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Review

Leveraging brassinosteroids towards the next Green Revolution

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The use of gibberellin-related dwarfing genes significantly increased grain yield during the Green Revolution. Brassinosteroids (BRs) play a vital role in regulating agronomic traits and stress resistance. The potential of BR-related genes in crop improvement has been well demonstrated, positioning BRs as crucial targets for the next agricultural biotechnological revolution. However, BRs exert pleiotropic effects on plants, and thus present both opportunities and challenges for their application. Recent research suggests promising strategies for leveraging BR regulatory molecules for crop improvement, such as exploring function-specific genes, identifying beneficial alleles, inducing favorable mutations, and optimizing spatial hormone distribution. Advancing our understanding of the roles of BRs in plants is imperative to implement these strategies effectively.

The potential of BRs for the next Green Revolution

The Green Revolution of the 1960s unintentionally utilized gibberellin (GA)-related genes to breed dwarf varieties of crops, resulting in a substantial increase in global grain yields [1,2]. However, the compromised GA functions led to reduced nitrogen use efficiency (NUE), causing the Green Revolution varieties (GRVs) to heavily rely on nitrogen fertilizers and resulting in significant environmental consequences [3,4]. In addition, given that the world population now surpasses 8 billion, there is an urgent need to produce more food from a limited amount of arable land. Furthermore, growing concerns about global food security are amplified by climate change worldwide. These circumstances necessitate solutions, preferably through molecular design approaches, to develop the next generation of GRVs – high-yielding, stable crops that require fewer resources such as fertilizers, pesticides, water, and labor.

In this context, BRs, a group of polyhydroxylated plant steroid hormones that were discovered two decades later than the Green Revolution, emerge as a promising avenue for achieving the goals of the next Green Revolution. BRs play vital roles in regulating essential traits for crop breeding, some of which are not significantly modulated by GA [5]. BRs are also involved in responses to environmental conditions such as light, water, and nutrient availability, as well as various stressors including salt, drought, cold, and heat. Extensive research conducted over recent decades has primarily focused on understanding BR homeostasis and signaling pathways in model plants such as arabidopsis (*Arabidopsis thaliana*) and rice (*Oryza sativa*). The utilization of BR-related genes has proved to be effective in improving various major crop species [6–10], highlighting the enormous potential of BRs in driving the next Green Revolution.

In this updated review, building upon an earlier work [5], we present an overview of the latest advances in understanding BR signaling and its functions in crops. In addition, we summarize the current status of molecular design using BR genes and propose strategies for their effective utilization. Given the imbalances in research coverage among different species, our primary focus is

Highlights

Brassinosteroids (BR) signaling pathway is believed to be largely conserved among different species, although some components, steps, or events may differ between rice and *Arabidopsis thaliana*.

BRs play essential roles in regulating both yield and stress-related traits, as well as in environmental adaptability, and thus hold promise for producing high-yielding and stable crops with less input.

BR genes have been shown to be valuable for crop improvement employing various approaches. One prevalent method is to enhance the planting density of rice, maize, and wheat.

Strategies such as exploring function-specific genes, identifying beneficial alleles, inducing favorable mutations, and optimizing the spatial hormone distribution can facilitate the utilization of BR genes towards the next Green Revolution.

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on major cereal crops, with a particular emphasis on rice, while also incorporating relevant studies from other species for discussion purposes. For clarity, we include the abbreviated species name before each gene/protein name, where applicable.

An update on the BR signaling pathway in rice

BR recognition in rice predominantly involves the leucine-rich repeat receptor-like kinase (LRR-RLK) OsBRI1, in conjunction with coreceptor SERK-family proteins (OsSERKs), located on the cell membrane. The signal is subsequently transmitted downstream through a range of protein families, including BSK kinases (OsBSKs), PPKL phosphatases (OsPPKLs), GSK3/SHAGGY-like kinases (OsGSKs), and BZR-family transcription factors (OsBZRs), via processes such as phosphorylation and dephosphorylation. Although the majority of BR signaling events in this pathway are conserved between rice and arabidopsis, there may be specific variations in rice compared to arabidopsis (Box 1). Furthermore, some components predicted from arabidopsis BR signaling, such as AtCDG kinases and AtBKI1, are currently unidentified in rice. Each step of this **primary signaling pathway** (see Glossary) can be regulated by additional modulators employing diverse mechanisms (Figure 1).

On the cell membrane, OsSLA1, an LRR-RLK that lacks detectable kinase activity, interacts with OsBRI1 and OsSERK1 (also known as OsBAK1), and facilitates their interaction [11]. By contrast, the wall-associated receptor kinase OsWAK11, which responds to changes in cell wall pectin methyl-esterification, phosphorylates OsBRI1, thus inhibiting the OsBRI1–OsSERK1 interaction [12]. This suggests the existence of a cell wall-derived cue that fine-tunes BR signaling, as observed in arabidopsis [13]. It is worth noting that the OsBRI1 receptor undergoes rigorous **protein quality control** in the endoplasmic reticulum, where misfolded or incompletely folded OsBRI1 is degraded through the ubiquitination system involving the E2–E3 enzyme pair OsSMG3 and OsDGS1 [14]. The elimination of faulty OsBRI1 may enhance the production or activity of mature OsBRI1, thereby promoting BR signaling.

Glossary

Feedback regulatory effect: also known as feedback regulation or feedback control, refers to the mechanism by which the output of a system is used to modulate the input to that system.
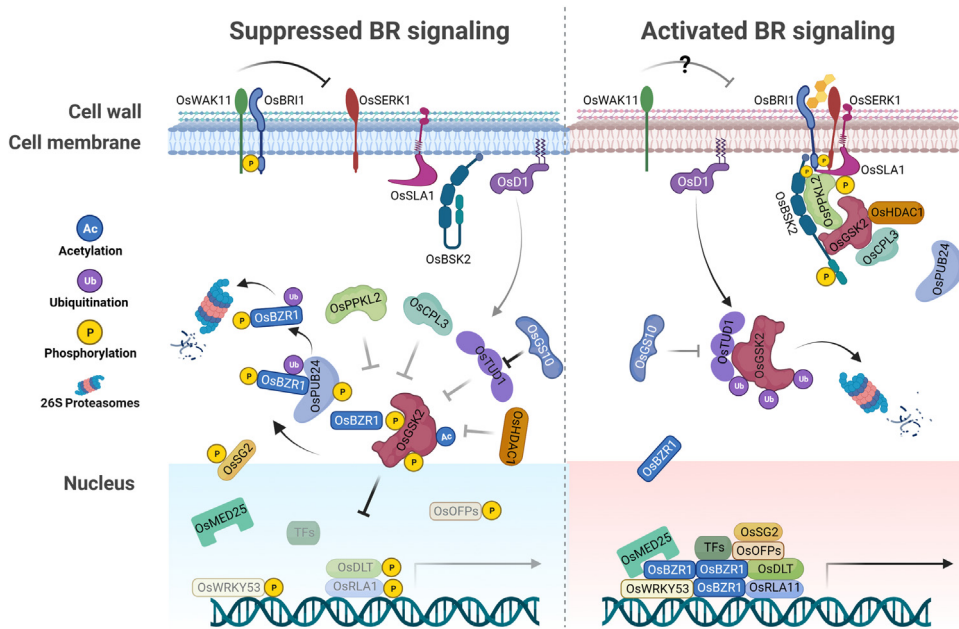**Internode organization pattern:** the arrangement and spacing of different internodes, which are the segments or sections of a plant stem located between two adjacent nodes.
Microtubule system: the dynamic network of intracellular microtubules that are composed of tubulin protein subunits, and which play essential roles in various cell activities such as cell division and cell shape maintenance.
Primary signaling pathway: the main pathway or key mechanism through which the signal is transmitted. In this context, it refers to the BRI1-mediated canonical brassinosteroid (BR) signaling pathway.
Protein quality control: a set of cellular mechanisms and processes that ensure the proper folding, assembly, and degradation of proteins to maintain their functional integrity.
Subspecies divergence: the process by which populations of a species differentiate over time, leading to the formation of distinct subspecies or geographic races.

Box 1. Non-conserved BR signaling steps in rice

The roles of PPKLs in BR signaling has garnered significant attention in both arabidopsis (AtBSU1, AtBSL1, AtBSL2, and AtBSL3) and rice (OsPPKL1, OsPPKL2, and OsPPKL3). Initially, AtBSU1 was identified as a BR signaling component with a hypothetical role in dephosphorylating AtBES1 (also known as AtBZR2) [85]. Subsequent studies revealed that AtBSU1 and AtBSL1 are actually responsible for dephosphorylating the GSK3-like kinase AtBIN2 [86], and AtPPKLs can form hetero-oligomers to exert their function [87]. Although genetic evidence supports functional redundancy among AtPPKLs, closer examination suggests that AtBSL2/3 might have unknown roles beyond BR signaling [21].

In rice, OsPPKL2 corresponds to OsBSL1, and OsPPKL1/3 corresponds to AtBSL2/3, whereas BSU1 is only present in Brassicaceae [21]. OsPPKL1 has been found to dephosphorylate OsGSK3, thereby stabilizing the BR signaling inhibitor [88]. In an *in vitro* kinase assay, OsBSK3 appears to inhibit the phosphatase activity of OsPPKL1 [19]. Another *in vitro* assay indicates that OsBSK1-1 may inhibit the kinase activity of OsGSK2 [17]. These findings suggest that there is a distinct BR signaling pathway in rice, that is unlike the well-established pathway in arabidopsis where AtBSK1 promotes AtBSU1 to suppress AtBIN2 [86]. It should be noted that, in line with the additional roles of AtBSL2/3, OsPPKL1 also significantly inhibits cytokinin signaling [80]. Given the dual regulation of two hormonal pathways by one protein, it becomes challenging to separate the dual roles of OsPPKL1 for further analysis. In addition, there is a suggestion that AtBSKs serve as scaffold proteins for integrating BR signaling components in arabidopsis [89]. Consistently, some OsBSKs can interact with both OsGSKs and OsPPKLs [18,89,90]. Considering the insights gained from AtPPKLs, the kinase activities and the roles of OsPPKLs require further clarification. The production and analysis of higher-order mutants will be essential for a comprehensive understanding of these complexities.

A notable difference lies in the step in which OsGSK2 is regulated by OsTUD1, in contrast to arabidopsis where the F-box ubiquitin ligase AtKIB1 is responsible for the degradation of AtBIN2 [24,91]. Interestingly, AtKIB1 and its closest rice homolog exhibit relatively low sequence similarity, whereas OsTUD1 and its arabidopsis homologs share a much higher protein similarity [24]. This raises the possibility that the arabidopsis homologs of OsTUD1 may similarly regulate AtBIN2, adding an intriguing perspective to explore.

Figure 1. Overview of the update on brassinosteroid (BR) signaling in rice. Without BRs or with low BRs, OsGSK2 phosphorylates OsBZR1 to retain it in the cytoplasm, and phosphorylates OsPUB24 to induce ubiquitination and degradation of OsBZR1 via the 26S proteasome (suppressed BR signaling). Perception of BRs by the OsBRI1–OsSERK1 receptor complex activates downstream BR signaling, where OsBSK2 is a scaffold protein that recruits OsPPKL2 and OsGSK2. OsPPKL2 then dephosphorylates OsGSK2, and then the BR signal induces OsTUD1 potentially through OsGPA1 to degrade OsGSK2, leading to the accumulation of OsBZR1 in the nucleus where it activates BR responses (activated BR signaling). Each step is regulated by additional components involving diverse mechanisms. On the membrane, OsBRI1–OsSERK1 interaction is modulated by OsWAK11, which responds to cell-wall status, and by OsSLA1. In the cytoplasm, the activity or stability of OsGSK2 is modulated by OsCPL3 and OsHADC1, and OsGS10 suppresses that of OsTUD1. In the nucleus, the activity of OsBZR1 is directly or indirectly affected by OsMED25, OsRLA1, and OsDLT, as well as a number of additional transcription factors (TFs), potentially forming a protein complex. Figure created with BioRender.com.

Several OsSERKs, including OsSERK1 [15] and OsSERK2 [16], as well as multiple OsBSKs such as OsBSK1-1 [17], OsBSK2 [18], and OsBSK3 [19], were shown to be required for rice BR signaling through mutant analysis. In addition, a comprehensive examination of higher-order mutants has confirmed the conserved and redundant functions of OsGSKs and OsBZRs in BR signaling [20]. However, the roles of OsPPKLs in BR signaling seem to be more diverse (Box 1). Nevertheless, it is highly probable that OsPPKL2 plays a conserved role in promoting BR signaling, similarly to AtPPKLs (known as AtBSU1 and AtBSLs) in arabidopsis [20,21]. Another phosphatase, OsCPL3, dephosphorylates OsGSK2 to destabilize the protein [22]. In addition, the histone deacetylase OsHDAC1 interacts with and deacetylates OsGSK2 to inhibit its kinase activity [23]. Moreover, BRs can (potentially through the G protein subunit OsRGA1, also known as OsD1) induce the accumulation of the U-box ubiquitin ligase OsTUD1, which promotes the ubiquitination and degradation of OsGSK2 [24,25]. Furthermore, OsGS10, containing six tandem Armadillo repeats, interacts with OsTUD1 and inhibits this process [26]. Therefore, both the stability and activity of OsGSK2 – the central inhibitor of BR signaling – are tightly controlled by multiple mechanisms (Figure 1). However, it remains unclear how the various controls acting on OsGSK2 are integrated.

OsGSK2 phosphorylates several substrates to suppress various BR responses, and OsBZRs is considered to be the key transcription factors in BR signaling. OsGSK2 enhances the stability of

the U-box ubiquitin ligase OsPUB24, which promotes the ubiquitination and degradation of OsBZR1 [27]. Degradation of OsGSK2 induces the accumulation of OsBZR1 in the nucleus, thereby influencing the expression of downstream BR-responsive genes. The mediator subunit OsMED25 interacts with OsBZR1 to facilitate transcriptional regulation [28]. Other transcription factors or regulators, such as OsRLA1 (an AP2 transcription factor, also known as OsSMOS1), OsDLT (a GRAS-family protein, also known as OsGRAS32), OsWRKY53 (a WRKY family transcription factor), OsSG2 (RNase H-like protein), and some OsOFPs (Ovate family proteins), may also participate in this process directly or indirectly, potentially by forming a transcription protein complex [29–33]. Currently, comprehensive identification of BR-responsive genes in rice remains limited.

The functions of BR in regulating agronomic traits

Understanding the functions of BR in regulating key agronomic traits is significant for effectively utilizing BRs or BR-related genes. A common morphological characteristic observed in BR-defective mutants across different species is their dwarf status, compact architecture, and size reduction of various organs. However, given the fundamental role of steroid hormones in regulating cell growth and their extensive crosstalk with other phytohormones, BRs are involved in virtually every aspect of plant growth and development. Nonetheless, the functions of BRs exhibit some unique features associated with important agronomic traits in crops (Figure 2), thus highlighting the intrinsic value and significant potential of the hormone in agricultural biotechnology.
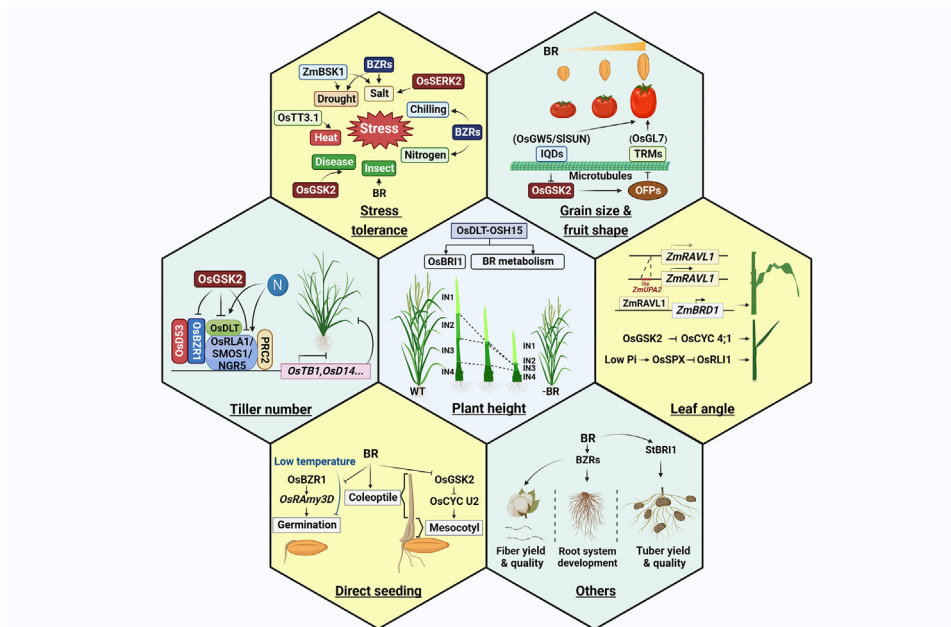
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Figure 2. Overview of updated brassinosteroid (BR) regulation of key traits. Progress in understanding BR regulation of key breeding traits and the main molecular events or pathways are indicated. BR regulation of tolerance to various stresses remains poorly understood (see Box 2 for more details). The regulation of fruit shape involves a mechanism similar to grain size. Regulation of germination, coleoptile, and mesocotyl is associated with direct seedling of rice, and thus is shown as a labor-saving trait. BR signaling is crucial in regulating fiber, root, and tuber development in other tissues or species. Abbreviations: IN, internode; IQD, IQ67 domain protein; N, nitrogen; Pi, inorganic phosphate; TRM, TONNEAU 1 recruiting motif protein; WT, wild type. Figure created with BioRender.com.

A BR deficit often leads to the specific shortening of the lower internodes, particularly the second internode located below the uppermost internode. An early study proposed that this unique **internode organization pattern** could be attributed to differential BR sensitivity, which is determined by the expression of *OsBRI1* in different internodes [34]. Supporting this hypothesis, it was recently found that OsDLT interacts with OSH15 (*Oryza sativa* homeodomain protein), which functions in the lower internodes, to regulate rice internode elongation by targeting and promoting the expression of *OsBRI1* [35]. Intriguingly, BR content exhibited contrasting trends in the young panicles and internodes of *dlt* or *osh15* mutants, or in the double mutant, that corresponded to the shortened internodes and increased grain sizes [35]. This unexpected observation indicates that the well-known **feedback regulatory effect** between BR signaling and BR metabolism differs between tissues, and can be either inhibitory or stimulatory. Therefore, BRs coordinate the elongation process of distinct internodes by orchestrating BR signaling and metabolism, thus providing insights into customized molecular designs for targeting specific internodes.

BRs play a significant role in regulating grain size, morphology, and fruit shape, which are the crucial yield-determining and quality-associated traits. In species such as rice and tomato (*Solanum Lycopersicum*), augmenting the BR response promotes the longitudinal growth of seeds and fruits, whereas a weakened BR response results in short, rounder grains or flattened fruits [18,36]. This regulatory function involves proteins such as IQDs (IQ67 domain proteins), OFPs, and TRMs (TONNEAU 1 recruiting motif proteins), and is closely related to the **microtubule system** (reviewed in [37]). IQDs have been identified as a novel type of microtubule-associated proteins [38], and members of this gene family such as *OsGW5* in rice [39] and *SlSUN* in tomato [40] are essential domestication genes that enhance grain size and fruit length, respectively. OsGW5 inhibits the kinase activity of OsGSK2 [39], whereas SlBZR1.7 (a member of SlBZRs) directly promotes the expression of *SlSUN* [41]. SlOFPs interact with SlTRMs and inhibit the elongation of fruit organs by influencing the localization of SlTRMs on microtubules, and this represents a common mechanism underlying the regulation of plant organ shape [42,43]. In rice, another TRM protein, OsGL7 (also known as OsGW7), is also a key regulator of grain shape [44,45]. Notably, several studies in rice have collectively revealed that OsOFPs can be phosphorylated by OsGSK2 and interacts with OsDLT, as well as with OsBZR1 and OSH1, to modulate BR responses, including plant architecture and grain morphology [30,46–48]. This wealth of information strongly suggests that BRs influence plant development by impacting on microtubule dynamics. In support, a recent study showed that a mutation in *OsBHS1*, that encodes the microtubule-severing protein OsKINESIN13, leads to BR hypersensitivity in rice [49]. In addition, OsGSK2 phosphorylates the Mei2-like protein OsOML4 to suppress grain size [50]. Furthermore, BRs are also beneficial in enhancing the appearance of rice grains [51,52] and in promoting the accumulation of pigments, nutrients, and flavor compounds in tomato fruits [53].

Regulating leaf angle is among the specific roles of BRs in various crop species. An increase in BR function leads to a larger leaf angle, whereas reduced BR function results in upright leaves, which is a desirable trait for improving planting density and crop yield per unit area. In rice, this effect involves the U-type cyclin protein OsCYC U4;1, which is targeted by OsGSK2 at the protein level and by OsBZR1 at the transcriptional level, thereby affecting the development of the lamina joint and leaf bending [54]. In maize (*Zea mays*), two loci associated with leaf angle, *ZmUPA1* and *ZmUPA2*, were identified from teosinte, the wild ancestor of maize [9]. *ZmUPA1* encodes a functionally defective version of the BR synthase ZmBRD1 (also known as ZmBR6ox), whereas *ZmUPA2* corresponds to a 2 bp deletion polymorphism located 9.5 kb upstream of the B3 transcription factor gene *ZmRAVL1*. The transcription factor ZmDRL1 interacts with ZmLG1 and binds to the *ZmUPA2* site, leading to upregulation of *ZmRAVL1*, which in turn inhibits *ZmUPA1* and reduces BR content, thereby causing the leaves to stand upright [9]. In *Setaria italica*, a

mutation in *SiDPY1*, which encodes an LRR-RLK that interferes with the interaction between SiBRI1 and SiSERK1, leads to drooping leaves [55].

The tiller number in rice plays a crucial role in determining panicle number and ultimately grain yield. The OsDLT–OsRLA1–OsBZR1 transcriptional protein complex directly suppresses the expression of *OsTB1* (also known as *OsFC1*), a crucial regulator of tiller number [56]. Interestingly, the strigolactone signaling component OsD53 can interact with OsBZR1 to inhibit *OsTB1* and regulate tiller number [56].

BRs could also contribute to the practice of direct seeding in rice – an important labor-saving cultivation method. BR signaling promotes rice seed germination at low temperatures [57]. OsBZR1 directly activates the expression of α-amylase *OsRAmy3D* to facilitate seed germination [58]. In addition, BRs promote the elongation of rice mesocotyl. OsGSK2 phosphorylates the U-type cyclin OsCYC U2, leading to its degradation and inhibition of mesocotyl elongation [59]. Furthermore, BRs stimulate coleoptile elongation, a response commonly used to assess BR sensitivity in rice.

BRs could play a significant role in root. In rice, BR-defective mutants typically exhibit shortened root growth [20]. In addition, BRs might positively regulate lateral root formation [23]. Different plant species could have distinctive traits where BRs also play a role. For instance, in cotton (*Gossypium hirsutum*) the BR signaling transcription factor GhBES1 (a member of GhBZRs) activates the expression of genes involved in cell elongation, such as *GhEXP3A*, to promote cotton fiber elongation, thus contributing to the yield and quality of cotton fibers [60]. In potato (*Solanum tuberosum*), the BR receptor StBRI1 plays a role in regulating the number and size of tubers [61]. These observations highlight the involvement of BRs in controlling distinct yield and quality traits in different species.

The functions of BRs in regulating the environmental plasticity of crops

Phytohormones, including BRs, play crucial roles in integrating internal developmental processes with external environmental cues in plants. BR responses have been shown to be modulated by nutrient availability, leading to alterations of plant architecture in rice. Nitrogen availability has a significant impact on tiller number in rice. OsNGR5 (also known as OsSMOS1/OsRLA1) has been identified as a nitrogen-responsive tillering regulator [62]. It binds to the upstream regions of tiller-inhibiting genes such as *OsD14* and *OsSPL14*, and recruits the PRC2 complex of histone methylation modifiers to inhibit their expression [62]. Another nitrogen-responsive tillering regulator, *OsTCP19*, targets and inhibits the expression of *OsDLT*, a positive regulator of tiller number [63]. Environmental cues such as low-phosphate conditions also influence leaf angle. Under phosphate starvation, the phosphate regulator OsSPX1 inhibits the activity of OsRLI1, a transcriptional activator of BR-responsive genes involved in lamina joint development, such as *OsBU1* and *OsBC1*. This repression of BR-responsive gene expression leads to altered leaf bending and leaf angle in response to phosphate availability [64]. These studies demonstrate the involvement of BR signaling transcription factors in mediating the regulation of plant morphological changes by nutrient signals.

BRs play crucial roles in regulating responses and adaptations to various abiotic and biotic stresses in plants. Genetic and chemical treatment analyses have revealed the involvement of GSK-targeting proteins or BZR-targeting genes in mediating these functions (Box 2). However, it is important to note that there may be conflicting reports regarding these functions, as mentioned earlier [5]. In addition to their role in stress responses, BRs have also been found to regulate insect behavior, where they act as a deterrent to feeding and oviposition by insects such as *Plutella xylostella* on plants [65]. Moreover, the BR synthesis gene *OsBR6ox* (also

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Box 2. An update on the roles of BRs in regulating crop stress tolerance

BRs have potential to enhance salt tolerance in crops. Overexpression of *ZmBSK1* in maize [92], *OsSERK2* in rice [16], and *SlBZR1* in tomato [93] has been found to improve salt tolerance. BRs may play a role in promoting drought tolerance, as evidenced by the overexpression of *TaBZR2* in wheat [94] and *ZmBSK1* in maize [95]. However, conflicting roles of BRs in this context have also been reported [96,97]. BR plays a crucial role in regulating heat tolerance, as demonstrated by the severe heat hypersensitivity resulting from *HvBRI1* mutation in *uzu* barley [71]. In addition, OsTT3.1/OsDGS1, a BR signaling component, contributes to thermotolerance [14,72]. In terms of chilling stress, SlBZR1 directly activates the expression of *SlNCED1*, a key gene involved in abscisic acid synthesis, thereby promoting chilling tolerance [98]. BRs play a positive role in coping with low nitrogen stress because OsBZR1 activates the expression of the ammonium transporter gene *OsAMT1;2* to facilitate ammonium uptake [99]. Under nitrogen-starved conditions, SlBZR1 directly activates the expression of tomato autophagy genes *SlATG2/6* to facilitate nutrient redistribution [100].

In the realm of biotic stresses, BRs inhibit plant resistance to rice black-streaked dwarf virus (RBSDV). OsGSK2 phosphorylates OsJAZ4, a crucial suppressor of jasmonic acid (JA) signaling, thereby modulating protein stability and subsequently suppressing the JA response to inhibit plant immunity [101]. Interestingly, OGSK2 can also phosphorylate OsMYC2, a key transcription factor involved in JA signaling, to regulate protein stability [102], representing an additional molecular pathway through which BRs suppress the JA response and plant immunity. However, unlike the case of RBSDV, BRs enhance resistance against rice stripe virus (RSV). Further analysis has revealed that RSV infection strongly suppresses BR synthesis, suggesting that the virus some-how hijacks the BR pathway to suppress plant immunity and facilitate infection [102]. It appears that, although BRs evidently antagonize JA to maintain a balance plant growth and immunity, specific viruses such as RSV could exploit this to their advantage.

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known as *OsBRD1*) has been shown to promote the degradation of pesticides such as atrazine and acetochlor [66]. These findings highlight the potential application of BR-related genes in reducing the use of chemical pesticides and mitigating environmental stress.

The utilization of BR genes for crop improvement

Dense planting is one of the major modern approaches for cultivating high-yielding crops. Upright leaves resulting from BR deficiency, which could avoid leaves shading each other, particularly under high-density planting conditions, can benefit this approach. Compared to GA deficiency, BR deficiency enables the simultaneous achievement of semi-dwarfism and a more compact plant architecture. Indeed, studies in different crops have demonstrated the effectiveness of this strategy. In rice, utilizing the weak BR mutant *osdwarf4* enhanced yield by ~32% compared to the wild type under dense planting conditions [6]. In maize, introducing the wild *UPA2* allele or editing *ZmRAVL1* enables yield increases of ~18% under high-density planting [9]. Very recently, a zinc-finger E3 ligase TaZnF was shown to positively regulate BR signaling by mediating the ubiquitination and degradation of TaBKI1, a homolog of AtBKI1 in wheat (*Triticum aestivum*) [10]. Significantly, deletion of *TaZnF-B* and *TaRht-B* (on B subgenomes), the Green Revolution gene, enhances wheat yield at different planting densities. Notably, under high-density planting conditions, the modified GRV had a yield increase of ~12% and significantly improved the lodging resistance [10]. The success of this strategy in three major crops strongly supports BR signaling as being one of the most promising targets for the next Green Revolution.

Because BRs promote GA synthesis, they should also positively regulate NUE, as supported by a BR treatment experiment in maize [67]. In rice, OsGRF4 (a target of OsGSK2) promotes grain size as well as carbon and nitrogen metabolism, whereas OsNGR5 (also a target of OsGSK2) enhances the tillering response to nitrogen [62,68,69]. Although increasing the expression of *OsNGR5* or *OsGRF4* alone significantly improves productivity, their combination further increases grain yield [62]. In addition, introducing the favorable allele of *OsTCP19* has also been found to significantly improve rice yield [63]. These findings suggest that BRs can be utilized for improving NUE in crops, although the precise molecular mechanisms underlying BR regulation of NUE remain largely unknown.

Challenges in harnessing BR genes for crop improvement

The pleiotropic nature of BR signaling offers the potential to simultaneously engineer multiple desirable traits in crops. However, some of these effects may result in conflicting characteristics, such as leaf angle and grain development, posing practical challenges for application [5]. Furthermore, the evaluation of BR utilization mentioned earlier primarily focused on yield under normal conditions, without considering resistance factors. Given the diverse roles of BRs in various stresses, BR-defective plants might be vulnerable when confronted with complex and harsh field conditions or when exposed to severe weather. The heat sensitivity observed in *uzu* barley (*Hordeum vulgare*) serves as a pertinent example in this regard [70,71]. Taking into account the involvement of its homolog *OsTT3.1* (also known as *OsDGS1*) in thermotolerance [72], deletion of *TaZnF-B* could potentially impact on the heat tolerance of wheat. Can we effectively employ BR genes to improve multiple traits by leveraging their pleiotropy, and can they be used to design crops with both high and stable yields? It is important to note that, although BR functions are pleiotropic, this may not necessarily apply to BR genes. Once accessible BR genes or their alleles become available, we can readily apply them to modify traits or design crops using advanced genome-editing techniques, transgenic methods, or conventional breeding approaches. Based on the current body of knowledge about phytohormones, we propose four strategies to address this question (Figure 3). We aim to introduce these concepts without delving into intricate methodologies, as illustrated in the examples discussed below.
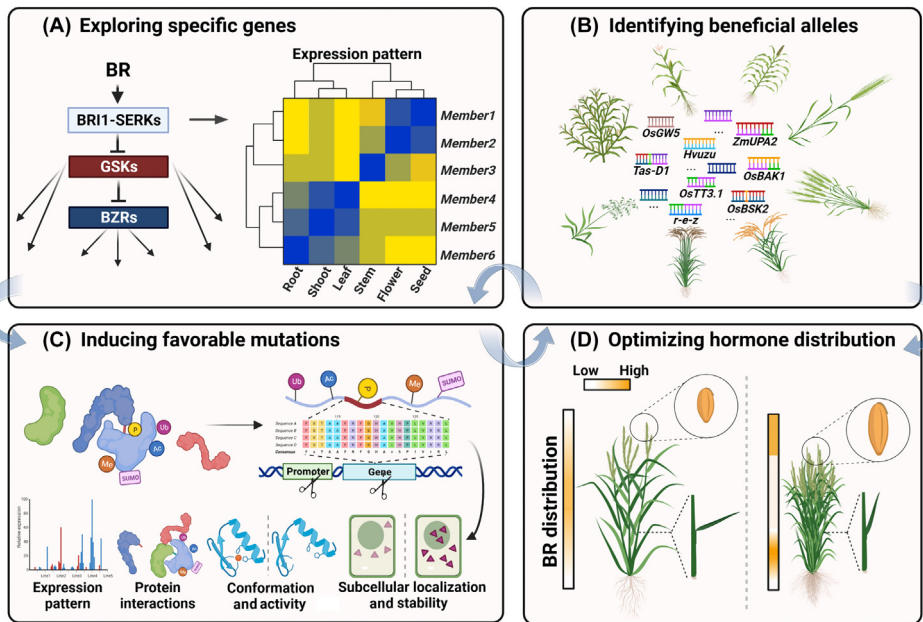
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Figure 3. Strategies for utilizing brassinosteroid (BR) molecules. (A) Identification of function-specific genes that contribute to branched BR pathways (downward arrows), the specific members of gene families, or genes with specific expression patterns would enable the manipulation of specific crop traits. (B) Identifying beneficial variations from the diversity of natural germplasm resources such as wild ancestors, relatives, landraces, and cultivars. (C) Introducing favorable mutations that do not exist in nature by using genome-editing tools based on knowledge of gene regulatory elements, protein modification sites, or any other functional sequences that could affect expression patterns, interactions, conformation, activity, subcellular localization, or stability. (D) Optimizing the spatial distribution of the hormones by manipulating transporters or metabolic genes to relocate hormones from less crucial locations to vital positions according to the desired outcome. Abbreviations: Ac, acetylation; Me, methylation; P, phosphorylation; SUMO, SUMOylation (small ubiquitin-like modifier addition); Ub, ubiquitin. Figure created with BioRender.com.

Exploring function-specific genes

Genes with specific functions have the potential to regulate individual traits with precision, thus minimizing undesirable effects. The functional specificity of BR signaling is governed by multiple molecular mechanisms. First, the primary BR signaling pathway can branch out to elicit various BR responses, where specific components in these branches modulate particular traits. For instance, the GA pathway can be considered as a downstream branch of BR signaling that specifically regulates plant height [73]. Second, within a given BR gene family, the members often exhibit both functional redundancy and differentiation. Manipulating a specific member or a combination of different members can improve a particular trait or even confer multiple beneficial effects. An example of this is seen in rice, where simultaneous editing of four *OsGSKs* generates a series of mutants, some of which simultaneously exhibit enlarged grain size and a compact structure [20]. Third, genes with specific expression patterns can have distinct functions in the context of BR-related genes. This expression specificity is frequently observed within gene families. For instance, mutants of *OSH15*, also known as *d6*, exhibit specific shortening of lower internodes in rice owing to the regional expression specificity of the gene [35].

Identifying beneficial variations from natural germplasm resources

The crucial functions of BRs in regulating essential traits suggest that variations in BR genes are likely to play a pivotal role in shaping the phenotypic diversity observed in natural plant populations. Furthermore, BR genes should also be prime targets for crop domestication and selection. Indeed, a recent study showed that natural variation in *OsBSK2* contributes to the codivergence of multiple traits between rice subspecies [18]. Additional variations in *OsBRI1*, *OsBAK1*, *OsPPKL1*, *OsGSK3*, and *OsBZR3* have been identified that have the possibility of contributing to **subspecies divergence** [18]. Coincidentally, variation in *AtBSK3* has been found to determine root elongation in different arabidopsis varieties in response to nitrogen availability [74]. However, our overall understanding of this aspect remains very limited. The success stories of *TaZnF-B* and *ZmUPA2* underscore the significance of identifying natural variations in BR genes. It is worth noting that, although the large deletion of *TaZnF-B* and *TaRht1-B* represents a rare allele [10], *ZmUPA1* and *ZmUPA2* were discovered in teosinte, the wild relative of maize [9]. These findings highlight the immense value of diverse germplasm resources, including wild ancestors, relatives, landraces, and modern cultivars, which remain to be fully explored.

Introducing favorable mutations based on protein/gene knowledge

Through extensive molecular genetic research, it is likely that most valuable genetic resources, such as the Green Revolution genes, have already been unconsciously utilized through intensive breeding practices in major crops. To revolutionize crop improvement it may be necessary to create advantageous mutations that do not exist naturally. Fortunately, advanced genome-editing technologies such as the CRISPR/Cas9 system offer the ability to precisely engineer protein/gene sequences, and provide a powerful tool for screening favorable alleles. For example, by editing the upstream open reading frame of *OsDLT*, a range of rice plants with different BR sensitivities and plant heights were successfully generated [75]. However, to achieve precise trait engineering in a rational-design manner, sufficient knowledge of the regulatory sites within proteins or genes is a prerequisite. This approach seems to be more feasible for BR signaling proteins because many of them undergo protein modification or regulation of stability. For instance, amino acid changes in or near the TREE motif of GSKs have been identified in different crops that enhance GSK protein stability [24,76–79]. However, the mechanism underlying these effects remains incompletely understood. A more compelling example is the elucidation of the function of amino acid D364 in OsPPKL1, which was found to be crucial for interrupting the H-D phosphorelay of cytokinin signaling [80]. By editing the region around D364, a series of semi-dominant, non-frameshift genotypes were created, resulting in gradual increases in grain size [80]. Screening these

genotypes on other backgrounds could enable the identification of the most suitable grain size for yield improvement. Assuming that we fully understand all the protein modification sites of OsGSK2, for instance, we could engineer the central BR signaling inhibitor to generate numerous forms with diverse kinase activity, protein stability, or functional specificities. This could enable the conferment of a desired trait or multiple beneficial traits by decoupling different BR-regulated traits.

Optimizing the spatial distribution of the hormones

Overexpression of the putative auxin transporter gene *OsBG1*, driven by its native promoter, has been shown to enhance grain size and yield in rice [81]. Similarly, overexpression of *OsAGO2*, an epigenetic regulator of the cytokinin transporter gene *OsBG3*, improved rice yield and salt resistance by modulating the distribution of cytokinins within the plant [82]. These studies highlight the promising prospects of optimizing the spatial distribution of hormones for crop enhancement. Compared to manipulating overall hormone levels, this strategy offers a more cost-effective and efficient approach to harnessing the benefits of hormones for crop improvement.

However, unlike other hormones such as auxin and cytokinin, our current understanding of BR transport is extremely limited (reviewed in [83]). In addition to the transport mechanism, another approach to modifying hormone distribution involves manipulating the genes responsible for BR homeostasis regulation. As mentioned earlier, mutations in genes such as *OsDLT* and *OSH15* have been found to alter the pattern of BR distribution, resulting in increased grain size but decreased culm length [35]. Correspondingly, OSH1 can strongly bind to several BR catabolic genes to induce BR inactivation, thereby maintaining shoot apical meristem development [84]. These findings highlight the significant role of BR catabolic genes in determining BR distribution. Therefore, reallocating hormones from less crucial locations to more vital positions represents one of the most promising strategies for effectively utilizing hormones in crop improvement.

Concluding remarks and future perspectives

We are entering an exciting era of molecular design where the manipulation of BR genes holds promise for shaping desired plant architecture in crops. However, we must also acknowledge the challenges posed by an increasingly arid and warmer future, accompanied by unpredictable and severe weather events. Furthermore, growing global concern has turned towards environmental deterioration. In light of these challenges, there is an urgent need to develop innovative, high-yielding, and stable crops that are resource-efficient. The pleiotropic nature of BR signaling presents promising avenues for tackling these challenges, but concurrently poses complexities in terms of harnessing BR genes. In our pursuit of a new Green Revolution, it is crucial to delve deeper into several unresolved issues (see Outstanding questions). By expanding our understanding of the functions of BR genes and their role in stress responses, we can unlock their potential to develop crops resilient to environmental challenges such as heat, drought, and extreme weather events. Moreover, the exploration of BR interactions with other hormones and the integration of various cues in a context-dependent manner takes on particular significance in developing the next generation of GRVs. Furthermore, by leveraging molecular design to optimize crop architecture and resource utilization as well as cultivation methods, we can work towards creating high-yielding crops that minimize resource inputs, thereby contributing to sustainable agriculture in a changing world.

Outstanding questions

How is the spatial distribution of BRs determined in plants? Are there BR transporters?

What are the actual roles of BRs in regulating drought tolerance, and what are the possible explanations for the conflicting reports?

How do BRs regulate temperature adaptability? Centrally, how do BRs promote thermotolerance?

How do BRs modulate developmental plasticity in response to various environmental cues, including favorable conditions or adverse stresses?

How are the different modifications to key BR signaling components, such as OsGSK2 and OsBZR1, integrated to control BR signaling?

Can we extensively identify the substrates of GSK3-like kinases, and how is the organization of the transcriptional factor complex dynamically regulated to determine the BR signaling output?

How do BRs modulate the microtubule system, and what is the biological significance of this modulation?

What are the roles of BRs at the cellular level? For instance, how do BRs regulate meristem activity?

How do BRs contribute to the diversity of natural plant populations and the domestication and selection of crops?

Can we finally utilize knowledge about BRs to develop the next GRVs, or precisely design any desirable crop in a freestyle manner?

Declaration of interests

The authors declare no competing interests.

References

1. Peng, J. *et al.* (1999) 'Green revolution' genes encode mutant gibberellin response modulators. *Nature* 400, 256–261
2. Sasaki, A. *et al.* (2002) Green revolution: a mutant gibberellin-synthesis gene in rice – new insight into the rice variant that helped to avert famine over thirty years ago. *Nature* 416, 701–702
3. Liu, Q. *et al.* (2022) Improving crop nitrogen use efficiency toward sustainable Green Revolution. *Annu. Rev. Plant Biol.* 73, 523–551
4. Hu, B. *et al.* (2023) Genetic improvement toward nitrogen-use efficiency in rice: lessons and perspectives. *Mol. Plant* 16, 64–74
5. Tong, H. and Chu, C. (2018) Functional specificities of brassinosteroid and potential utilization for crop improvement. *Trends Plant Sci.* 23, 1016–1028
6. Sakamoto, T. *et al.* (2006) Erect leaves caused by brassinosteroid deficiency increase biomass production and grain yield in rice. *Nat. Biotechnol.* 24, 105–109
7. Morinaka, Y. *et al.* (2006) Morphological alteration caused by brassinosteroid insensitivity increases the biomass and grain production of rice. *Plant Physiol.* 141, 924–931
8. Zhang, Y.C. *et al.* (2013) Overexpression of microRNA OsmiR397 improves rice yield by increasing grain size and promoting panicle branching. *Nat. Biotechnol.* 31, 848–852
9. Tian, J.G. *et al.* (2019) Teosinte ligule allele narrows plant architecture and enhances high-density maize yields. *Science* 365, 658–664
10. Song, L. *et al.* (2023) Reducing brassinosteroid signalling enhances grain yield in semi-dwarf wheat. *Nature* 617, 118–124
11. Song, Y. *et al.* (2022) OsSLA1 functions in leaf angle regulation by enhancing the interaction between OsBRI1 and OsBAK1 in rice. *Plant J.* 110, 1111–1127
12. Yue, Z.L. *et al.* (2022) The receptor kinase OsWAK11 monitors cell wall pectin changes to fine-tune brassinosteroid signaling and regulate cell elongation in rice. *Curr. Biol.* 32, 2454–2466
13. Wolf, S. *et al.* (2014) A receptor-like protein mediates the response to pectin modification by activating brassinosteroid signaling. *Proc. Natl. Acad. Sci. U. S. A.* 111, 15261–15266
14. Li, J. *et al.* (2023) An endoplasmic reticulum-associated degradation-related E2-E3 enzyme pair controls grain size and weight through the brassinosteroid signaling pathway in rice. *Plant Cell* 35, 1076–1091
15. Yuan, H. *et al.* (2017) 08SG2/OsBAK1 regulates grain size and number, and functions differently in Indica and Japonica backgrounds in rice. *Rice* 10, 25
16. Dong, N. *et al.* (2020) Regulation of brassinosteroid signaling and salt resistance by SERK2 and potential utilization for crop improvement in rice. *Front. Plant Sci.* 11, 621859
17. Tian, P. *et al.* (2023) BRASSINOSTEROID-SIGNALING KINASE1-1, a positive regulator of brassinosteroid signalling, modulates plant architecture and grain size in rice. *J. Exp. Bot.* 74, 283–295
18. Yin, W.C. *et al.* (2022) The divergence of brassinosteroid sensitivity between rice subspecies involves natural variation conferring altered internal auto-binding of OsBSK2. *J. Integr. Plant Biol.* 64, 1614–1630
19. Tian, P. *et al.* (2022) OsBSK3 positively regulates grain length and weight by inhibiting the phosphatase activity of OsPPKL1. *Plants* 11, 1586
20. Liu, D. *et al.* (2021) Diversification of plant agronomic traits by genome editing of brassinosteroid signaling family genes in rice. *Plant Physiol.* 187, 2563–2576
21. Maselli, G.A. *et al.* (2014) Revisiting the evolutionary history and roles of protein phosphatases with kelch-like domains in plants. *Plant Physiol.* 164, 1527–1541
22. Gong, L.P. *et al.* (2022) OsCPL3 is involved in brassinosteroid signaling by regulating OsGSK2 stability. *J. Integr. Plant Biol.* 64, 1560–1574
23. Hou, J. *et al.* (2022) The histone deacetylase 1/GSK3/SHAGGY-like kinase 2/BRASSINAZOLE-RESISTANT 1 module controls lateral root formation in rice. *Plant Physiol.* 189, 858–873
24. Liu, D. *et al.* (2022) The U-box ubiquitin ligase TUD1 promotes brassinosteroid-induced GSK2 degradation in rice. *Plant Commun.* 4, 100450
25. Hu, X.M. *et al.* (2013) The U-box E3 ubiquitin ligase TUD1 functions with a heterotrimeric G alpha subunit to regulate brassinosteroid-mediated growth in rice. *PLoS Genet.* 9, e1003391
26. Chen, E. *et al.* (2023) Armadillo repeat only protein GS10 negatively regulates brassinosteroid signaling to control rice grain size. *Plant Physiol.* 192, 967–981
27. Min, H.J. *et al.* (2019) OsBZR1 turnover mediated by OsSK22-regulated U-box E3 ligase OsPUB24 in rice BR response. *Plant J.* 99, 426–438
28. Ren, Y. *et al.* (2020) *Oryza sativa* mediator subunit OsMED25 interacts with OsBZR1 to regulate brassinosteroid signaling and plant architecture in rice. *J. Integr. Plant Biol.* 62, 793–811
29. Qiao, S. *et al.* (2017) The RLA1/SMOS1 transcription factor functions with OsBZR1 to regulate brassinosteroid signaling and rice architecture. *Plant Cell* 29, 292–309
30. Xiao, Y.H. *et al.* (2020) GSK2 stabilizes OFP3 to suppress brassinosteroid responses in rice. *Plant J.* 102, 1187–1201
31. Hirano, K. *et al.* (2017) SMALL ORGAN SIZE 1 and SMALL ORGAN SIZE 2/DWARF AND LOW-TILLERING form a complex to integrate auxin and brassinosteroid signaling in rice. *Mol. Plant* 10, 590–604
32. Huang, Y. *et al.* (2022) Ribonuclease H-like gene SMALL GRAIN2 regulates grain size in rice through brassinosteroid signaling pathway. *J. Integr. Plant Biol.* 64, 1883–1900
33. Tian, X. *et al.* (2021) OsWRKY53 integrates classic brassinosteroid signaling and the mitogen-activated protein kinase pathway to regulate rice architecture and seed size. *Plant Cell* 33, 2753–2775
34. Yamamuro, C. *et al.* (2000) Loss of function of a rice brassinosteroid insensitive1 homolog prevents internode elongation and bending of the lamina joint. *Plant Cell* 12, 1591–1605
35. Niu, M. *et al.* (2022) Rice DWARF AND LOW-TILLERING and the homeodomain protein OSH15 interact to regulate internode elongation via orchestrating brassinosteroid signaling and metabolism. *Plant Cell* 34, 3754–3772
36. Sierra-Orozco, E. *et al.* (2021) Identification and characterization of GLOBE, a major gene controlling fruit shape and impacting fruit size and marketability in tomato. *Hortic. Res.* 8, 138
37. Snouffer, A. *et al.* (2020) The shape of things to come: ovate family proteins regulate plant organ shape. *Curr. Opin. Plant Biol.* 53, 98–105
38. Kumari, P. *et al.* (2021) IQ67 DOMAIN proteins facilitate preprophase band formation and division-plane orientation. *Nat. Plants* 7, 739–747
39. Liu, J.F. *et al.* (2017) GW5 acts in the brassinosteroid signalling pathway to regulate grain width and weight in rice. *Nat. Plants* 3, 17043
40. Xiao, H. *et al.* (2008) A retrotransposon-mediated gene duplication underlies morphological variation of tomato fruit. *Science* 319, 1527–1530
41. Yu, T. *et al.* (2022) Regulation of tomato fruit elongation by transcription factor BZR1.7 through promotion of SUN gene expression. *Hortic. Res.* 9, uhac121
42. Zhang, B.Y. *et al.* (2023) A combinatorial TRM–OFP module bilaterally fine-tunes tomato fruit shape. *New Phytol.* 238, 2393–2409
43. Wu, S. *et al.* (2018) A common genetic mechanism underlies morphological diversity in fruits and other plant organs. *Nat. Commun.* 9, 4734
44. Wang, Y.X. *et al.* (2015) Copy number variation at the GL7 locus contributes to grain size diversity in rice. *Nat. Genet.* 47, 944–948
45. Wang, S.K. *et al.* (2015) The OsSPL16–GW7 regulatory module determines grain shape and simultaneously improves rice yield and grain quality. *Nat. Genet.* 47, 949–954
46. Xiao, Y. *et al.* (2017) Brassinosteroids regulate OFP1, a DLT interacting protein, to modulate plant architecture and grain morphology in rice. *Front. Plant Sci.* 8, 1698

47. Yang, C. *et al.* (2018) OsOFP19 modulates plant architecture by integrating the cell division pattern and brassinosteroid signaling. *Plant J.* 93, 489–501

48. Yang, C. *et al.* (2016) OVATE Family Protein 8 positively mediates brassinosteroid signaling through interacting with the GSK3-like kinase in rice. *PLoS Genet.* 12, e1006118

49. Zhang, Y.L. *et al.* (2022) The kinesin-13 protein BR HYPERSENSITIVE 1 is a negative brassinosteroid signaling component regulating rice growth and development. *Theor. Appl. Genet.* 135, 1751–1766

50. Lyu, J. *et al.* (2020) Control of grain size and weight by the GSK2–LARGE1/OML4 pathway in rice. *Plant Cell* 32, 1905–1918

51. Li, Q.F. *et al.* (2018) Seed-specific expression of OsDWF4, a rate-limiting gene involved in brassinosteroids biosynthesis, improves both grain yield and quality in rice. *J. Agric. Food Chem.* 66, 3759–3772

52. Zhang, X.F. *et al.* (2020) Phytohormone dynamics in developing endosperm influence rice grain shape and quality. *J. Integr. Plant Biol.* 62, 1625–1637

53. Hu, S.S. *et al.* (2020) Regulation of fruit ripening by the brassinosteroid biosynthetic gene SlCYP90B3 via an ethylene-dependent pathway in tomato. *Hortic. Res.* 7, 163

54. Sun, S. *et al.* (2015) Brassinosteroid signaling regulates leaf erectness in *Oryza sativa* via the control of a specific U-type cyclin and cell proliferation. *Dev. Cell* 34, 220–228

55. Zhao, M.C. *et al.* (2020) DROOPY LEAF1 controls leaf architecture by orchestrating early brassinosteroid signaling. *Proc. Natl. Acad. Sci. U. S. A.* 117, 21766–21774

56. Fang, Z.M. *et al.* (2020) Strigolactones and brassinosteroids antagonistically regulate the stability of the D53–OsBZR1 complex to determine FC1 expression in rice tillering. *Mol. Plant* 13, 586–597

57. Kim, S.H. *et al.* (2022) Brassinosteroid biosynthesis gene OsD2 is associated with low-temperature germinability in rice. *Front. Plant Sci.* 13, 985559

58. Xiong, M. *et al.* (2022) Brassinosteroids regulate rice seed germination through the BZR1–RAmy3D transcriptional module. *Plant Physiol.* 189, 402–418

59. Sun, S. *et al.* (2018) Natural selection of a GSK3 determines rice mesocotyl domestication by coordinating strigolactone and brassinosteroid signaling. *Nat. Commun.* 9, 2523

60. Zhu, L. *et al.* (2023) A conserved brassinosteroid-mediated BES1–CERP–EXPA3 signaling cascade controls plant cell elongation. *Cell Rep.* 42, 112374

61. Huang, S.H. *et al.* (2021) RNA interference knockdown of the brassinosteroid receptor BRI1 in potato (*Solanum tuberosum* L.) reveals novel functions for brassinosteroid signaling in controlling tuberization. *Sci. Hortic.* 290, 110516

62. Wu, K. *et al.* (2020) Enhanced sustainable green revolution yield via nitrogen-responsive chromatin modulation in rice. *Science* 367, eaaz2046

63. Liu, Y.Q. *et al.* (2021) Genomic basis of geographical adaptation to soil nitrogen in rice. *Nature* 590, 600–605

64. Ruan, W.Y. *et al.* (2018) An SPX–RLI1 module regulates leaf inclination in response to phosphate availability in rice. *Plant Cell* 30, 853–870

65. Yang, K. *et al.* (2020) A gustatory receptor tuned to the steroid plant hormone brassinolide in *Plutella xylostella* (Lepidoptera: Plutellidae). *eLife* 9, e64114

66. Qiao, Y.X. *et al.* (2022) OsBR6ox, a member in the brassinosteroid synthetic pathway facilitates degradation of pesticides in rice through a specific DNA demethylation mechanism. *Sci. Total Environ.* 838, 156503

67. Xing, J.P. *et al.* (2022) Brassinosteroids modulate nitrogen physiological response and promote nitrogen uptake in maize (*Zea mays* L.). *Crop J.* 10, 166–176

68. Che, R. *et al.* (2015) Control of grain size and rice yield by GL2-mediated brassinosteroid responses. *Nat. Plants* 2, 15195

69. Li, S. *et al.* (2018) Modulating plant growth-metabolism coordination for sustainable agriculture. *Nature* 560, 595–600

70. Chono, M. *et al.* (2003) A semidwarf phenotype of barley uzu results from a nucleotide substitution in the gene encoding a putative brassinosteroid receptor. *Plant Physiol.* 133, 1209–1219

71. Dockter, C. and Hansson, M. (2015) Improving barley culm robustness for secured crop yield in a changing climate. *J. Exp. Bot.* 66, 3499–3509

72. Zhang, H. *et al.* (2022) A genetic module at one locus in rice protects chloroplasts to enhance thermotolerance. *Science* 376, 1293–1300

73. Tong, H. *et al.* (2014) Brassinosteroid regulates cell elongation by modulating gibberellin metabolism in rice. *Plant Cell* 26, 4376–4393

74. Jia, Z.T. *et al.* (2019) Natural variation of BSK3 tunes brassinosteroid signaling to regulate root foraging under low nitrogen. *Nat. Commun.* 10, 2378

75. Xue, C.X. *et al.* (2023) Tuning plant phenotypes by precise, graded downregulation of gene expression. *Nat. Biotechnol.* Published online March 9, 2023. https://doi.org/10.1038/s41587-023-01707-w

76. Tong, H. *et al.* (2012) DWARF AND LOW-TILLERING acts as a direct downstream target of a GSK3/SHAGGY-like kinase to mediate brassinosteroid responses in rice. *Plant Cell* 24, 2562–2577

77. Hou, L.J. *et al.* (2022) *Zea mays* GSK2 gene is involved in brassinosteroid signaling. *Plant Growth Regul.* 97, 117–125

78. Li, B. *et al.* (2023) BnaC01.BIN2, a GSK3-like kinase, modulates plant height and yield potential in *Brassica napus*. *Theor. Appl. Genet.* 136, 29

79. Cheng, X.J. *et al.* (2020) A single amino acid substitution in STKc_GSK3 kinase conferring semispherical grains and its implications for the origin of *Triticum sphaerococcum*. *Plant Cell* 32, 923–934

80. Liu, D. *et al.* (2022) A cryptic inhibitor of cytokinin phosphorelay controls rice grain size. *Mol. Plant* 15, 293–307

81. Liu, L.C. *et al.* (2015) Activation of Big Grain1 significantly improves grain size by regulating auxin transport in rice. *Proc. Natl. Acad. Sci. U. S. A.* 112, 11102–11107

82. Yin, W. *et al.* (2020) ARGONAUTE2 enhances grain length and salt tolerance by activating BIG GRAIN3 to modulate cytokinin distribution in rice. *Plant Cell* 32, 2292–2306

83. Vukasinovic, N. and Russinova, E. (2018) BRexit: possible brassinosteroid export and transport routes. *Trends Plant Sci.* 23, 285–292

84. Tsuda, K. *et al.* (2014) Genome-wide study of KNOX regulatory network reveals brassinosteroid catabolic genes important for shoot meristem function in rice. *Plant Cell* 26, 3488–3500

85. Mora-Garcia, S. *et al.* (2004) Nuclear protein phosphatases with Kelch-repeat domains modulate the response to brassinosteroids in *Arabidopsis*. *Genes Dev.* 18, 448–460

86. Kim, T.W. *et al.* (2009) Brassinosteroid signal transduction from cell-surface receptor kinases to nuclear transcription factors. *Nat. Cell Biol.* 11, 1254–U233

87. Kim, E.-J. *et al.* (2016) Oligomerization between BSU1 family members potentiates brassinosteroid signaling in *Arabidopsis*. *Mol. Plant* 9, 178–181

88. Gao, X.Y. *et al.* (2019) Rice qGL3/OsPPKL1 functions with the GSK3/SHAGGY-kike kinase OsGSK to modulate brassinosteroid signaling. *Plant Cell* 31, 1077–1093

89. Ren, H. *et al.* (2019) BRASSINOSTEROID-SIGNALING KINASE 3, a plasma membrane-associated scaffold protein involved in early brassinosteroid signaling. *PLoS Genet.* 15, e1007904

90. Zhang, B. *et al.* (2015) OsBRI1 activates BR signaling by preventing binding between the TPR and kinase domains of OsBSK3 via phosphorylation. *Plant Physiol.* 170, 1149–1161

91. Zhu, J.Y. *et al.* (2017) The F-box protein KIB1 mediates brassinosteroid-induced inactivation and degradation of GSK3-like kinases in *Arabidopsis*. *Mol. Cell* 66, 648–657

92. Liu, L. *et al.* (2022) Overexpression of a *Zea mays* brassinosteroid-signaling kinase gene ZmBSK1 confers salt stress tolerance in maize. *Front. Plant Sci.* 13, 894710

93. Jia, C.G. *et al.* (2021) Tomato BZR/BES transcription factor SlBZR1 positively regulates BR signaling and salt stress tolerance in tomato and *Arabidopsis*. *Plant Sci.* 302, 110719

94. Cui, X.Y. *et al.* (2019) BES/BZR transcription factor TaBZR2 positively regulates drought responses by activation of TaGST1. *Plant Physiol.* 180, 605–620

95. Liu, L. *et al.* (2021) BRASSINOSTEROID-SIGNALING KINASE 1 phosphorylating CALCIUM/CALMODULIN-DEPENDENT PROTEIN KINASE functions in drought tolerance in maize. *New Phytol.* 231, 695–712

96. Nie, S.M. *et al.* (2019) Enhanced brassinosteroid signaling intensity via SlBRI1 overexpression negatively regulates drought resistance in a manner opposite of that via exogenous BR application in tomato. *Plant Physiol. Biochem.* 138, 36–47

97. Chen, J. *et al.* (2017) *Arabidopsis* WRKY46, WRKY54, and WRKY70 transcription factors are involved in brassinosteroid-regulated plant growth and drought responses. *Plant Cell* 29, 1425–1439

98. An, S.M. *et al.* (2023) Brassinosteroid signaling positively regulates abscisic acid biosynthesis in response to chilling stress in tomato. *J. Integr. Plant Biol.* 65, 10–24

99. Yang, S. *et al.* (2021) BZR1 regulates brassinosteroid-mediated activation of AMT1;2 in rice. *Front. Plant Sci.* 12, 665883

100. Wang, Y. *et al.* (2019) BZR1 mediates brassinosteroid-induced autophagy and nitrogen starvation in tomato. *Plant Physiol.* 179, 671–685

101. He, Y.Q. *et al.* (2020) The OsGSK2 kinase integrates brassinosteroid and jasmonic acid signaling by interacting with OsJAZ4. *Plant Cell* 32, 2806–2822

102. Hu, J.L. *et al.* (2020) Rice stripe virus suppresses jasmonic acid-mediated resistance by hijacking brassinosteroid signaling pathway in rice. *PLoS Pathog.* 16, e1008801