Designed Manipulation of the Brassinosteroid Signal to Enhance Crop Yield

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Brassinosteroid (BR), a plant steroid hormone, plays crucial role in modulating plant growth and development, which affect crop architecture and yield. However, BR application cannot highly benefit to agricultural production as expectation, because it regulates multiple processes in different tissues and leads to side effect. In addition, accurately modifying BR signal at transcriptional level is difficult. Effective manipulation of the BR signal and avoidance of side effects are required to enhance yield in different crops. Application of BR by spraying at specific developmental stages can enhance crop yield, but this method is impractical for use on a large scale. The accurate molecular design of crops would be much more helpful to manipulate the BR signal in specific organs and/or at particular developmental stages to enhance crop yield. This minireview summarizes the BR regulation of yield in different crops, especially horticultural crops, and the strategies used to regulate the BR signal to enhance crop yield. One popular strategy is to directly modulate the BR signal through modifying the functions of important components in the BR signal transduction pathway. Another strategy is to identify and modulate regulators downstream of, or in crosstalk with, the BR signal to manipulate its role in specific processes and increase crop yield. Efforts to accurately design a BR manipulation strategy will ultimately lead to effective control of the BR signal to avoid side effects and enhance crop yield.

Keywords: crop yield, brassinosteroid, manipulation, crop design, side effect

INTRODUCTION

High crop yield is an essential goal of agriculture and is important for food security. The yield of different crops depends on the specific organs harvested. Seed yield (grain yield) is important for cereal crops such as rice (*Oryza sativa*), wheat (*Triticum aestivum*), barley (*Hordeum vulgare*), and maize (*Zea mays*). Seed yield is also important for some non-cereal horticultural and oilseed crops such as pea (*Pisum sativum*), soybean (*Glycine max*), and canola (*Brassica napus*). Farmers harvest fruits and seeds for food or produce seedlings, oils, or other derivatives. Breeders and scientists try to balance the vegetative and reproductive growth of these crops to increase the total seed yield. In contrast, vegetables produce roots,

leaves, and stems for human consumption. For vegetables, the ideal conditions direct nutrition and energy into vegetative development and limit nutrient allocation to reproductive development.

The plant steroid hormone brassinosteroid (BR) functions in plant cell elongation and division, tissue differentiation, organogenesis, reproductive development, metabolism, photomorphogenesis, stress tolerance, and plant immunity (Morinaka et al., 2006; Sakamoto et al., 2006; Ahammed et al., 2012a; Ahammed et al., 2012b; Zhu et al., 2015; Zhou et al., 2015). In *Arabidopsis*, BR regulates seed development and affects the seed number and size/weight by transcriptional modulation of genes and pathways regulating ovule and seed development (Schruff et al., 2006; Ohto et al., 2009; Zhou et al., 2009; Huang et al., 2013; Jiang et al., 2013). In rice, BR regulates plant architecture and grain yield (Fujioka and Yokota, 2003). The gene products of *D11* and *BRD1* function in BR biosynthesis and affect rice plant height (Hong et al., 2002; Tanabe et al., 2005). The BR-deficient mutant *d2* has erect leaves (Hong et al., 2003), indicating that BR affects leaf bending. Increased BR levels lead to enhanced tiller number, larger spikes, and increased grain number (Wu et al., 2008). In addition, BR positively regulates grain size/weight (Hong et al., 2005; Tanabe et al., 2005; Morinaka et al., 2006; Sahni et al., 2016).

Although BR positively regulates seed/grain yield, its application cannot highly contribute to agricultural production as expectation. The genes in the BR biosynthesis and signal transduction pathways are expressed constitutively. BR biosynthesis can be regulated by light, circadian cycles, and BR itself (Bancos et al., 2002; Bancos et al., 2006). Rapid alkalinization factor1 (RALF1), TEOSINTE BRANCHED1/CYCLOIDEA/PROLIFERATING CELL FACTOR1 (TCP1), CESTA (CES), PHYTOCHROME INTERACTING FACTOR4 (PIF4), and COGWHEEL1 (COG1) were reported to influence DWF4 expression and BR content (Guo et al., 2010; Poppenberger et al., 2011; Bergonci et al., 2014; Gao et al., 2015; Wei et al., 2017). Regulating the activity of BR signal components also modulates BR signal. The BR receptor BRASSINOSTEROID INSENSITIVE1 (BRI1, a cell-surface receptor kinase) (Friedrichsen et al., 2000; Wang et al., 2001), BRI1-ASSOCIATED RECEPTOR KINASE1 (BAK1) (Li et al., 2002; Nam and Li, 2002), BR-signaling kinases1 (BSK1) (Tang et al., 2008), Constitutive Differential Growth1 (CDG1) (Muto et al., 2004; Kim et al., 2011), BRI1 suppressor 1 (BSU1) (Kim et al., 2009), BR-induced transcription factors BRASSINAZOLE RESISTANT1 (BZR1) (Wang et al., 2002) and BRI EMS SUPPRESSOR1 (BES1) (Yin et al., 2002), and PP2A (Tang et al., 2011) are positive regulator of BR signal, and increasing their activity leads to enhanced BR signal. BRI1 Kinase Inhibitor 1 (BKI1) (Wang and Chory, 2006), BRASSINOSTEROID INSENSITIVE2 (BIN2, a GSK3-like kinase) (Li et al., 2001; He et al., 2002; Li and Nam, 2002) and phosphopeptide-binding 14-3-3 proteins (Bai et al., 2007; Gampala et al., 2007) are negative regulators of BR signal, and increasing their activity leads to reduced BR signal. Analyses of the regulatory mechanism indicates that they are regulated mainly at the post-translational level, except for BR induced transcription factors, which could be regulated also at transcription level (Jiang et al., 2015).

In rice, the total grain yield is determined by the grain yield of individual plants (grain number and weight) and planting density (total panicle number per unit area). BR positively regulates grain number and weight by contributing to better nutrition and higher efficiency of carbohydrate transportation from source to sink, but it negatively regulates panicle number because it induces leaf bending and lodging (Choe et al., 2001; Wu et al., 2008). A slightly reduced BR signal may enhance total yield though increasing planting density and lodging resistance, but these benefits may easily be out-weighed by decreased reproductive development. These negative side effects discourage the use of BR in agricultural production. The accurate molecular design of crops would be much more helpful to manipulate BR signaling in specific organs and/or at particular developmental stages to enhance crop yield.

REGULATION OF YIELD BY BRASSINOSTEROID IN DIFFERENT CROPS

Brassinosteroid regulation of yield in rice has been well summarized, especially in rice (Tong and Chu, 2018; Castorina and Consonni, 2020). BR regulation can affect the yield in other crops. For example, in maize, the BR signal at the seedling stage affects field traits and yield (Hu et al., 2017). Application of BR was found to significantly increase maize plant height during the early weeks after treatment, but it positively or negatively regulated yield depending on different genotypes or developmental stages (Holá et al., 2010). The maize gene *ZmRAVL1* was shown to regulate BR C-6 oxidase1 and alter the endogenous BR content and leaf angle, which enhanced yield by increasing plant density (Tian et al., 2019). In barley, loss of function of BR-biosynthesis genes (*BR-6-oxidase*, *CPD*, and *DIMINUTO*) and BR-signaling gene (*HvBRI1*) lead to dwarf phenotype, indicated BR signal modification would be potential genetic building blocks for breeding strategies with sturdy and climate-tolerant barley cultivars (Chono et al., 2003; Dockter et al., 2014; Janeczko et al., 2016). Comparing to poaceae crops, the functions and regulatory mechanism of BR signal in the yield regulation of horticulture crops still less studied. In cotton, fiber growth was promoted by treatment with BR, but inhibited by treatment with the BR biosynthesis inhibitor brassinazole (BRZ) (Shi et al., 2006). In tomato, the BR biosynthetic gene *DWF* affects fruit yield by regulating architecture, and the BR level was found to be positively correlated with carotenoid accumulation in the fruits (Li et al., 2016). BR was found to promote tomato yield through enhanced autophosphorylation of SlBRI1, and increased plant expansion, leaf area, fruit weight, and number of fruits per cluster (Wang et al., 2019). Brassinolide (BL), 28-homobrassinolide (28-hBL), and 24-epibrassinolide (24-eBL) treatments stimulated both the growth and yield of tomato plants (Vardhini and Rao, 2001). In pea, the level of biologically active BRs (BL and castasterone, CS) were found to

peak during seed development, indicating that BR is important for seed development (Nomura et al., 2007). The seed yield, seed number, and seed protein contents of pea were shown to increase in response to 10 μM EBL treatment (Shahid et al., 2011). In soybean, 24-eBL treatment of seeds before sowing positively influenced seed parameters, including weight (Procházka et al., 2016). In lettuce, treatments with BR analogs increased production as a result of an increase in weight (enhanced diameter and length) (Zhang et al., 2007). Spraying with the BR analog DI-31 increased the yield of field-grown pepper due to an increase in the number of fruits per plant (Serna et al., 2012). In cucumber, BR was found to play an essential role during early fruit development. Application of EBL in a cultivar without parthenocarpic capacity induced cell division and parthenocarpic growth, whereas BRZ treatment in a cultivar with natural parthenocarpic capacity inhibited fruit set and subsequently fruit growth. This inhibitory effect could be rescued by application of EBL (Fu et al., 2008). In fenugreek, 0.50 ppm BR applied as a foliar spray increased seed yield by 14.6%, compared with that of the mock control (Godara et al., 2017). In radish, BR stimulated radish growth, which was associated with increased levels of carbohydrates, soluble proteins, vitamins, ascorbic acid, and niacin (Vardhini et al., 2011). Exogenous application of 24-eBL and 28-hBL significantly ameliorated the total protein content in *Brassica juncea* seedlings, as compared with untreated control seedlings (Sirhindi et al., 2009). Treatment of the onion cultivar Alice with synthetic BL increased the mass of individual bulbs and yield, and reduced the negative impact of water deficit (Doležalová et al., 2016). Spraying with BR at 0.1 ppm at the second and fourth leaf stages increased the yield of watermelon, and significantly affected quality parameters including total soluble solids, total sugars, and lycopene content (Susila et al., 2012). In strawberry, BR treatments increased fruit yield by 9%–34%, and increased the proportion of marketable fruits (Salazar et al., 2016). A spray treatment of yellow passionfruit plants with the BR analog BB-16 increased the number of fruits per plant, and the treatment in the three consecutive weeks after the appearance of the first flowers was found to be the most efficient (Gomes et al., 2006).

Besides direct regulation, BR can also indirectly influence yield *via* its effects on crop growth and development, architecture, and stress tolerance. A BR-deficient dwarf mutant of faba bean (*Vicia faba* L.), Rineri, was characterized by dark-green leaves and reduced plant height, internode and petiole length, shoot weight, and number of branches. This gene was identified as a homolog of *Arabidopsis dwf1* and pea *lkb* (Fukuta et al., 2004). In groundnut, BR treatment alone and in combination with BA affected *in vitro* establishment and promoted various physiological parameters that affect growth (Verma et al., 2011). Priming of lucerne seeds with BL significantly increased the shoot fresh weight, shoot dry weight, root dry weight, root length, and root vigor. The primed seeds also showed reduced malondialdehyde accumulation, suggesting that a suitable concentration of BL can improve germination and

seedling growth in saline soils (Zhang et al., 2007). In potato, treatment with 0.5 μM gibberellic acid (GA), 0.1 μM napthaleneacetic acid (NAA), and 0.1 μM EBL was found to be the best combination for increasing node number, branch number, leaf number, shoot length, and survival in culture (Basera et al., 2018). Application of BR advanced maturity of winter rapeseed by 4 to 8 days (Wan et al., 2017). Grape berry ripening was promoted by application of BR and delayed by application of BRZ (Symons et al., 2006). Treatment with EBL also significantly promoted the activities of acidic and neutral invertases and sucrose synthase (sucrolytic) at various stages of grape ripening, and increased the soluble sugars content in the berries (Xu et al., 2015). Treatment with BR reduced decay of jujube fruits, possibly because of its effects to induce disease resistance and delay senescence (Zhu et al., 2010).

IMPROVING TREATMENT EFFICIENCY

As mentioned above, the traditional BR treatment method is direct spraying onto crop plants, especially onto reproductive organs, to affect seed yield. Spraying is a more popular method than the addition of BR to the growth medium, because BR is not effectively transported over long distances *in vivo* (Montoya et al., 2005). Therefore, the addition of BR to the growth medium may affect roots, but it cannot be expected to affect other organs. However, spraying has various limitations. It is expensive, inefficient, unstable, and it cannot be conducted on a large scale. A simple and stable method with higher efficiency (Li et al., 2018) was developed to do BR treatment, but it cannot be used conveniently for production. Instead, it is more suitable for research on BR-specific regulation of reproductive development. Some BR analogs are available as commercial pesticides that can be applied by spraying with a relatively low cost (e.g., HBL, Panpan Industry Co. Ltd., Cas. No. 74174-44-0). However, pesticides are generally impure with low efficiency and have unclear side effects. Therefore, practical and highly efficient methods are still needed. For crops without established transformation systems, exogenous BR treatment is still a practical method. However, just as there are concerns about pesticide residues, there are concerns about residues of directly applied hormones. Interestingly, after treated externally by BR, residues of common organophosphorus, organochlorine, and carbamate pesticides 30% to 70% decreased on tomato, rice, tea, broccoli, cucumber, strawberry, and other plants (Zhou et al., 2015), indicating BR also functioned in increasing crop quality.

EFFECTIVE MODULATION OF BRASSINOSTEROID SIGNAL IN SPECIFIC ORGANS TO ENHANCE YIELD

Previous studies have reported that enhancing the BR signal by overexpression of BR biosynthesis genes can contribute to

improved nutritional status, increased carbohydrate transport from source to sink tissues, and higher grain yield. However, an overall increase in BR content can also result in larger laminar joints and increased height, leading to reduced lodging resistance and decreased planting density (Choe et al., 2001; Wu et al., 2008). A higher level of BR from exogenous treatment does not necessarily increase the BR signal *in planta*. Therefore, effective modulation of the BR signal in specific organs is needed to enhance yield.

Overexpression of BR biosynthesis genes can increase the BR content and signal in many tissues and organs, but can also lead to feedback inhibition of the BR signal (Kim et al., 2006). Although tissue-specific promoters have been used to drive expression of BR biosynthesis genes, the BR signal in specific organs is not regulated effectively (Wu et al., 2008). Modifying the functions of components of the BR signal transduction

pathway is an alternative strategy to influence the BR signal. Phenotypic analysis illustrated that modifications of BRI1, BIN2, and BZR1/BES1 are highly efficient to modulate BR signal. The gain-of-function mutant of BIN2, *bin2-1*, shows phenotypes associated with reduced BR signaling, similar to *bri1* (Li et al., 2001). And the gain-of-function mutant of BZR1, *bzr1-1D*, shows phenotypes associated with enhanced BR signaling (Wang et al., 2002; He et al., 2005). In further analyses, point mutations of the sequences of *bin2-1* and *bzr1-1D* were sufficient to decrease or increase BR signaling. And these two gain-of-function coding sequences derived by a tissue specific and high transcribed promoter were sufficient to reduce or enhance BR signal in the specific tissue. The transformation systems are designed for modulating the priority of the BR signal in reproductive development, and these systems in *Arabidopsis* have been successively established (**Figure 1**; Zu et al., 2019).
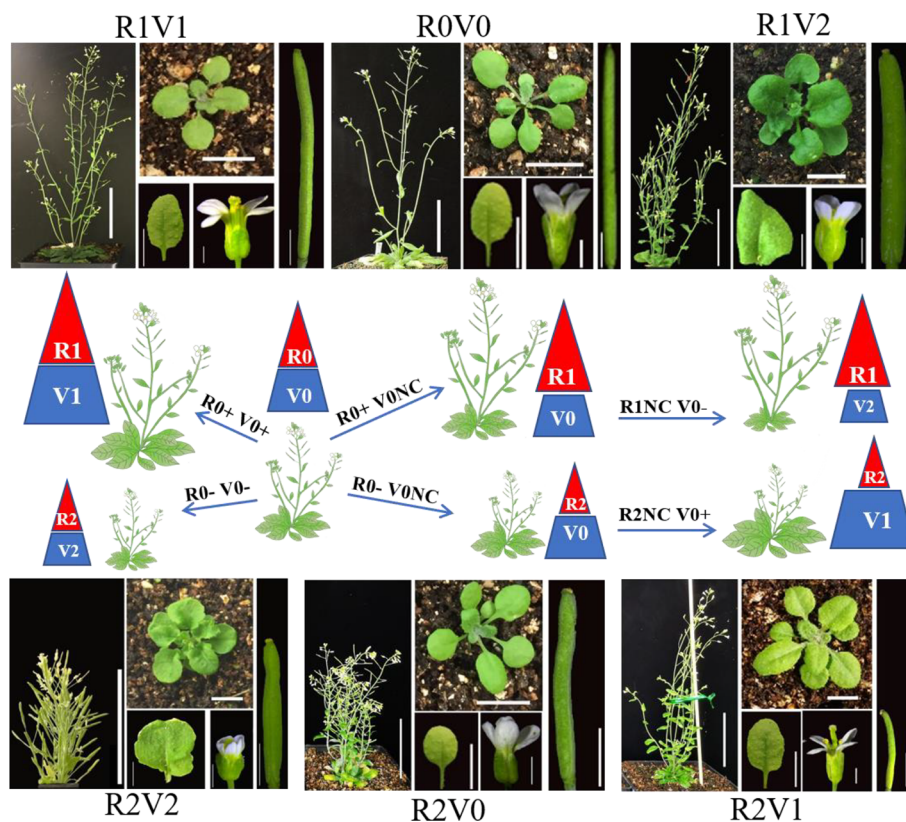

FIGURE 1 | Designed manipulation of brassinosteroid signal to enhance seed and leaf biomass of Arabidopsis. R, reproductive organs; V, vegetative organs; +, increase; −, decrease; NC, no change. 0, original BR signal and normal organ size; 1, enhanced BR signal and increased organ size; 2, reduced BR signal and decreased organ size. R+ and R− indicate increased and decreased size of reproductive organs respectively, which lead to R1 and R2. V+ and V− indicate increased and decreased size of vegetative organs, respectively, which lead to V1 and V2, respectively. R0NC and V0NC indicate no change in the size of reproductive and vegetative organs, respectively. R1V2 suggests enhanced BR signal and increased size of reproductive organs, but reduced BR signal and decreased size of vegetative organs, which is the optimum combination of total seed production of the unit area. R2V1 suggests enhanced BR signal and increased size of vegetative organs, and reduced BR signal and decreased size of reproductive organs, which is the optimum combination of leaf production with enhanced lodging resistance. Adult plants, bar = 5 cm. Rosette leaves, bar = 1 cm. Floral organs, bar = 2 mm. Siliques, bar = 2 mm. The pictures in **Figure 1** reorganized from the author's article of Zu et al., 2019, Frontiers in Plant Science.

The optimization of seed mass requires transformation with a combination of a reproductive tissue-specific promoter and coding sequences to increase the BR signal, in a genetic background with slightly reduced BR signal (with enough nutrition accumulation). In this system, the enhanced BR signal generated in transformants is targeted to reproductive organs without affecting vegetative organs, thus avoiding side effects (**Figure 1**). The optimization of leaf mass requires transformation with a combination of a reproductive tissue-specific promoter and coding sequences that decrease the BR signal in a genetic background with enhanced BR signal. The reduced BR signal generated in transformants is targeted to reproductive organs without affecting vegetative organs. The growth of the inflorescence stem is inhibited, lodging is reduced, and nutrition is directed to leaf growth (**Figure 1**). These two systems also could be used in research on BR-specific regulation of plant reproductive development. Trialing new combinations of other tissue-specific promoters and genetic backgrounds may provide new systems to enhance the biomass of aimed organs. Because BR signal transduction and BR regulation of growth and development are essentially conserved in *Arabidopsis*, rice, soybean, and other plants (Nakamura et al., 2006; Sakamoto et al., 2006; Bai et al., 2007; Tanaka et al., 2009; Zhang et al., 2016), systems that are effective for *Arabidopsis* could be expanded to other crops to enhance crop yield, especially eudicots. *Arabidopsis* BR-related mutants are useful genetic tools to identify the regulators of BR signals in other species.

IDENTIFICATION AND FUNCTIONAL CHARACTERIZATION OF REGULATORS INVOLVED IN BR-REGULATED PLANT GROWTH AND DEVELOPMENT

Besides directly manipulating the components of the BR signal transduction pathway, modifying regulators downstream of, or in crosstalk with, the BR signal may also help to increase crop yield through gently manipulating the BR signal. These regulators are mainly identified in rice. ILI1, IBH1 (Zhang et al., 2009), CSA (Zhu et al., 2015) function downstream of BR signaling and are involved in BR regulation of growth and development. GRF4 (Duan et al., 2015) and BU1 (Tanaka et al., 2009) are involved in other signaling pathways but also function in BR-mediated regulation of rice growth and yield. Some of them primarily regulate specific processes but do not affect others, suggesting that they could be useful modules for crop design. The new identified player, OsGATA7 regulates rice architecture more than grain yield (Zhang et al., 2018). The genome-edited lines of *OsGATA7* showed condensed architecture but only slightly decreased the accumulation of photosynthate. Therefore, the total yield was increased *via* increased planting density, even though the grain yield per plant was slightly decreased. Most of the above genes have homologs in *Arabidopsis*, and their functions are conserved in BR signaling and BR regulation of growth and development

(Zhang et al., 2009; Luo et al., 2010). Thus, strategies developed for *Arabidopsis* may be applicable to other crops to enhance crop yield.

FUTURE DIRECTIONS

Taken together, there are three ways to modulate BR signal and subsequently enhance crop yield. First, improving treatment efficiency in some horticultures without efficient transformation. Second, directly modulating BR signal in specific organs by tissue specific promoters and modifying coding sequences of BR signaling components. Third, identifying new regulators involved in BR-responsive plant growth and development and manipulating BR signal in specific organs by modifying new regulators. Even transgenic crops are limited in some species, and in some countries, the investigation of known components and new regulators involving in BR regulation of crop yield also give clues to screen putative candidate varieties containing good natural mutation. Accurate manipulation of the BR signal will avoid side effects and enhance crop yield. The coding sequences functioned in effective enhancing BR signal (like *bzr1-1D* and other modified sequences of new regulators) in reproductive organs and properly reducing BR signal (like *bin2-1* and *OsGATA7* editing sequence, as well as other modified sequences of new regulators) in vegetative organs contribute to the increased total seed/grain yield. Besides the BR signal, other signals can also be manipulated *via* molecular design. For example, increasing cytokinin signal in panicle enhance grain number (Ashikari et al., 2005), and it would be an effective module to enhance grain yield. Practical and highly efficient treatment methods are still needed for some crops that lack transformation systems. The overall aim of the molecular design is to develop smart crops with enhanced yield.

AUTHOR CONTRIBUTIONS

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