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Regulation of Shoot Branching by Strigolactones and Brassinosteroids: Conserved and Specific Functions of *Arabidopsis* BES1 and Rice BZR1

Shoot branching results from axillary bud initiation and bud outgrowth and determines plant architecture and productivity. Strigolactones (SLs) are a class of terpenoid phytohormones that were identified to inhibit bud outgrowth in 2008. The core SL signaling pathway has been established in the last decade. Following the identification of the α/β hydrolase DWARF14 (D14) as a putative SL receptor, independent studies have proposed a variety of models explaining how SLs are perceived and D14 is activated (reviewed in detail by Burger and Chory, 2020). SLs bind to D14 and are hydrolyzed into their active form, which activates D14 and facilitates its interaction with the F-box protein DWARF 3 (D3) in rice, or its *Arabidopsis* ortholog MORE AXILLARY GROWTH2 (MAX2) (Burger and Chory, 2020). The F-box protein then recruits and ubiquitinates downstream substrates. Two substrates of D3/MAX2 have been independently identified, namely the transcription repressor DWARF53 (D53) in rice and the basic Helix Loop Helix (bHLH) transcription factor BRI1-EMS SUPPRESSOR1 (BES1) in *Arabidopsis*. SLs induce the ubiquitination and degradation of D53 and BES1, which is dependent on the receptor complex D14/AtD14-D3/MAX2, to inhibit shoot branching. In addition, the *Arabidopsis* orthologs of D53, known as SUPPRESSOR OF MAX2 1-Like genes (SMXLs), also play a conserved role in SL signaling (reviewed by Machin et al., 2020). However, some key questions regarding the core SL signaling pathway remain to be resolved. First, do the two independently identified targets of D3/MAX2, D53/SMXLs and BES1, cooperate to transduce SL signals regulating shoot branching, and how? Second, although the core SL signaling components involved in the control of shoot branching are conserved between rice and *Arabidopsis*, does the rice ortholog of BES1, OsBZR1, act in SL signaling regulating rice tillering (equivalent to shoot branching in *Arabidopsis*)? Third, as OsBZR1/BES1 play critical roles in brassinosteroid (BR) signaling, do BRs themselves regulate shoot branching/tillering in *Arabidopsis* and rice, and how?

Two recent studies have addressed some of these points and revealed the conserved transcriptional cascade at the core of the SL signaling pathway regulating shoot branching in both rice and *Arabidopsis* (Fang et al., 2019; Hu et al., 2019). Just like the *Arabidopsis* ortholog BES1, SLs promote the degradation of OsBZR1 to inhibit tillering in rice, and this depends on the receptor complex D14-D3 (Wang et al., 2013; Fang et al., 2019). Therefore, both OsBZR1/BES1 and D53/D53-like SMXLs, as positive regulators of shoot branching, negatively transduce SL signals downstream of D14/AtD14- D3/MAX2 (Machin et al., 2020). In addition, these two studies demonstrated that the rice gene *FINE CULM1* (*FC1*) and its *Arabidopsis* ortholog *BRANCHED1* (*BRC1*), which serve as local key switches for bud outgrowth, are epistatic to OsBZR1/BES1 and D53/SMXLs. Furthermore, OsBZR1/BES1 and D53/SMXLs genetically depend on each other to repress *FC1/BRC1* expression for shoot branching regulation in both rice and *Arabidopsis* (Fang et al., 2019; Hu et al., 2019). D53/D53-like SMXLs are class I Clp ATPases with an EAR motif that act as transcriptional repressors via the recruitment of TOPLESS (TPL) and TOPLESS-related proteins (TPRs). TPL/TPR proteins form tetrameric corepressors, each monomer of which can bind the EAR-containing peptide. Recently, the EAR-2 motif of D53 was shown to induce oligomerization of TPR2 tetramers, resulting in D53-mediated stabilization of the TPR2 corepressor-nucleosome complex to modify chromatin and repress transcription (Ma et al., 2017). However, because they lack the ability to bind DNA directly, D53/SMXLs require cofactors that specifically bind the promoters of target genes for chromatin modification and transcription inhibition (Ma et al., 2017; Song et al., 2017). Two recent studies provide biochemical evidence that OsBZR1/BES1 can act as such a cofactor, as it interacts with and recruits D53/D53-like SMXLs to the *FC1/BRC1* promoter, where it directs transcriptional repression in rice and *Arabidopsis*. This transcriptional inhibition depends on direct DNA binding by OsBZR1/BES1 and transcriptional repression imposed by the EAR motif of D53/SMXLs (Fang et al., 2019; Hu et al., 2019). Therefore, these two studies highlight the conserved roles of the D53/SMXLs–OsBZR1/BES1 module in SL-regulated shoot branching in rice and *Arabidopsis* (Figure 1).

In addition to the above-mentioned roles in SL signaling, OsBZR1 and BES1 play critical roles in BR signaling; BES1 was originally identified as an integral component of the BR signaling pathway (Yin et al., 2005). Interestingly, two recent studies discovered that BR signaling controls shoot branching differently via OsBZR1/BES1 in monocots and dicots. In *Arabidopsis*, BR signaling mainly controls the phosphorylation status of BES1, and only non-phosphorylated BES1 can trigger the downstream BR-related transcription network (Yin et al., 2005). SL signaling mainly induces the degradation of BES1 by MAX2, regardless of its phosphorylation status, to inhibit shoot branching (Wang et al., 2013). The recent study by Hu et al. (2019) further demonstrated that both phosphorylated and non-phosphorylated forms of BES1 interact with SMXLs, and that BRs do not affect the ability of BES1 to bind to the *BRC1* promoter and therefore do not modulate *BRC1* expression. These results provided an explanation as to why BR signaling components upstream of BES1 have no effect on shoot branching or *BRC1* expression (Wang et al., 2013; Hu et al., 2019).

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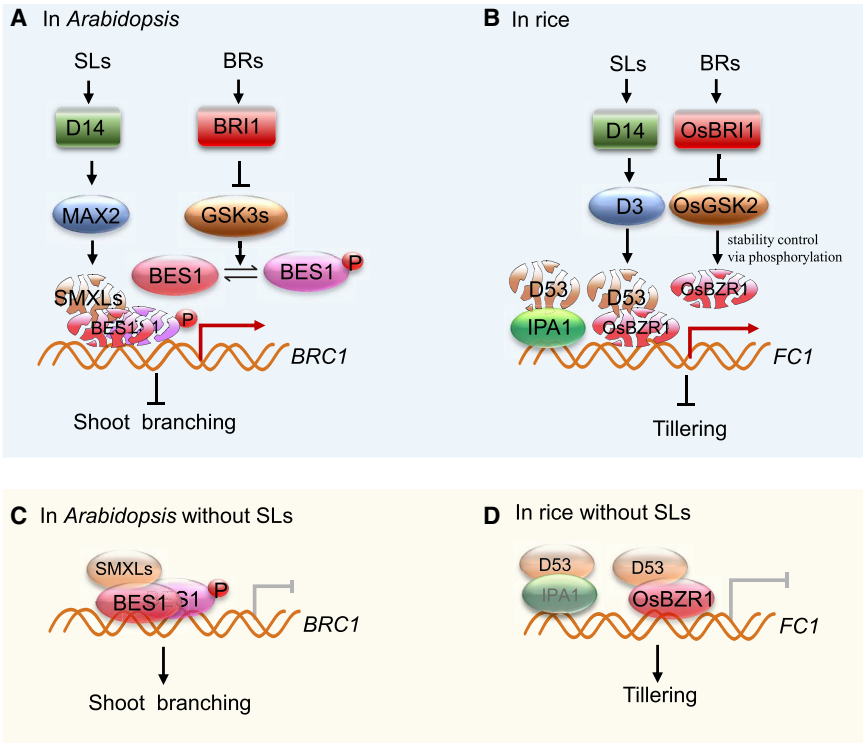

Figure 1. The SL and BR Signaling Pathways Regulate Shoot Branching via BES1/OsBZR1 in *Arabidopsis* and Rice.

(A) In *Arabidopsis*, SL signaling inhibits branching through AtD14-MAX2-SMXLs-BES1-BRC1, a pathway conserved in monocots. BR signaling alters the phosphorylation status of BES1 but has no effect on shoot branching.

(B) In rice, SL signaling induces the degradation of D53 and OsBZR1 to relieve the inhibition of *FC1* and the repression of IPA1, which promotes the transcription of *FC1* and inhibits tillering. BR signaling promotes tillering, which depends on the D53-OsBZR1 module, by increasing the accumulation of OsBZR1.

(C) Without SL signals in *Arabidopsis*, the SMXLs-BES1 complex accumulates and inhibits *BRC1* expression to promote shoot branching.

(D) Without SL signals in rice, the D53-OsBZR1 complex accumulates and inhibits *FC1* expression, and the accumulation of D53 inhibits the transcriptional activity of IPA1 to promote tillering. Modified from Fang et al. (2019) and Hu et al. (2019).

In contrast, in rice, the BR-regulated phosphorylation status of OsBZR1 determines its protein stability and its transduction of BR signals, while SL signaling inhibits rice tillering by promoting OsBZR1 degradation (Fang et al., 2019). Therefore, BR signaling likely regulates tillering via the regulation of OsBZR1 stability. The comprehensive genetic and biochemical analysis carried out by Fang et al. (2019) demonstrated that the SL and BR signaling pathways antagonistically control rice tillering through the regulation of the stability of the D53-OsBZR1 module, which directly modulates *FC1* expression. Therefore, rice OsBZR1 and *Arabidopsis* BES1 differentially regulate shoot branching in these two species based on their specific integration of BR signals (Figure 1A and 1B).

In addition, *Arabidopsis* BES1 and homologs act redundantly in SL-mediated branching; for instance, BES1 and its homologs interact with MAX2 and SMXLs (Wang et al., 2013; Hu et al., 2019), and this explains why the T-DNA insertion line *bes1-1*, in which only *BES1* expression is abolished, displayed no obvious alteration of branching (Bennett et al., 2016). Indeed, a *BES1-RNAi* line, with decreased transcript levels of *BES1* and the *BES1* homologs *BZR1, BEH2*, and *BEH3*, exhibited reduced branching (Wang et al., 2013). Furthermore, *OsBZR1* and *BES1* are highly expressed in axillary buds (Fang et al., 2019; Hu et al., 2019), an expression pattern that overlaps with that of other SL signaling components, including *D3/MAX2*, *D14/AtD14*, and *D53/D53-like SMXLs* (Stirnberg et al., 2007; Arite et al., 2009; Zhou et al., 2013), suggesting that OsBZR1 and BES1 might transduce the SL signal in buds. Although a hextuple mutant lacking *BES1* and all five *BES1* homologs is available in *Arabidopsis* (Chen et al., 2019), its serious defective vegetative and reproductive phenotypes makes any interpretation in the context of shoot branching challenging.

Recently, IDEAL PLANT ARCHITECTURE1 (IPA1), a member of the SQUAMOSA PROMOTER BINDING PROTEIN-LIKE (SPL) family of transcription factors, was identified as a positive regulator that acts downstream of D53 in SL signaling (Song et al., 2017). D53 interacts with and inhibits the transcriptional activity of IPA1 (Song et al., 2017). Therefore, the SL signaling pathway in rice may involve two types of transcription factors, OsBZR1 and IPA1, both acting as D53 partners but differing in how they transduce SL signals for transcriptional regulation of tillering (Figure 1B and 1D). In the presence of SL, degradation of the negative module D53-OsBZR1 is induced, leading to the alleviation of a repressive chromatin state at the *FC1* locus. In parallel, D53 degradation also allows the activation of IPA1, a positive component of SL signaling, which may trigger an SL-related transcriptional network, including induction of *FC1* expression, thereby causing inhibition of tillering (Figure 1B). In the absence of SLs, the accumulated D53-OsBZR1 complex inhibits *FC1* expression via chromatin modifications mediated by the TPL-related complex, while accumulated D53 also maintains repression of IPA1, resulting in transcriptional inhibition (Figure 1D). However, whether and how D53-OsBZR1 and D53-IPA1 coordinately inhibit the SL-triggered transcription networks remain to be investigated. For example, do OsBZR1 and IPA1 genetically interact, and what are their direct targets in SL signaling? In addition, orthologs of D53 in poplar and grass (*Dasypyrum villosum*) also function in SL-mediated branching (Katyayini et al., 2019; Bazhenov et al., 2020), while IPA1 and IPA1-related SPLs have been reported to play a role in SL-regulated tillering in rice and wheat (Liu et al., 2017; Song et al., 2017). It would be interesting to investigate whether the function of D53-OsBZR1 and D53-IPA1 in SL signaling is conserved in other plant species.

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