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Short Article

Strigolactone/MAX2-Induced Degradation of Brassinosteroid Transcriptional Effector BES1 Regulates Shoot Branching

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SUMMARY

Strigolactones (SLs), a class of the most recently identified terpenoid phytohormones, play essential roles in plant development, specifically in suppressing shoot branching. MAX2, a subunit of an SCF E3 ligase and a positive regulator that inhibits shoot branching, is likely a key SL signaling component. Here, we provide genetic and biochemical evidence to demonstrate that BES1 interacts with MAX2 and acts as its substrate to regulate SL-responsive gene expression. Additional AtD14, a putative receptor of SLs, can promote BES1 degradation. Knockdown of *BES1* and its homologs dramatically suppressed the branching phenotype of *max2-1* mutant. These results portray an SL signaling cascade from the putative receptor to downstream transcription factors. In addition, we demonstrate that the SL and brassinosteroid (BR) signaling pathways distinctly regulate the same transcription factor, BES1, to control specific developmental processes.

INTRODUCTION

Strigolactones (SLs) have long been recognized as symbiotic signals responsible for induction of seed germination of root parasite plants and as branching factors for symbiotic arbuscular mycorrhizal (AM) fungi (Cook et al., 1966; Akiyama et al., 2005). Recently, genetic and physiological evidence demonstrated that plants produce SLs, which function as phytohormones to repress shoot branching (Umehara et al., 2008; Gomez-Roldan et al., 2008). Mutants deficient in SL biosynthesis or signaling in diverse species including *Arabidopsis thaliana* (more axillary shoot [*max*]), *Pisum sativum* (*ramosus*, *rms*), *Oryza sativa* (dwarf [*d*] or high tillering dwarf [*htd*]), and *Petunia hybrida* (decreased apical dominance [*dad*]), have similar enhanced branching phenotypes (Beveridge and Kyozuka, 2010; Domagalska and Leyser, 2011). The first identified putative signaling component is more axillary growth locus 2 (MAX2) in *Arabidopsis*. The enhanced shoot branching phenotype of *max2* and the results of reciprocal grafting experiments demonstrated that MAX2 acts as a positive regulator that mediates SL responses (Stirnberg et al., 2002; Booker et al., 2005; Gomez-Roldan et al., 2008). MAX2 encodes an F box protein, a subunit of the S phase kinase-associated protein 1-cullin-F box (SCF) type ubiquitin E3 ligase, and is mainly expressed in parenchyma cells of xylem (Stirnberg et al., 2007). It has been recently reported that, in *Arabidopsis*, AtD14, an α/β-fold hydrolase, acting as a putative SL receptor, can bind to GR24, a synthetic analog of SLs (Zhao et al., 2013; Kagiyama et al., 2013), and decreased apical dominance 2 (DAD2), an ortholog of AtD14 in petunia, has the capacity to recruit PhMAX2A, an ortholog of MAX2, in the presence of GR24 (Hamiaux et al., 2012). SLs also promote membrane trafficking-mediated PIN1 depletion (Crawford et al., 2010; Shinohara et al., 2013), but PIN1 unlikely serves a direct MAX2 target due to their different subcellular localization (Stirnberg et al., 2007; Crawford et al., 2010). In addition, *BRANCHED1* (*BRC1*), encoding a TCP transcription factor, is specifically expressed in axillary nodes to inhibit branch outgrowth and may act downstream in the SL signaling pathway (Aguilar-Martínez et al., 2007). However, because AtBRC1 and PsBRC1 act as positive regulators to inhibit shoot branching, BRC1 protein may not be a degradation target of MAX2 (Aguilar-Martínez et al., 2007; Braun et al., 2012). Therefore, the substrates of MAX2 and the nature of downstream components of SL signaling pathway in *Arabidopsis* remain mysteries.

BES1 (*bri1*-EMS-suppressor 1), a positive regulator in brassinosteroid (BR) signaling pathway, acts as a downstream transcription factor to directly regulate BR-responsive gene expression (Yin et al., 2002), and phosphorylation and dephosphorylation of BES1 is a major way to regulate its activity (Yin et al., 2002; Vert and Chory, 2006). *bes1-D* (En2 background), a gain-of-function mutant of *BES1*, in which both phosphorylated and dephosphorylated BES1 were greatly accumulated due to a single mutation (proline to leucine at position 233) in its PEST domain (polypeptide sequences enriched in proline (P), glutamate (E), serine (S), and threonine (T), responsive for protein degradation) (Rechsteiner and Rogers, 1996), showed enhanced BR signaling outputs (Yin et al., 2002). However, a recent study suggested that genes differentially regulated in *bes1-D* are dramatically different from these BR-responsive genes, and chromatin immunoprecipitation coupled with *Arabidopsis* tilling arrays (ChIP-chip) experiments also indicated that many BES1-targeted genes are not BR-responsive genes

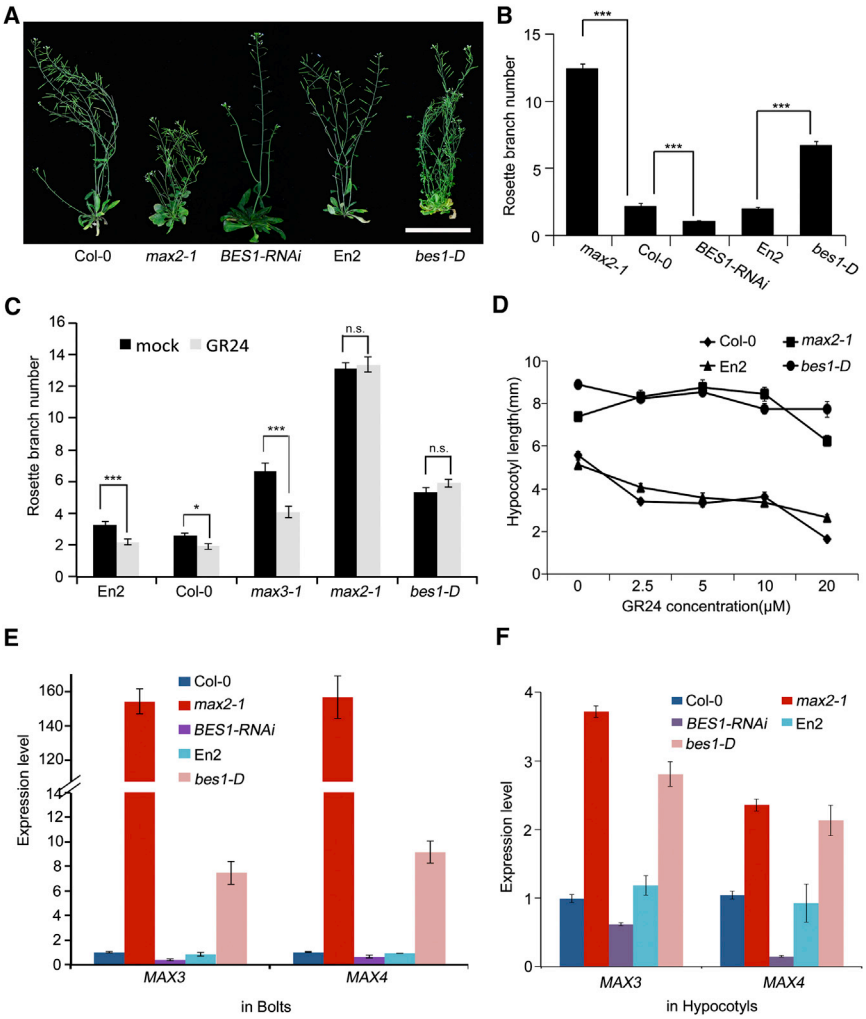

Figure 1. *bes1-D* Exhibits More Rosette Branches and Is Insensitive to SLs

(A) Shoot branching phenotype of the 50-day-old plants. Scale bar represents 10 cm.

(B) Shoot branch number of the 50-day-old plants. Data are means ± SE (n > 15).

(C) GR24 sensitivity assays in shoot branching.

(D) GR24 sensitivity assays in hypocotyl length. Data are means ± SE (n > 30).

(E) Relative expression levels of *MAX3* and *MAX4* in basal cauline internodes of the 7-week-old plants. The expression levels of *MAX3* and *MAX4* in Col-0 and En2 were defined as "1." Data are means ± SE (n = 3).

(F) Relative expression levels of *MAX3* and *MAX4* in hypocotyls of the 4-day-old seedlings grown under weak light. The expression levels of *MAX3* and *MAX4* in Col-0 and En2 were defined as "1." Data are means ± SE (n = 3).

Student's t tests were used to determine significant levels of the indicated comparisons. Significant levels: ***p < 0.001; *p < 0.05; n.s., no significance. See also Figure S1.

(Yu et al., 2011). Therefore, BES1 may also directly participate in other signaling pathways.

Interestingly, in this study, we observed that *bes1-D* had significantly enhanced rosette branching phenotype, and the *BES1-RNAi* line (Yin et al., 2005) had only one bolt. Furthermore, we found that BES1 and its homologs can interact with MAX2, and BES1 protein acts as a substrate of MAX2 for degradation. The reduced expression of *BES1* and its homologs can dramatically suppress the enhanced branching phenotype in *max2* mutant. Taken together, these data support that BES1 is a direct target of MAX2 and acts as a negative regulator of SL signaling pathway to promote shoot branching.

RESULTS AND DISCUSSION

BES1-Related Mutants Display Altered Shoot Branching Phenotype

We found that *bes1-D*, a BES1 gain-of-function mutant in *Arabidopsis*, exhibited the phenotype of enhanced shoot branching (Figure 1A). In addition, plants expressing *bes1-D* driven by a *MAX2* promoter showed a phenotype with dramatically increased axillary bud, leaf numbers in rosette centers, and rosette branch number, which was greatly similar to *max2-1* (Figures S1A and S1B available online). Furthermore, we observed that a *BES1-RNAi* line (Yin et al., 2005) in which transcript levels of *BES1* and *Brassinazole-resistant 1* (*BZR1*) were significantly reduced (Figure S1C), had only one bolt (Figures 1A and 1B). However, we did not observe a greatly altered branching phenotype in other BR-related mutants, including the BR-insensitive mutants *bri1-301* and *bin2-1*, a *BRI1* overexpression line (*BRI1-OX*), and a triple knockout line (*bin2-3bil1bil2*) of *BIN2* and its two homologs (Figure S1D), suggesting that under normal growing conditions the regulatory mechanism of BES1 by upstream BR signaling may not significantly alter the branching phenotype.

SL Responses Are Dramatically Compromised in *bes1-D* Mutant

SLs are widely recognized as shoot branching regulators, and a loss-of-function mutant of a critical component in SL signaling pathway, *max2-1*, has long hypocotyl and increased branch number, which are largely similar to *bes1-D*. Therefore, we tested whether the altered branching phenotypes in the *BES1*-related mutants were regulated by SLs, and found that, unlike the wild-type and a SL-deficient mutant *max3-1*, *bes1-D* mutant was insensitive to the applied GR24, a synthetic analog of SLs inhibiting shoot branching, similar to *max2-1* (Figure 1C). It is known that SLs inhibit hypocotyl elongation, and *max2-1* mutant displays a longer hypocotyl (Stirnberg et al., 2002; Tsuchiya et al., 2010). Therefore, we measured the sensitivity of *bes1-D* to SLs in hypocotyl elongation and found that *bes1-D* and *max2-1* exhibited normal hypocotyl elongation, even in the presence of GR24 (Figure 1D). However, wild-type seedlings grown on 20 μM GR24 were only ~30% the height of seedlings grown without GR24 (Figure 1D). GR24 can still significantly inhibit