10.1016/j.cca.2011.04.038

Bhutia, S. K., Panda, P. K., Sinha, N., Praharaj, P. P., Bhol, C. S., Panigrahi, D. P., et al. (2019). Plant lectins in cancer therapeutics: targeting apoptosis and autophagy-dependent cell death. *Pharmacol. Res.* 144, 8–18. doi: 10.1016/j.phrs.2019.04.001

Bohn, L., Meyer, A. S., and Rasmussen, S. K. (2008). Phytate: impact on environment and human nutrition. A challenge for molecular breeding. *J. Zhejiang Univ. Sci. B* 9, 165–191. doi: 10.1631/jzus.B0710640

Campion, B., Sparvoli, F., Doria, E., Tagliabue, G., Galasso, I., Fileppi, M., et al. (2009). Isolation and characterisation of an lpa (low phytic acid) mutant in common bean (*Phaseolus vulgaris* L.). *Theor. Appl. Genet.* 118, 1211–1221. doi: 10.1007/s00122-009-0975-8

Carbas, B., Machado, N., Oppolzer, D., Ferreira, L., Brites, C., Rosa, E. A. S., et al. (2020). Comparison of near-infrared (NIR) and mid-infrared (MIR) spectroscopy for the determination of nutritional and antinutritional parameters in common beans. *Food Chem.* 306:125509. doi: 10.1016/j.foodchem.2019.125509

Choi, W. C., Parr, T., and Lim, Y. S. (2019). The impact of four processing methods on trypsin-, chymotrypsin- and alpha-amylase inhibitors present in underutilised legumes. *J. Food Sci. Technol.* 56, 281–289. doi: 10.1007/s13197-018-3488-0

Cominelli, E., Confalonieri, M., Carlessi, M., Cortinovis, G., Daminati, M., Porch, T., et al. (2018). Phytic acid transport in *Phaseolus vulgaris*: a new low phytic acid mutant in the PvMRP1 gene and study of the PvMRPs promoters in two different plant systems. *Plant Sci.* 270, 1–12. doi: 10.1016/j.plantsci.2018.02.003

Cominelli, E., Pilu, R., and Sparvoli, F. (2020). Phytic acid and transporters: what can we learn from low phytic acid mutants? *Plants* 9:69. doi: 10.3390/plants9010069

Conti, M. V., Guzzetti, L., Panzeri, D., De Giuseppe, R., Coccetti, P., Labra, M., et al. (2021). Bioactive compounds in legumes: implications for sustainable nutrition and health in the elderly population. *Trends Food Sci. Technol.* 117, 139–147. doi: 10.1016/j.tifs.2021.02.072

Cusworth, G., Garnett, T., and Lorimer, J. (2021). Legume dreams: the contested futures of sustainable plant-based food systems in Europe. *Glob. Environ. Chang.* 69:102321. doi: 10.1016/j.gloenvcha.2021.102321

Duhan, A., Khetarpaul, N., and Bishnoi, S. (2001). Saponin content and trypsin inhibitor activity in processed and cooked pigeon pea cultivars. *Int. J. Food Sci. Nutr.* 52, 53–59. doi: 10.1080/09637480020027200

Duranti, M. (2006). Grain legume proteins and nutraceutical properties. *Fitoterapia* 77, 67–82. doi: 10.1016/j.fitote.2005.11.008

El-Araby, M. M., El-Shatoury, E. H., Soliman, M. M., and Shaaban, H. F. (2020). Characterization and antimicrobial activity of lectins purified from three Egyptian leguminous seeds. *AMB Express* 10:90. doi: 10.1186/s13568-020-01024-4

Embaby, H. E.-S. (2010). Effect of heat treatments on certain antinutrients and in vitro protein digestibility of peanut and sesame seeds. *Food Sci. Technol. Res.* 17, 31–38. doi: 10.3136/fstr.17.31

Emire, S., and Rakshit, S. (2007). Effect of processing on antinutrients and in vitro protein digestibility of kidney bean (*Phaseolus vulgaris* L.) varieties grown in East Africa. *Food Chem.* 103, 161–172. doi: 10.1016/j.foodchem.2006.08.005

Ferreira, H., Pinto, E., Gil, A. M., and Vasconcelos, M. W. (2022). "Chapter 15—potential role of pulses in the development of functional foods modulating inflammation and oxidative stress," in *Current Advances for Development of Functional Foods Modulating Inflammation and Oxidative Stress*. eds. B. Hernández-Ledesma and C. Martínez-Villaluenga (Cambridge, Massachusetts, United States: Academic Press), 287–309.

Ferreira, H., Vasconcelos, M., Gil, A. M., and Pinto, E. (2021). Benefits of pulse consumption on metabolism and health: a systematic review of randomized controlled trials. *Crit. Rev. Food Sci. Nutr.* 61, 85–96. doi: 10.1080/10408398.2020.1716680

Filosa, S., Di Meo, F., and Crispi, S. (2018). Polyphenols-gut microbiota interplay and brain neuromodulation. *Neural Regen. Res.* 13, 2055–2059. doi: 10.4103/1673-5374.241429

Ganesan, K., and Xu, B. (2017). Polyphenol-rich lentils and their health promoting effects. *Int. J. Mol. Sci.* 18:2390. doi: 10.3390/ijms18112390

Gautam, A. K., Gupta, N., Narvekar, D. T., Bhadkariya, R., and Bhagyawant, S. S. (2018). Characterization of chickpea (*Cicer arietinum* L.) lectin for biological activity. *Physiol. Mol. Biol. Plants* 24, 389–397. doi: 10.1007/s12298-018-0508-5

Gautam, A. K., Sharma, D., Sharma, J., and Saini, K. C. (2020). Legume lectins: potential use as a diagnostics and therapeutics against the cancer. *Int. J. Biol. Macromol.* 142, 474–483. doi: 10.1016/j.ijbiomac.2019.09.119

Guillamón, E., Pedrosa, M. M., Burbano, C., Cuadrado, C., Sánchez, M. D. C., and Muzquiz, M. (2008). The trypsin inhibitors present in seed of different grain legume species and cultivar. *Food Chem.* 107, 68–74. doi: 10.1016/j.foodchem.2007.07.029

Guo, Z., Barimah, A. O., Yin, L., Chen, Q., Shi, J., El-Seedi, H. R., et al. (2021). Intelligent evaluation of taste constituents and polyphenols-to-amino acids ratio in matcha tea powder using near infrared spectroscopy. *Food Chem.* 353:129372. doi: 10.1016/j.foodchem.2021.129372

Gupta, R. K., Gangoliya, S. S., and Singh, N. K. (2015). Reduction of phytic acid and enhancement of bioavailable micronutrients in food grains. *J. Food Sci. Technol.* 52, 676–684. doi: 10.1007/s13197-013-0978-y

Gutierrez, N., Avila, C. M., Moreno, M. T., and Torres, A. M. (2008). Development of SCAR markers linked to zt-2, one of the genes controlling absence of tannins in faba bean. *Aust. J. Agric. Res.* 59, 62–68. doi: 10.1071/AR07019

Hadidi, M., Ibarz, A., and Pagan, J. (2020). Optimisation and kinetic study of the ultrasonic-assisted extraction of total saponins from alfalfa (*Medicago sativa*) and its bioaccessibility using the response surface methodology. *Food Chem.* 309:125786. doi: 10.1016/j.foodchem.2019.125786

Han, I. H., and Baik, B.-K. (2006). Oligosaccharide content and composition of legumes and their reduction by soaking, cooking, ultrasound, and high hydrostatic pressure. *Cereal Chem.* 83, 428–433. doi: 10.1094/CC-83-0428

Herrera, M. D., Acosta-Gallegos, J. A., Reynoso-Camacho, R., and Pérez-Ramírez, I. F. (2019). Common bean seeds from plants subjected to severe drought, restricted- and full-irrigation regimes show differential phytochemical fingerprint. *Food Chem.* 294, 368–377. doi: 10.1016/j.foodchem.2019.05.076

Hu, F. B. (2002). Dietary pattern analysis: a new direction in nutritional epidemiology. *Curr. Opin. Lipidol.* 13, 3–9. doi: 10.1097/00041433-200202000-00002

Hummel, M., Hallahan, B. F., Brychkova, G., Ramirez-Villegas, J., Guwela, V., Chataika, B., et al. (2018). Reduction in nutritional quality and growing area suitability of common bean under climate change induced drought stress in Africa. *Sci. Rep.* 8:16187. doi: 10.1038/s41598-018-33952-4

Iannetta, P. P. M., Hawes, C., Begg, G. S., Maaß, H., Ntatsi, G., Savvas, D., et al. (2021). A multifunctional solution for wicked problems: value-chain wide facilitation of legumes cultivated at bioregional scales is necessary to address the climate-biodiversity-nutrition nexus. *Front. Sust. Food Syst.* 5:692137. doi: 10.3389/fsufs.2021.692137

Jukema, J. W., Liem, A. H., Dunselman, P. H., van der Sloot, J. A., Lok, D. J., and Zwinderman, A. H. (2005). LDL-C/HDL-C ratio in subjects with cardiovascular disease and a low HDL-C: results of the RADAR (rosuvastatin and atorvastatin in different dosages and reverse cholesterol transport) study. *Curr. Med. Res. Opin.* 21, 1865–1874. doi: 10.1185/030079905X74952

Kalpanadevi, V., and Mohan, V. R. (2013). Effect of processing on antinutrients and in vitro protein digestibility of the underutilized legume, *Vigna unguiculata* (L.) Walp subsp. unguiculata. *LWT Food Sci. Technol.* 51, 455–461. doi: 10.1016/j.lwt.2012.09.030

Kårlund, A., Paukkonen, I., Gómez-Gallego, C., and Kolehmainen, M. (2021). Intestinal exposure to food-derived protease inhibitors: digestion physiology- and gut health-related effects. *Healthcare* 9:1002. doi: 10.3390/healthcare9081002

Karnpanit, W., Coorey, R., Clements, J., Nasar-Abbas, S. M., Khan, M. K., and Jayasena, V. (2016). Effect of cultivar, cultivation year and dehulling on raffinose family oligosaccharides in Australian sweet lupin (*Lupinus angustifolius* L.). *Int. J. Food Sci. Technol.* 51, 1386–1392. doi: 10.1111/ijfs.13094

Kataria, A., Chauhan, B. M., and Gandhi, S. (1988). Effect of domestic processing and cooking on the antinutrients of black gram. *Food Chem.* 30, 149–156. doi: 10.1016/0308-8146(88)90152-5

Kaur, D., and Kapoor, A. C. (1992). Nutrient composition and antinutritional factors of rice bean (*Vigna umbellata*). *Food Chem.* 43, 119–124. doi: 10.1016/0308-8146(92)90224-P

Khamassi, K., Faysal, B., Hobbs, D., Irigoyen, J., Stoddard, F., O'Sullivan, D., et al. (2013). A baseline study of vicine–convicine levels in faba bean (*Vicia faba* L.) germplasm. *Plant Genet. Resour.* 11, 250–257. doi: 10.1017/S1479262113000105

Khazaei, H., Purves, R. W., Hughes, J., Link, W., O'Sullivan, D. M., Schulman, A. H., et al. (2019). Eliminating vicine and convicine, the main anti-nutritional factors restricting faba bean usage. *Trends Food Sci. Technol.* 91, 549–556. doi: 10.1016/j.tifs.2019.07.051

Kouris-Blazos, A., and Belski, R. (2016). Health benefits of legumes and pulses with a focus on Australian sweet lupins. *Asia Pac. J. Clin. Nutr.* 25, 1–17. doi: 10.6133/apjcn.2016.25.1.23

Król-Grzymała, A., and Amarowicz, R. (2020). Phenolic compounds of soybean seeds from two European countries and their antioxidant properties. *Molecules* 25:2075. doi: 10.3390/molecules25092075

Kumar, S., and Pandey, G. (2020). Biofortification of pulses and legumes to enhance nutrition. *Heliyon* 6:e03682. doi: 10.1016/j.heliyon.2020.e03682

Lagarda-Diaz, I., Guzman-Partida, A. M., and Vazquez-Moreno, L. (2017). Legume lectins: proteins with diverse applications. *Int. J. Mol. Sci.* 18:1242. doi: 10.3390/ijms18061242

Lam, S. K., and Ng, T. B. (2010). Isolation and characterization of a French bean hemagglutinin with antitumor, antifungal, and anti-HIV-1 reverse transcriptase activities and an exceptionally high yield. *Phytomedicine* 17, 457–462. doi: 10.1016/j.phymed.2009.07.017

Liu, A., Contador, C. A., Fan, K., and Lam, H.-M. (2018). Interaction and regulation of carbon, nitrogen, and phosphorus metabolisms in root nodules of legumes. *Front. Plant Sci.* 9:1860. doi: 10.3389/fpls.2018.01860

Liu, Y.-M., Shahed-Al-Mahmud, M., Chen, X., Chen, T.-H., Liao, K.-S., Lo, J. M., et al. (2020). A carbohydrate-binding protein from the edible lablab beans effectively blocks the infections of influenza viruses and SARS-CoV-2. *Cell Rep.* 32:108016. doi: 10.1016/j.celrep.2020.108016

Luzzatto, L., and Arese, P. (2018). Favism and glucose-6-phosphate dehydrogenase deficiency. *N. Engl. J. Med.* 378, 60–71. doi: 10.1056/NEJMra1708111

Macarulla, M. T., Medina, C., De Diego, M. A., Chávarri, M., Zulet, M. A., Martínez, J. A., et al. (2001). Effects of the whole seed and a protein isolate of faba bean (*Vicia faba*) on the cholesterol metabolism of hypercholesterolaemic rats. *Br. J. Nutr.* 85, 607–614. doi: 10.1079/BJN2000330

Maphosa, Y., and Jideani, V. (2017). "The role of legumes in human nutrition," *Functional Food- Improve Health through Adequate Food*. ed. M. C. Hueda (London, United Kingdom: IntechOpen).

Marventano, S., Izquierdo Pulido, M., Sánchez-González, C., Godos, J., Speciani, A., Galvano, F., et al. (2017). Legume consumption and CVD risk: a systematic review and meta-analysis. *Public Health Nutr.* 20, 245–254. doi: 10.1017/S1368980016002299

Mayer Labba, I.-C., Frøkiær, H., and Sandberg, A.-S. (2021). Nutritional and antinutritional composition of fava bean (*Vicia faba* L., var. minor) cultivars. *Food Res. Int.* 140:110038. doi: 10.1016/j.foodres.2020.110038

Meletis, J. (2012). Favism. A brief history from the "abstain from beans" of pythagoras to the present. *Arch. Hellenic Med.* 29, 258–263.

Mirali, M., Purves, R. W., and Vandenberg, A. (2017). Profiling the phenolic compounds of the four major seed coat types and their relation to color genes in lentil. *J. Nat. Prod.* 80, 1310–1317. doi: 10.1021/acs.jnatprod.6b00872

Mirmiran, P., Hosseinpour-Niazi, S., and Azizi, F. (2018). Therapeutic lifestyle change diet enriched in legumes reduces oxidative stress in overweight type 2 diabetic patients: a crossover randomised clinical trial. *Eur. J. Clin. Nutr.* 72, 174–176. doi: 10.1038/ejcn.2017.113

Mitchell, T., Kumar, P., Reddy, T., Wood, K. D., Knight, J., Assimos, D. G., et al. (2019). Dietary oxalate and kidney stone formation. *Am. J. Pphysiol. Renal Physiol.* 316, F409–F413. doi: 10.1152/ajprenal.00373.2018

Mulimani, V. H., Rudrappa, G., and Supriya, D. (1994). α-Amylase inhibitors in chick pea (*Cicer arietinum* L). *J. Sci. Food Agric.* 64, 413–415. doi: 10.1002/jsfa.2740640404

Mullins, A. P., and Arjmandi, B. H. (2021). Health benefits of plant-based nutrition: focus on beans in cardiometabolic diseases. *Nutrients* 13:519. doi: 10.3390/nu13020519

Mus, F., Crook, M. B., Garcia, K., Garcia Costas, A., Geddes, B. A., Kouri, E. D., et al. (2016). Symbiotic nitrogen fixation and the challenges to its extension to nonlegumes. *Appl. Environ. Microbiol.* 82, 3698–3710. doi: 10.1128/AEM.01055-16

Muzquiz, M., Varela, A., Burbano, C., Cuadrado, C., Guillamón, E., and Pedrosa, M. M. (2012). Bioactive compounds in legumes: pronutritive and antinutritive actions. Implications for nutrition and health. *Phytochem. Rev.* 11, 227–244. doi: 10.1007/s11101-012-9233-9

Nciri, N., Cho, N., El Mhamdi, F., Ben Ismail, H., Ben Mansour, A., Sassi, F. H., et al. (2015). Toxicity assessment of common beans (*Phaseolus vulgaris* L.) widely consumed by tunisian population. *J. Med. Food* 18, 1049–1064. doi: 10.1089/jmf.2014.0120

Nissar, J., Ahad, T., Naik, H., and Hussain, S. (2017). A review phytic acid: as antinutrient or nutraceutical. *J. Pharm. Phytochem.* 6, 1554–1560.

Panda, P. K., Mukhopadhyay, S., Behera, B., Bhol, C. S., Dey, S., Das, D. N., et al. (2014). Antitumor effect of soybean lectin mediated through reactive oxygen species-dependent pathway. *Life Sci.* 111, 27–35. doi: 10.1016/j.lfs.2014.07.004

Petroski, W., and Minich, D. M. (2020). Is there such a thing as "anti-nutrients"? A narrative review of perceived problematic plant compounds. *Nutrients* 12:2929. doi: 10.3390/nu12102929

Piecyk, M., Wołosiak, R., Drużynska, B., and Worobiej, E. (2012). Chemical composition and starch digestibility in flours from polish processed legume seeds. *Food Chem.* 135, 1057–1064. doi: 10.1016/j.foodchem.2012.05.051

Polak, R., Phillips, E. M., and Campbell, A. (2015). Legumes: health benefits and culinary approaches to increase intake. *Clin. Diabetes* 33, 198–205. doi: 10.2337/diaclin.33.4.198

Popova, A., and Mihaylova, D. (2019). Antinutrients in plant-based foods: a review. *Open Biotechnol. J.* 13, 68–76. doi: 10.2174/1874070701913010068

Pulkkinen, M., Coda, R., Lampi, A.-M., Varis, J., Katina, K., and Piironen, V. (2019). Possibilities of reducing amounts of vicine and convicine in faba bean suspensions and sourdoughs. *Eur. Food Res. Technol.* 245, 1507–1518. doi: 10.1007/s00217-019-03282-4

Raes, K., Knockaert, D., Struijs, K., and Camp, J. (2014). Role of processing on bioaccessibility of minerals: influence of localization of minerals and anti-nutritional factors in the plant. *Trends Food Sci. Technol.* 37, 32–41. doi: 10.1016/j.tifs.2014.02.002

Rivas-Vega, M. E., Goytortúa-Bores, E., Ezquerra-Brauer, J. M., Salazar-García, M. G., Cruz-Suárez, L. E., Nolasco, H., et al. (2006). Nutritional value of cowpea (*Vigna unguiculata* L. Walp) meals as ingredients in diets for Pacific white shrimp (*Litopenaeus vannamei* Boone). *Food Chem.* 97, 41–49. doi: 10.1016/j.foodchem.2005.03.021

Robinson, G. H. J., Balk, J., and Domoney, C. (2019). Improving pulse crops as a source of protein, starch and micronutrients. *Nutr. Bull.* 44, 202–215. doi: 10.1111/nbu.12399

Samtiya, M., Aluko, R. E., and Dhewa, T. (2020). Plant food anti-nutritional factors and their reduction strategies: an overview. *Food Prod. Process. Nutr.* 2:6. doi: 10.1186/s43014-020-0020-5

Sánchez-Chino, X., Jiménez-Martínez, C., Dávila-Ortiz, G., Álvarez-González, I., and Madrigal-Bujaidar, E. (2015). Nutrient and nonnutrient components of legumes, and its chemopreventive activity: a review. *Nutr. Cancer* 67, 401–410. doi: 10.1080/01635581.2015.1004729

Sangronis, E., and Machado, C. J. (2007). Influence of germination on the nutritional quality of *Phaseolus vulgaris* and *Cajanus cajan*. *LWT Food Sci. Technol.* 40, 116–120. doi: 10.1016/j.lwt.2005.08.003

Santos, C., Benkeblia, N., and Vasconcelos, M. (2017). "Strategies for enhancing phytonutrient content in plant-based foods," *Phytonutritional Improvement of Crops*. ed. N. Benkeblia (Hoboken, New Jersey, United States: Wiley-Blackwell), 203–232.

Shang, R., Wu, H., Guo, R., Liu, Q., Pan, L., Li, J., et al. (2016). The diversity of four anti-nutritional factors in common bean. *Hortic. Plant J.* 2, 97–104. doi: 10.1016/j.hpj.2016.06.001

Sharma, A., and Sehgal, S. (1992). Effect of processing and cooking on the antinutritional factors of faba bean (*Vicia faba*). *Food Chem.* 43, 383–385. doi: 10.1016/0308-8146(92)90311-O

Shi, L., Arntfield, S. D., and Nickerson, M. (2018). Changes in levels of phytic acid, lectins and oxalates during soaking and cooking of Canadian pulses. *Food Res. Int.* 107, 660–668. doi: 10.1016/j.foodres.2018.02.056

Shi, L., Mu, K., Arntfield, S. D., and Nickerson, M. T. (2017). Changes in levels of enzyme inhibitors during soaking and cooking for pulses available in Canada. *J. Food Sci. Technol.* 54, 1014–1022. doi: 10.1007/s13197-017-2519-6

Shi, J., Xue, S. J., Ma, Y., Li, D., Kakuda, Y., and Lan, Y. (2009). Kinetic study of saponins B stability in navy beans under different processing conditions. *J. Food Eng.* 93, 59–65. doi: 10.1016/j.jfoodeng.2008.12.035

Siddhuraju, P., and Becker, K. (2005). Nutritional and antinutritional composition, in vitro amino acid availability, starch digestibility and predicted glycemic index of differentially processed mucuna beans (*Mucuna pruriens* var. utilis): an under-utilised legume. *Food Chem.* 91, 275–286. doi: 10.1016/j.foodchem.2004.02.044

Siener, R., Seidler, A., and Honow, R. (2020). Oxalate-rich foods. *Food Sci. Technol.* 41, 169–173. doi: 10.1590/fst.10620

Singh, B., Singh, J. P., Singh, N., and Kaur, A. (2017). Saponins in pulses and their health promoting activities: a review. *Food Chem.* 233, 540–549. doi: 10.1016/j.foodchem.2017.04.161

Smýkal, P., Coyne, C. J., Ambrose, M. J., Maxted, N., Schaefer, H., Blair, M. W., et al. (2015). Legume crops phylogeny and genetic diversity for science and breeding. *Crit. Rev. Plant Sci.* 34, 43–104. doi: 10.1080/07352689.2014.897904

Sparvoli, F., Laureati, M., Pilu, R., Pagliarini, E., Toschi, I., Giuberti, G., et al. (2016). Exploitation of common bean flours with low antinutrient content for making nutritionally enhanced biscuits. *Front. Plant Sci.* 7:928. doi: 10.3389/fpls.2016.00928

Sridhar, K., and Sahadevan, S. (2006). Nutritional and antinutritional significance of four unconventional legumes of the genus *Canavalia*—a comparative study. *Food Chem.* 99, 267–288. doi: 10.1016/j.foodchem.2005.07.049

Srikanth, S., and Chen, Z. (2016). Plant protease inhibitors in therapeutics-focus on cancer therapy. *Front. Pharmacol.* 7:470. doi: 10.3389/fphar.2016.00470

Stagnari, F., Maggio, A., Galieni, A., and Pisante, M. (2017). Multiple benefits of legumes for agriculture sustainability: an overview. *Chem. Biol. Technol. Agric.* 4:2. doi: 10.1186/s40538-016-0085-1

Świeca, M., and Baraniak, B. (2014). Influence of elicitation with H_2O_2 on phenolics content, antioxidant potential and nutritional quality of *Lens culinaris* sprouts. *J. Sci. Food Agric.* 94, 489–496. doi: 10.1002/jsfa.6274

Vashishth, R., Semwal, A. D., Naika, M., Sharma, G. K., and Kumar, R. (2021). Influence of cooking methods on antinutritional factors, oligosaccharides

and protein quality of underutilized legume *Macrotyloma uniflorum*. *Food Res. Int.* 143:110299. doi: 10.1016/j.foodres.2021.110299

Winham, D., and Hutchins, A. (2007). Baked bean consumption reduces serum cholesterol in hypercholesterolemic adults. *Nutr. Res.* 27, 380–386. doi: 10.1016/j.nutres.2007.04.017

Zhu, Z., Jiang, W., and Thompson, H. J. (2012). Edible dry bean consumption (*Phaseolus vulgaris* L.) modulates cardiovascular risk factors and diet-induced obesity in rats and mice. *Br. J. Nutr.* 108, S66–S73. doi: 10.1017/S0007114512000839

Frontiers in
Plant Science

Cultivates the science of plant biology and its applications

The most cited plant science journal, which advances our understanding of plant biology for sustainable food security, functional ecosystems and human health.

Discover the latest Research Topics

See more →

frontiers

Frontiers in
Plant Science

frontiers | Research Topics