Short title: BR signal-dependent root responses to B deficiency

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**Boron deficiency-induced root growth inhibition is mediated by
brassinosteroid signalling regulation in *Arabidopsis***

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One-sentence summary: B deficiency reduces the accumulation of brassinolide by reducing *BR6ox1* and *BR6ox2* mRNA level to down-regulate BR signalling and modulate root elongation.

Author contributions:

C.Z., M.H. and F.X. together designed the experiments. C.Z. performed most of the experiments unless indicated as follows. C.Z. conducted RNA-seq experiments and analyzed RNA-seq data with M.H. and N.Y. C.Z. and S.W. performed the confocal microscopy. C.W., G.D., H.C., and L.S. provided the discussion on the work and edited the manuscript. C.Z., M.H., L.C., and F.X. wrote the manuscript, and all authors approved it.

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ABSTRACT

Brassinosteroid (BR) is a pivotal phytohormone involved in regulating root development. Boron (B) is an essential micronutrient for plant growth and development, and root growth of plants is rapidly inhibited under B deficiency condition, but the mechanisms are still elusive. Here, we demonstrate that BR plays crucial roles in these processes. We identify BR-related processes underlying B deficiency at the physiological, genetic, molecular/cell biological and transcriptome levels, and provide strong evidences that B deficiency can affect BR signalling, thereby altering root growth. RNA-sequencing analysis reveals a high co-regulation between BR-regulated genes and B deficiency-responsive genes. We found that low B negatively regulates BR signalling to control BR signalling-dependent root elongation, *bes1-D* exhibits insensitivity to low B stress, and *bri1-301* mutants fails to respond to B depletion. Exogenous eBL application can rescue the inhibition of root growth under B deficiency condition, and application of BR biosynthesis inhibitor BRZ aggravates root growth inhibition of wild-type under B deficiency condition. B deficiency reduces the nuclear signal of BES1. We further found that B deficiency reduces the accumulation of brassinolide (BL) by reducing *BR6ox1* and *BR6ox2* mRNA level to down-regulate BR signalling and modulate root elongation. Altogether, our results uncover a role of BR signalling in root elongation under B deficiency.

Keywords: Boron deficiency, Brassinosteroid signalling, BL, *BR6ox1*, *BR6ox2*, *Arabidopsis*

INTRODUCTION

Warington gave the first definitive proof of boron (B) requirement for a higher plant in 1923 (Warington, 1923). Since then, B has been identified an essential micronutrient for higher plants. The main known function of B in higher plants is to cross-link the apiosyl residues of two neighbouring monomeric RG-II (monorhamnogalacturonan-II, mRG-II) molecules for forming a dimeric B-dRG-II pectin complex (Kobayashi *et al.*, 1996). This role of B in cell walls was confirmed by the *Arabidopsis mur1* dwarf mutant with brittle stems, which could be rescued with added B (O'Neill *et al.*, 2001).

Boron deficiency is a prevalent micronutrient deficiency in agricultural production worldwide, which leads to crop yield loss and quality decline (Shorrocks, 1997). B deficiency leads to a wide range of morphological, and physiological symptoms, such as apical growth inhibition, leaf expansion reduction, terminal bud necrosis, flower abortion and fruit shedding (Goldbach, 1997; Brown *et al.*, 2002). Among these symptoms, one of the earliest responses to B deficiency for plants is the inhibition or cessation of root elongation (Dell *et al.*, 1997). Reduction of root growth under B deficiency has been reported in many dicots, including *Arabidopsis*, *Brassica napus* and orange (Takano *et al.*, 2006; Hua *et al.*, 2016; Wu *et al.*, 2017). Graminaceous plants demand less B, but graminaceous mutants of B transporter genes display conspicuous inhibition of root elongation (Nakagawa *et al.*, 2007; Durbak *et al.*, 2014; Shao *et al.*, 2018). Two protein families are involved in B transport, the NIP influx channels and the BOR efflux transporters (Miwa & Fujiwara, 2010). However, the mechanisms through which B deficiency inhibits root growth are still enigmatic.

Brassinosteroids (BRs) are a class of polyhydroxylated steroid phytohormones in plants. BRs regulate a wide range of physiological and developmental processes including cell elongation, cell division, senescence, root hair initiation, stomata development, vascular differentiation, reproductive development and photomorphogenesis (Clouse & Sasse, 1998; Divi & Krishna, 2009; Wei & Li, 2016). BRs also interact with many other hormones and environmental factors to modulate plant growth and development (Nemhauser *et al.*, 2006; Zhang *et al.*, 2009; Yang *et al.*, 2011). In addition, BRs promote the expression of cell wall synthesis genes and

94 affect the expression of P450 genes involved in BR biosynthesis and catabolism
 95 (Goda *et al.*, 2002). BRs are synthesized from phytosterols through multiple, mostly
 96 oxidative reactions. Campesterols (CR) is thought to be the first molecule specifically
 97 for the BR biosynthesis, which is firstly converted to campestanol (CN), then to
 98 castasterone (CS), and finally to brassinolide (BL) (Fujioka *et al.*, 1995; Choi *et al.*,
 99 1996; Choi *et al.*, 1997). Several cytochrome P450 enzymes play important roles in
 100 the BR biosynthesis, such as DEETIOLATED2 (DET2), CONSTITUTIVE
 101 PHOTOMORPHOGENESIS AND DWARFISM (CPD), ROTUNDIFOLIA3 (ROT3),
 102 CYP90D1, DWARF4 (DWF4), BRASSINOSTEROID-6-OXIDASE1 (BR6ox1), and
 103 BR6ox2. CPD catalyzes C-23 hydroxylation of BRs (Ohnishi *et al.*, 2012), and
 104 DWF4 is contribute to multiple C-22 hydroxylation (Choe *et al.*, 1998). Both BR6ox1
 105 and BR6ox2 catalyze multiple C-6 oxidation, while BR6ox2 catalyzes the
 106 lactonization of CS to BL (Bishop *et al.*, 1999; Kim *et al.*, 2005). Some artificial
 107 chemicals inhibitors of BR biosynthesis have been reported. For example,
 108 brassinazole (BRZ) can directly inhibit the catalytic activity of DWF4 (Asami *et al.*,
 109 2000).

110 BRs are perceived at the plasma membrane by three leucine-rich repeat
 111 receptor-like kinase (LRR-RLKs) BRASSINOSTEROID INSENSITIVE 1 (BRI1) (Li
 112 & Chory, 1997). BRs bind to the extracellular leucine-rich repeat domain of BRI1,
 113 which phosphorylates BRI KINASE INHIBITOR 1 (BKI1) as a downstream negative
 114 regulator of BRI (Wang & Chory, 2006), resulting in the release of BKI1 from the
 115 intracellular BRI1 kinase domain, allowing BRI1 to recruit its co-receptor
 116 BRI1-ASSOCIATED RECEPTOR KINASE 1 (BAK1), another LRR-RLK (Li *et al.*,
 117 2002; Nam & Li, 2002). At downstream of BRI1 and BAK1, members of the
 118 BRASSINOSTEROID SIGNALING KINASE (BSK) cytoplasmic kinase family
 119 initiate a phosphorylation-and-dephosphorylation cascade (Kim *et al.*, 2009).
 120 Reversible phosphorylation and dephosphorylation cascade leads to nuclear
 121 accumulation of two unphosphorylated transcription factors,
 122 BRASSINAZOLE-RESISTANT 1 (BZR1) and BRI1-EMS-SUPPRESSOR 1 (BES1)
 123 (Wang *et al.*, 2002; Yin *et al.*, 2002). BZR1 and BES1 can regulate thousands of target

124 genes to mediate plant growth and development (Sun *et al.*, 2010; Yu *et al.*, 2011).
 125 The hypophosphorylated state of *bes1-D* and *bzr1-D* dominant mutants results in
 126 constitutive activated BR signalling and constitutive BR responses (Wang *et al.*, 2002;
 127 Yin *et al.*, 2002).

128 BRs regulate growth and development of plant roots in a dose-dependent manner
 129 (Clouse *et al.*, 1996; Mussig *et al.*, 2003). Application of low concentration of BL
 130 can facilitate root growth, but high concentration of BL inhibited root elongation
 131 (Gonzalez-Garcia *et al.*, 2011). Mutants impaired in BR biosynthesis, such as *cpd* and
 132 *det2*, showed much shorter roots compared to those of wild-type plants (Chory *et al.*,
 133 1991; Fujioka *et al.*, 1997). Both *bri1* and *bak1* which were the mutants defective in
 134 BR signal transduction, also showed significantly shortened roots (Clouse *et al.*, 1996;
 135 Li *et al.*, 2002). Recent studies found that BZR1 and BES1 activity conferred to root's
 136 insensitivity to Pi deprivation through repressing LPR1 expression and affecting Fe
 137 distribution (Singh *et al.*, 2014; Singh *et al.*, 2018). It was also found that BSK3 as a
 138 major determinant for primary root length response to low N condition (Jia *et al.*,
 139 2019).

140 Here, we report that B deficiency impacts on BR-dependent root growth through
 141 reducing the accumulation of BL. We show that B deficiency results in reduced root
 142 meristem size and mature cell length. The *bes1-D* constitutive BR response mutant
 143 showed significantly longer primary root than wild-type under B deficiency condition,
 144 while *bri1-301* failed to respond to B depletion. B deficiency down-regulated *BR6ox1*
 145 and *BR6ox2* to reduce the accumulation of BL to inhibit BR-dependent root growth.
 146 Overall, our data highlight the important role of BR signalling in the adaptation to B
 147 deficiency condition.

148

149

150 **RESULTS**

151 **Influence of B deficiency on root growth**

152 To evaluate the impact of B depletion upon root growth in *Arabidopsis*, we performed
153 a root sensitivity assay by monitoring primary root growth and developmental
154 parameters of roots in medium of gradient B concentrations. Primary root length of
155 wild-type plants (En2) was significantly inhibited when B concentration was
156 decreased from 100 μ M to 0.05 μ M (Fig. 1A and B). The root growth was decelerated
157 rapidly with the decrease of B concentration within 3 days after germination (Fig.
158 1C).

159
160 It is known that the root growth inhibition is the result of a decrease in cell size and/or
161 cell number. To investigate whether the inhibition under B deficiency condition
162 results from the decreased cell number and/or cell size, we measured the number of
163 meristematic cells and the length of fully elongated root cells. Roots of wild-type
164 grown under B deficiency displayed reduced root meristem size, reduced number of
165 meristematic cells (Fig. 1D, E and H), and decreased expression of the *CYCBI;1*,
166 which can be regarded as the marker gene of cyclin cell cycle (Fig. 1G). A significant
167 decrease of mature cell length was also found for plants grown at B deficiency
168 condition (Fig. 1F and I). Taken together, these observations indicate that B
169 deficiency results in reduced number of meristematic cells, root meristem size and
170 mature cell length in roots, and thus reduces root growth.

171

172 **Genome-wide root responses to B deficiency**

173 To shed light on the pathways mediating root growth responses to B deficiency, we
174 performed whole-genome RNA sequencing (RNA-seq) analysis to compare root
175 transcriptomes of 9-day-old wild-type plants grown under adequate and depleted B
176 conditions. We found that 4,441 genes showed significantly differential expression in
177 wild-type plants between the depleted B condition versus adequate B condition (Fig.
178 2B), 2,360 of these genes were strongly (>2-fold) up-regulated or down-regulated
179 (Supplemental Table S1). The top gene ontology (GO) terms (ranked by Correct

p-Value) among the differential expression genes showed that B deficiency directly and/or indirectly influences a range of biological processes, including oxidation-reduction, cell wall organization, lipid metabolic and steroid biosynthetic process (Fig. 2A). Increased H₂O₂ fluorescence and O₂⁻ fluorescence in the root with the fluorescent probes 3'-(*p*-hydroxyphenyl) fluorescein (HPF) and dihydroethidium (DHE) were observed under B deficiency (Supplemental Fig. S1).

Since several recent studies demonstrated that BRs played an important role in root growth (Gonzalez-Garcia *et al.*, 2011; Hacham *et al.*, 2011; Chaiwanon *et al.*, 2015; Jaillais *et al.*, 2016), we compared our B deficiency-responsive genes with a recent root-specific BR-regulated gene data set generated by RNA-seq (Chaiwanon *et al.*, 2015). Interestingly, 45.9% of the genes regulated by B deficiency were also regulated by BRs in the root (Fig. 2B and Supplemental Table S2), indicating a high degree of co-regulation between them. Furthermore, we found that more than 60% of the co-regulated genes responded to B deficiency and BRs in opposite directions (Fig. 2C and Supplemental Fig. S2A). It has been reported that activated BR signalling appears to inhibit at least five BR biosynthetic genes, as well as *BR1* through negative feedback, while positively regulated downstream BR signalling by activating transcription of *BSU1* and inhibiting *BIN2* (Tanaka *et al.*, 2005; Sun *et al.*, 2010). Indeed, *BR1*, *BAK1*, *BIN2* and *BZR1* were accumulated more in mRNA level in the wild type plants under B deficiency, and reduced mRNA accumulation for *BSU1*, no significant changes for *BES1* were observed in our RNA-seq result (Supplemental Fig. S2C-H). CONSTITUTIVE PHOTOMORPHOGENESIS AND DWARF (CPD) was up-regulated by B deficiency (Supplemental Fig. S2B). What's more, the content of BL, the most active BR, was reduced under B deficiency in the wild type (En2) (Fig. 2D). Based on these results, we speculate that B deficiency is likely to negatively regulate BR signalling to affect root growth.

BR signalling is involved in root responses to boron deficiency

BRs regulate root growth by regulating the elongation of differentiated cells and

meristem size (Gonzalez-Garcia *et al.*, 2011; Hacham *et al.*, 2011; Fridman *et al.*, 2014). To investigate the possible role of BRs in root responses to B deficiency, we grew BR signalling-related mutants under adequate (100 μ M) and depleted (0.1 μ M) B conditions and scored their primary root length. The *bes1-D* mutant, which over-accumulates the dephosphorylated active BR-regulated transcription factor BES1 (Yin *et al.*, 2002), showed significantly longer primary root length than wild-type En2 under 0.1 μ M B condition (Fig. 3A and B) and the difference was even more significant under 0.05 μ M B condition (Fig. 3D; Supplemental Fig. S3A and B) or treated for longer time (17 DAG) under 0.1 μ M B condition (Supplemental Fig. S3C and D). Quantification of root growth in the medium with B deficiency showed that *bes1-D* roots exhibited greater growth rate than wild-type under B deficiency (Fig. 3C). Cellular analysis revealed that *bes1-D* displayed longer mature cells, larger meristem and more meristematic cells than wild-type under B deficiency (Fig. 3E-G). To test whether BZR1 activity also impedes root response to low B stress, we established plant lines harbouring the same stabilizing mutation in BZR1 (*pBZR1-bzr1-D*). Like *bes1-D*, *pBZR1-bzr1-D* also showed significantly longer primary root length than wild-type under B deficiency condition (Supplemental Fig. S3E and F).

To strengthen the genetic evidence supporting the role of BR signalling in B deficiency mediated root growth, we used BR receptor mutant *bri1-301* (Xu *et al.*, 2008), which showed shorter root than wild-type plants under B sufficient condition (Fabregas *et al.*, 2013). However, the *bri1-301* mutant failed to respond to B depletion (Fig. 4A-D). These genetic evidences clearly suggest that BR signalling positively impacts on root responses to decreased B concentration.

BRs regulate plant root growth and development in a dose-dependent manner (Roddick *et al.*, 1993; Clouse *et al.*, 1996; Mussig *et al.*, 2003). If B deficiency downregulated BR signalling to inhibit root elongation, exogenous application of 24-epibrassinolide (eBL), a bioactive BR, should rescue the B deficiency-induced

phenotype. Indeed, wild-type root growth was significantly enhanced in the presence of 0.01 nM eBL, which was similar to the length of *bes1-D* under B deficiency (Fig. 4E and F). While application of BR biosynthesis inhibitor BRZ aggravated root growth inhibition of wild-type under B deficiency condition, BRZ treatment did not affect *bes1-D* root's insensitivity to B deficiency (Fig. 4G and H). BRs increase nuclear accumulation of both BES1 and BZR1 (Wang *et al.*, 2002; Yin *et al.*, 2002). In *p35s-BES1-GFP* plants, we found that nuclear signal was reduced significantly under B deficiency, while increased nuclear signal was observed following exogenous eBL application (Fig. 4I and J). Taken together, these results indicate that B deficiency downregulates BR signalling to inhibit root growth.

BR signalling impacts neither B transport nor translocation

The root growth of *nip5;1* mutant was severely inhibited under B deficiency condition, and enhanced expression of *NIP5;1* and *BOR1* could improve the resistance to low B stress in *Arabidopsis* (Miwa *et al.*, 2006; Kato *et al.*, 2009). To assess whether BES1 activity can trigger *AtNIP5;1* gene expression, we analysed the relative expression of B transport or translocation genes in roots and shoots. In wild-type, the transcription level of *AtNIP5;1* in root was greatly higher under B deficiency condition than under B sufficient condition (Fig. 5A), consistent with previous studies (Takano *et al.*, 2011). However, lower expression was observed in root of *bes1-D* plants under B deficiency condition (Fig. 5A). We also analysed other B-related genes, including *AtNIP6;1*, *AtNIP7;1*, *AtBOR1*, *AtBOR2*, and *AtBOR4*. Except *AtBOR1* had higher expression level in shoot of *bes1-D* mutant than wild-type under both B sufficient and deficient conditions, no obvious changes were observed in the transcription levels of *AtNIP6;1*, *AtNIP7;1*, *AtBOR2* and *AtBOR4* between the mutant and wild-type plants (Fig. 5B-F).

To indicate whether the expression changes of B-related genes are associated with the endogenous B homeostasis, we performed inductively coupled plasma-optical emission spectrophotometry (ICP-OES) analysis to quantify the B concentration and accumulation in both wild-type and *bes1-D* plants grown under adequate and depleted

270 B conditions. We found *bes1-D* had higher B concentration than wild-type in root but
271 lower in shoot (Fig. 5G), and it seemed to confer to the lower expression of *AtNIP5;1*
272 in root of *bes1-D* plants under B deficiency condition (Takano *et al.*, 2011). The
273 difference in B concentration is likely to be caused by the different dry mass of
274 *bes1-D* and wild-type (Fig. 5H). Furthermore, no significant differences in B
275 accumulation were detected between wild-type and *bes1-D* mutant (Fig. 5I). Taken
276 together, the insensitivity of *bes1-D* to B deficiency results from *BES1* acting
277 independently of B transport or translocation pathway.

278

279 **Boron deficiency reduces the accumulation of BL**

280 The mechanisms that B deficiency impinges BR signalling may be diverse. Based on
281 the findings, we hypothesized that B deficiency reduces BR activity and/or levels to
282 inhibit root growth. To address this hypothesis, we analyzed the relative expression
283 levels of genes involved in BR biosynthesis in wild-type and *bes1-D* mutant under B
284 sufficient and deficiency condition with qRT-PCR. Consistent with the result of
285 RNA-seq, the expression levels of *CPD* genes were higher under B deficiency than
286 sufficient B supply (Fig. 6A and Supplemental Fig. S2B). Interestingly, both *BR6ox1*
287 and *BR6ox2* genes were down-regulated under B deficiency, in contrast with the
288 negative feedback of reduced BR signalling (Fig. 6B and C; Supplemental Fig. S2I
289 and J). *BR6ox2* involved in mediating the conversion of CS to BL in the rate-limiting
290 final step of BRs biosynthesis (Kim *et al.*, 2005). In *bes1-D* mutant, the expression
291 levels of these genes were consistently lower than the wild type (Fig. 6A-C), which is
292 consistent with the known negative feedback regulation of *BES1* on their expression
293 (Tanaka *et al.*, 2005).

294

295 The significant changes in expression of BR biosynthesis genes prompted us to
296 determine the endogenous levels of BRs in wild type and *bes1-D* plants. Several BR
297 derivatives in both wild-type and *bes1-D* seedlings grown in B sufficient and
298 deficiency conditions were analysed, including the most active BR, BL, and its
299 immediate precursor, castasterone (CS), and the precursors of CS,

300 6-deoxocastasterone (6DCS). Indeed, BL levels were decreased in both wild-type and
301 *bes1-D* plants under B deficiency condition (Fig. 6G and Supplemental Table S3), and
302 *bes1-D* mutant accumulated less BL than wild-type, in agreement with that BL
303 biosynthesis was under a negative feedback regulation of BES1 activity (Tanaka *et al.*,
304 2005). No difference was found in CS level, but there were more 6DCS accumulated
305 in wild-type under B deficiency (Fig. 6E and F; Supplemental Table S3). Both 6DCS
306 and CS are synthesized from their corresponding higher order precursors (e.g.
307 campesterol) by BR-specific synthetases like CPD, BR6ox1 and BR6ox2 (Miklós
308 Szekeres *et al.*, 1996; Shimada *et al.*, 2003) (Fig. 6D). The accumulation of 6DCS
309 was probably due to the up-regulation of *CPD* gene and down-regulation of *BR6ox1*
310 and *BR6ox2* genes in wild-type under B deficiency, and the reduced *BR6ox2* gene
311 expression conferred to the lower level of BL in wild-type under B deficiency.
312 *BR6ox1* and *BR6ox2* should have been up-regulated by weakened BR signal through
313 feedback pathway theoretically (Tanaka *et al.*, 2005). It's obvious that B deficiency
314 disrupted this feedback, but the regulation network is unknown. Altogether, our data
315 indicate that B deficiency impinges on BR signalling and BR-signalling dependent
316 root growth at the transcriptional level of *BR6ox1* and *BR6ox2* genes.

317

318

DISCUSSION

Although there's been almost one century of research on its biology, B is still a much unknown micronutrient. B deficiency causes the inhibition of primary root growth rapidly (Fig. 1A-C), but mechanisms of root responses to B deficiency are poorly understood. Here, we used genetic, genomic, biochemical, and cell biological approaches to demonstrate that B deficiency impinges on BR signalling at the level of BL to control BR signalling-dependent root elongation.

The phytohormone BRs play pivotal roles in the regulation of plant growth and development with respect to environmental variability (Clouse & Sasse, 1998; Divi & Krishna, 2009). BRs regulate root growth by regulating the elongation of differentiated cells and meristem size with a dose-dependent manner (Chory *et al.*, 1991; Gonzalez-Garcia *et al.*, 2011; Fridman *et al.*, 2014). B deficiency affects cell division and expansion, and finally results in root growth inhibition (Fig. 1D-I). We hypothesize that BR signalling may be involved in root responses to B deficiency. In agreement, first, 45.9% of the genes regulated by B deficiency in root were also regulated by BRs (Fig. 2B). We further analysed the expression patterns of these genes. Interestingly, more than 60% of the co-regulated genes response to B deficiency and BRs in the opposite directions (Fig. 2C and Supplemental Fig. S2A). Therefore, we speculate that B deficiency negatively regulates BR signalling to inhibit root growth. Second, we confirmed our hypothesis with different BR signalling mutants. Our data showed that both constitutive BR response mutant *bes1-D* and BR receptor mutant *bril-301* were insensitive to B deficiency (Fig. 3A-D and Fig. 4A-D), and the enhanced root length of *bes1-D* mutant was the consequence of increased root cell expansion and proliferation (Fig. 3E-G). Third, exogenous application of eBL could rescue the root growth inhibition of wild-type to reach the length of *bes1-D* under B deficiency (Fig. 4E and F). While application of the BR biosynthesis inhibitor BRZ made wild-type plants more sensitive to B deficiency (Fig. 4G and H). Moreover, the nuclear signal of BES1-GFP was decreased under B deficiency, and could be restored by eBL treatment (Fig. 4I and J). In conclusion, our work provides

complementary evidence demonstrating that B deficiency downregulates BR signalling to control the BR signalling-dependent root growth.

351

To further explore the effects of B deficiency on BR signal, we examined the homeostasis of endogenous BRs and the expression activity of BR synthetases genes. Under B deficiency, BL concentration in WT was decreased significantly (Fig. 6G), resulting in the up-regulation of *CPD* through negative feedback (Fig. 6A and Supplemental Fig. S2B). The up-regulation of *CPD* led to the increase of 6DCS (Fig. 6D and E). However, CS, the derivative of 6DCS, did not increase under B deficiency, which could be explained by the decrease of *BR6ox1* and *BR6ox2* expression (Fig. 6B-D, F and Supplemental Fig. S2I-J). The reduced expression of *BR6ox2* also contributed to the decline of BL concentration (Fig. 6C, D and G). In line with this supposition, BL concentration was even lower along with lower transcription activity of *BR6ox2* in *bes1-D*.

363

We speculated whether BR can trigger B-related genes expression to improve root elongation under B deficiency. The transcription level of *AtNIP5;1* in *bes1-D* is lower than wild-type in root (Fig. 5A), which is probably due to the higher concentration of B in *bes1-D* (Fig. 5G), because *NIP5;1* mRNA is accumulated under low B conditions but not under high B conditions, mediated by uORF of *NIP5;1* UTR region (Takano *et al.*, 2006). There's no significant difference in other B-related genes involved in B uptake and transport (Fig. 5B-F). Taken together, BES1 regulates primary root length under low B stress not by influencing the expression of B-related genes.

372

Previously, evidence about the involvement of auxin, ethylene, and cytokinin in the responses of root growth to B deficiency in *Arabidopsis* has been reported (Abreu *et al.*, 2014; Juan *et al.*, 2015; Poza-Viejo *et al.*, 2018). Crosstalk between BR and other hormones has been observed at the physiological and transcriptional levels (Goda *et al.*, 2004; Nemhauser *et al.*, 2004; Hardtke *et al.*, 2007; Gendron *et al.*, 2008). Decreased BR signalling under B deficiency may have the ability to regulate other

phytohormones signalling. Future work will elucidate whether reduced BR signalling under B deficiency interact with other phytohormones. We also noticed the primary root length of *bes1-D* was obviously longer than wild-type under B deficiency condition, but it reached only half the primary root length of wild-type under normal B condition (Fig. 3A). This may owe to the 54.1% genes regulated by B deficiency but not by BR (Fig. 2B). Future functional studies of the B deficiency regulated genes will further advance our understanding of the plant responses to B deficiency.

It is well known that the BL concentration in reproductive organs is significantly higher than that in vegetative organs, and the reproductive development of many crops, such as *Brassica napus*, is hypersensitive to B deficiency. Is the decrease of BL under B deficiency more considerable in reproductive organs? Does that suggest the potential role of BR signalling in B nutrition of plant reproduction? It needs further studies to answer these questions. Together, our work provides complementary evidence demonstrating that BRs involve in B deficiency-induced root reduction. Under B insufficient condition, the down-regulation of *BR6ox1* and *BR6ox2* leads to decreased BL biosynthesis, thus reduces BR signal and inhibits root elongation (Fig. 7). This is the framework of mechanism underlying BR's involvement in root responses to B deficiency, but a deeper and more detailed understanding of the regulation pathway requires future studies.

MATERIALS AND METHODS

Plant material, growth conditions, and experiment treatments

The mutants and transgenic lines used in our study were previously reported, including *bes1-D* (Yin *et al.*, 2002), *bril-301* (Xu *et al.*, 2008), *CYCBI;1:GUS* (Gonzalez-Garcia *et al.*, 2011). *pBZR1-bzr1-D* and *p35S-BES1-GFP* transgenic plants were generated in *Arabidopsis* ecotypes En2 and Col-0 background through agrobacterium-mediated floral dipping method, respectively (Clough *et al.*, 1998). Table S4 describes the primers used for constructing of these BR transgenic plants. Seeds were sterilized with 1% (w/v) NaClO for 10 min and rinsed completely in

409 ultrapure water. Two days after vernalization at 4°C, the seeds were germinated on
410 solid vertical MGRL medium (Fujiwara *et al.*, 1992) containing 1% sucrose and 1%
411 Gellan Gum supplemented with 100 µM or 0.1 µM B unless otherwise indicated. For
412 chemical and hormone treatments, the seeds were planted on relevant supplemented
413 MGRL medium and analyzed after 9 to 11 d, unless otherwise indicated. eBL and
414 BRZ were dissolved in 100% dimethyl sulfoxide. BRZ was added at concentration of
415 100 nM and 500 nM. eBL was added at a concentration of 0.01 nM unless indicated
416 otherwise. All plants grown at a temperature regime of 22/20°C (light/dark) under an
417 16/8 h (light/dark) photoperiod with a light intensity of 300–320 µmol·m⁻²·s⁻¹.

418

419 **RNA extraction and expression analysis**

420 Nine-day-old En2 and *bes1-D* mutant grown on MGRL solid medium with 100 µM B
421 were transferred to 100 or 0.1 µM B medium for 24 h, and then harvested for RNA
422 extraction with the RNA extraction kit (Promega, Madison, WI, United States),
423 followed with reverse transcription using ReverTra Ace qPCR RT Master Mix with
424 gDNA Remover kit (TOYOBO). Quantitative real-time PCR assays were performed
425 under a Real-time PCR Detection System of QuantStudioTM6 Flex (ABI, USA) in a
426 384-well plate format using SYBR Green PCR (TOYOBO, Osaka, Japan). *UBQ5* and
427 *ACTIN* genes were used as two reference genes. Relative expression values were
428 analyzed through the method of $\Delta\Delta$ threshold cycle. Three biological replicates were
429 performed for all reactions. The primers for qRT-PCR are listed in Supplemental
430 Table S4.

431

432 **Root growth parameters**

433 For the analysis of root growth rate, the position of root tips was recorded at the same
434 time from 1 to 10 or 11-day-olds, and the root elongation in 24 h was measured. For
435 sensitivity assays, the seeds were planted on MGRL medium containing different B
436 concentrations as shown in the text. After 9 or 10 d, the primary root length was
437 calculated on acquired images of seedlings by the ImageJ software. For mature and
438 meristem zone cell size analysis, root tips of seedlings were imaged under a Leica

439 TCS SP8 confocal laser scanning microscopes (www.leica-microsystems.com/home/).
 440 The roots were soaked with 10 μ M FM4-64 about 3 min before observation. FM4-64
 441 was viewed at an excitation wavelength of 488 nm, and the emission light was
 442 collected at 556 nm. Meristem size was defined as the distance from the quiescent
 443 center to the boundary of meristem where the cell of cortical is twice the size of the
 444 prior cell. Mature zone cell size was acquired via measuring the length of epidermal
 445 cells which showed the first root hair bulge. The cell divisions of root tips were
 446 observed with transgenic plants *CYCBI;1:GUS*. GUS histochemical staining was
 447 carried out using 5-bromo-4-chloro-3-indolyl-b-D-glucuronide as substrate
 448 (Gonzalez-Garcia *et al.*, 2011). The ROS staining was performed according to the
 449 Jens Müller *et al.* (Müller *et al.*, 2015).

450

451 **RNA-Seq analysis**

452 Six samples of mRNA (wild type En2 grown on MGRL medium containing 100 μ M
 453 or 0.1 μ M B, in three biological repetitions) were collected from roots of 9-day-old
 454 seedlings. The RNA-Seq experiments were carried out by Novogene. Library
 455 construction was implemented according to Illumina instructions and sequenced on a
 456 HiSeq 2000, to obtain 125 bp/150 bp paired-end reads. These reads were mapped to
 457 the *Arabidopsis thaliana* TAIR10 genome using Hisat2 v2.0.4. Transcript coordinates
 458 from the TAIR10 reference set were used to guide the alignment process. After
 459 alignment, HTSeq v0.9.1 was used to count the reads numbers mapped to each gene.
 460 The differential expression genes (DEGs) were analyzed by the DESeq R package
 461 (1.18.0). The Benjamini and Hochberg's approach were used to adjust the resulting
 462 P-values for controlling the false discovery rate. Genes with an adjusted P-value
 463 <0.05 found by DESeq were defined as differentially expressed. The GSeq R
 464 package was used to implement Gene Ontology (GO) enrichment analysis of
 465 differentially expressed genes.

466

467 **Boron measurement**

468 Seedlings of En2 and *bes1-D* were germinated in MGRL media for 11 d under 0.1 and

100 μ M B conditions. Roots and shoots, harvested separately, were dried at 105°C for 30 min and then developed into constant weight at 65°C. Total dry weight and number of plants were measured for counting B concentration and accumulation. The samples were ground into powders with carnelian mortar. Adding 10 mL of 1 M HCl to shake in 250 rpm shaker for 2 hours, the B was lixiviated out. Lixivate solution were filtered with filter paper and diluted 2 to 6 times. The B concentration was measured by inductively coupled plasma-optical emission spectrophotometry (Thermo Scientific).

Confocal microscopy

The T₃ homozygous seeds of *35S:BES1:GFP* were planted on MGRL medium for 4 days under 100 μ M B conditions, and then transferred to adequate B (100 μ M B), deficient B (0.1 μ M B) and deficient B supplemented with 1 nM eBL medium for 24 h. Root tips of the seedlings were viewed on a Leica TCS SP8 confocal laser scanning microscopes (www.leica-microsystems.com/home/). GFP was viewed at an excitation and emission wavelength of 488 nm. For comparing the protein levels directly, the laser intensity and detection settings were controlled constantly. Fluorescence measurements in roots were performed using Leica LAS AF Lite software.

BR analysis

For BR content, seedlings of En2 and *bes1-D* were germinated in MGRL media for 11 days under 0.1 and 100 μ M B conditions, and whole plant were harvested. The determination of BR was performed by ProNetsBio. Samples of 200 mg freeze drying weight were grinded to powder in liquid nitrogen and extracted with ice-cold 80% methanol for 2 h and BL (OlChem) , CS (OlChem), 6DCS (Sigma) as internal standards. After centrifugation, the samples were purified by Bond Elut preloaded columns and elution with methanol, and then further purified on strata-X columns and elution with methanol. Methanol solution was dried with nitrogen, dissolved in methanol, filtered through 0.22 μ m membrane and then analyzed by HPLC-MS/MS.

499 Statistical analysis

500 The values are means $\pm$ SD. Significant differences between treatments were analyzed
501 based on two tailed unpaired Student's *t*-tests, and *P*-values $p < 0.05$ were considered
502 statistically significant. In multiple comparisons, Tukey-test was implemented at $p \leq$
503 0.05.

504 SUPPLEMENTAL DATA

505 **Supplemental Figure S1.** The ROS staining of wild-type En2 plants grown on
506 MGRL media for 10 d under 100 and 0.1 μ M B conditions.

507 **Supplemental Figure S2.** The BR-related genes response to boron deficiency in root
508 in transcriptomes.

509 **Supplemental Figure S3.** BES1 and BZR1 activity confers root insensitivity to B
510 deficiency.

511 **Supplemental Table S1.** Differential expression of *Arabidopsis* root genes at 0.1 μ M
512 and 100 μ M H₃BO₃.

513 **Supplemental Table S2.** BR and B deficiency co-regulated genes in the root.

514 **Supplemental Table S3.** BR concentration in whole seedlings of wild- type En2 and
515 mutant *bes1-D* grown in sufficient B (+B) and deficient B (-B) conditions for 11 d.

516 **Supplemental Table S4.** Primers used for cloning and qRT-PCR.

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520 (Chinese Academy of Sciences) for providing the seeds of *CYCBI;1::GUS* transgenic
521 plants.

522 FIGURE LEGENDS

523 **Figure 1. B deficiency impacts on root elongation.**

524 (A) Phenotype of wild-type plants grown at decreasing concentrations of B for 11 d.
525 Scale bar, 10 mm.

(B) Primary root lengths of wild-type plants grown at decreasing concentrations of B for 11 d (mean $\pm$ s.d., $n = 30$). The asterisks indicate a statistically significant difference (t -test, $P < 0.001$).

(C) Root grown rate of wild-type grown at decreasing concentrations of B for 11 d (mean $\pm$ s.d., $n = 30$). DAG, Days after germination.

(D-F) Quantification of meristem size (D) (mean $\pm$ s.d., $n > 6$), number of meristematic cells (E) (mean $\pm$ s.d., $n > 6$) and mature cortex cell size (F) (mean $\pm$ s.d., $n > 40$) of wild-type root grown as in (H) and (I). The asterisks indicate a statistically significant difference (t -test, $P < 0.001$).

(G) The expression of *pCYCB1;1:GUS* plants grown on MGRL media for 6 d under 100 and 0.1 μ M B conditions. Scale bar, 50 μ m.

(H) Confocal microscopy image of 6-day-old wild-type. Arrows represent the meristem boundary where cortical cells double their size. Scale bar, 50 μ m.

(I) Confocal microscopy image of wild-type root grown as in (A). Scale bar, 100 μ m.

Figure 2. Genome-wide responses to boron deficiency in root.

(A) Top 20 (ranked by false discovery rate (FDR < 0.05)) significantly enriched GO terms derived from genes that are regulated by B deficiency.

(B) Venn diagrams representing the overlap between B deficiency-regulated genes and published BR-regulated genes in roots.

(C) Hierarchical cluster analysis of log₂ fold change value of BR-B deficiency co-regulated genes.

(D) The BL content of wild type En2 under 0.1 and 100 μ M B conditions.

Figure 3. BES1 activity confers root insensitivity to B deficiency.

(A) Phenotype of wild-type (En2) and *bes1-D* plants grown on MGRL media for 10 d under 0.1 and 100 μ M B conditions. Scale bar, 10 mm.

(B) Primary root length and (C) root growth rate of En2 and *bes1-D* plants grown as in (A) (mean $\pm$ s.d., $n \geq 45$). The asterisks indicate a statistically significant difference (t -test, $P < 0.001$).

(D) Root sensitivity of wild-type (En2) and *bes1-D* to increasing concentrations of B (mean $\pm$ s.d., $n \geq 45$). The asterisks indicate a statistically significant difference (*t*-test, $P < 0.001$).

(E-G) Mature cortex cell size (E) (mean $\pm$ s.d., $n > 40$), meristem size (F) (mean $\pm$ s.d., $n > 6$), number of meristematic cells (G) (mean $\pm$ s.d., $n > 6$). The asterisks indicate a statistically significant difference (*t*-test, $P < 0.001$).

Figure 4. BR signaling is involved in root responses to boron deficiency.

(A) Phenotype of wild-type (Col-0) and *bri1-301* plants grown on MGRL media for 9 days under 0.1 and 100 μ M B conditions. Scale bar, 10 mm.

(B) Primary root length of wild-type (Col-0) and *bri1-301* plants grown as in (A) (mean $\pm$ s.d., $n \geq 30$). The asterisks indicate a statistically significant difference (*t*-test, $P < 0.001$).

(C) Root growth rate of wild-type (Col-0) and *bri1-301* plants grown on MGRL media for 11 d under 0.1 and 100 μ M B conditions (mean $\pm$ s.d., $n \geq 30$).

(D) Root sensitivity of wild-type (Col-0) and *bri1-301* to increasing concentrations of B (mean $\pm$ s.d., $n \geq 40$). The asterisks indicate a statistically significant difference (*t*-test, $P < 0.001$).

(E) Phenotype of wild-type (En2) and *bes1-D* plants grown on MGRL media for 9 d under 0.1 and 100 μ M B conditions in the absence and presence of 0.01 nM eBL. Scale bar, 10 mm.

(F) Primary root length of En2 and *bes1-D* seedlings grown as (E) (mean $\pm$ s.d., $n \geq 40$). The asterisks indicate a statistically significant difference (*t*-test, $P < 0.001$).

(G) Primary root length of En2 and *bes1-D* seedlings grown as (H) (mean $\pm$ s.d., $n > 25$). The different letters above the columns indicate significant differences between all genotypes and all growth conditions (Tukey- test, $p \leq 0.05$).

(H) Phenotype of wild-type (En2) and *bes1-D* plants grown on MGRL media for 10 days under 0.1 and 100 μ M B conditions in the presence or absence of the BR biosynthesis inhibitor BRZ are shown. Scale bar, 10 mm.

(I) A confocal image. Snapshots of the BES1-GFP signal, seedling transferred from adequate B medium to adequate B, B deficiency and B deficiency supplemented with eBL conditions for 24 h, respectively. Scale bar, 50µm.

(J) Fluorescent signal measured in the seedling grown as (I) (mean ± s.d., n = 50). The asterisks indicate a statistically significant difference (*t*-test, *P* < 0.001).

Figure 5. Relative expression of B transport-related genes, B homeostasis and dry weight in En2 and *bes1-D*.

(A-F) Relative expressions of *NIP5;1* (A), *NIP6;1*(B), *NIP7;1* (C), *BOR1* (D), *BOR2* (E), *BOR4* (F) in root and shoot under 100 and 0.1 µM B (mean ± s.d., n=3).

(G-I) B concentration, dry weight and B accumulation of En2 and *bes1-D* plants grown on MGR media for 10 days under 0.1 and 100 µM B conditions (mean ± s.d., n=4). The asterisk shows a significant difference. *, *P* <0.05 with two-tailed Student's *t* test. **, *P* <0.01 with two-tailed Student's *t* test. ***, *P* <0.001 with two-tailed Student's *t* test.

Figure 6. Low B stress triggers a reduction of BL.

(A-C) Analysis of relative expression level of BR biosynthesis genes in roots of En2 and *bes1-D* plants grown 100 and 0.1 µM B (mean ± s.d., n=3).

(D) Structures of BR molecules.

(E-G) Content of detected 6DCS (E), CS (F) and BL(G) are shown (mean ± s.d., n=3). The asterisk shows a significant difference. *, *P* <0.05 with two-tailed Student's *t* test.

Figure 7. A model of boron deficiency-induced root growth inhibition is mediated by BRs in *Arabidopsis*.

The BR biosynthesis genes, including *CPD*, is up-regulated by low B stress. *BR6ox1* and *BR6ox2*, are down-regulated by low B stress. The coloured red and green of rectangle indicate that gene are up-regulated and down-regulated under B deficiency, respectively. Under B sufficiency (left), the high concentration of BL result in nuclear

618 accumulation of two unphosphorylated transcription factors, BZR1 and BES1, leading
619 to BR-responsive gene expression and increasing root length. Compared with B
620 sufficient conditions, B deficiency (right) reduced BL content by down-regulating the
621 expression of *BR6ox1* and *BR6ox2*. BRI1 perceived low BL concentration lead to BR
622 signaling decreased, BES1 and BZR1 were phosphorylated, and BR-responsive genes
623 were inhibited, leading to decreasing root length.

624

625

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Parsed Citations

- Abreu I, Poza L, Bonilla I, Bolaños L (2014)** Boron deficiency results in early repression of a cytokinin receptor gene and abnormal cell differentiation in the apical root meristem of *Arabidopsis thaliana*. *Plant Physiol Bioch* 77: 117-121.
 Pubmed: [Author and Title](#)
 Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)
- Asami T, Min YK, Nagata N, Yamagishi K, Takatsuto S, Fujioka S, Murofushi N, Yamaguchi I, Yoshida S (2000)** Characterization of brassinazole, a triazole-type brassinosteroid biosynthesis inhibitor. *Plant Physiol* 123(1): 93-99.
 Pubmed: [Author and Title](#)
 Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)
- Bell RW, Rerkasem B (1997)** Boron in soils and plants. *Developments in Plant & Soil Sciences* 76.
 Pubmed: [Author and Title](#)
 Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)
- Bishop GJ, Nomura T, Yokota T, Harrison K, Noguchi T, Fujioka S, Takatsuto S, Jones JG, Kamiya Y (1999)** The tomato DWARF enzyme catalyses C-6 oxidation in brassinosteroid biosynthesis. *Proc. Natl. Acad. Sci* 96(4): 1761-1766.
 Pubmed: [Author and Title](#)
 Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)
- Brown PH, Bellaloui N, Wimmer MA, Bassil ES, Ruiz J, Hu H, Pfeffer H, Dannel F, Römheld V (2002)** Boron in plant biology. *Plant Biol.* 4: 205-223.
 Pubmed: [Author and Title](#)
 Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)
- Camacho-Cristóbal JJ, Martín-Rejano EM, Begoña Herrera-Rodríguez M, Navarro-Gochicoa MT, Rexach J, González-Fontes A (2015)** Boron deficiency inhibits root cell elongation via an ethylene/auxin/ROS-dependent pathway in *Arabidopsis* seedlings. *J. Exp. Bot* 66(13): 3831.
 Pubmed: [Author and Title](#)
 Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)
- Chaiwanon J, Wang ZY (2015)** Spatiotemporal brassinosteroid signaling and antagonism with auxin pattern stem cell dynamics in *Arabidopsis* roots. *Curr. Biol* 25: 1031-1042.
 Pubmed: [Author and Title](#)
 Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)
- Choe S, Dilkes BP, Fujioka S, Takatsuto S, Feldmann SKA (1998)** The DWF4 gene of *Arabidopsis* encodes a cytochrome P450 that mediates multiple 22 alpha-hydroxylation steps in brassinosteroid biosynthesis. *Plant Cell* 10(2): 231-243.
 Pubmed: [Author and Title](#)
 Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)
- Choi YH, Fujioka S, Harada A, Yokota T, Takatsuto S, Sakurai A (1996)** A brassinolide biosynthetic pathway via 6-deoxocastasterone. *Phytochemistry* 43(3): 593-596.
 Pubmed: [Author and Title](#)
 Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)
- Choi YH, Fujioka S, Nomura T, Harada A, Yokota T, Takatsuto S, Sakurai A (1997)** An alternative brassinolide biosynthetic pathway via late C-6 oxidation. *Phytochemistry* 44(4): 609-613.
 Pubmed: [Author and Title](#)
 Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)
- Chory J, Nagpal P, Peto CA (1991)** Phenotypic and genetic analysis of *det2*, a new mutant that affects light-regulated seedling development in *Arabidopsis*. *Plant Cell* 3: 445-459.
 Pubmed: [Author and Title](#)
 Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)
- Clough SJ, Bent AF (1998)** Floral dip: a simplified method for *Agrobacterium*-mediated transformation of *Arabidopsis thaliana*. *Plant J* 16: 735-743.
 Pubmed: [Author and Title](#)
 Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)
- Clouse SD, Langford M, McMorris TC (1996)** A brassinosteroid-insensitive mutant in *Arabidopsis thaliana* exhibits multiple defects in growth and development. *Plant Physiol* 111: 671-678.
 Pubmed: [Author and Title](#)
 Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)
- Clouse SD, Sasse JM (1998)** Brassinosteroids: essential regulators of plant growth and development. *Annu. Rev. Plant Phys* 49: 427-451.
 Pubmed: [Author and Title](#)
 Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)
- Divi UK, Krishna P (2009)** Brassinosteroid: a biotechnological target for enhancing crop yield and stress tolerance. *N. Biotechnol* 26: 131-136.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

Durbak AR, Phillips KA, Pike S, O'Neill MA, Mares J, Gallavotti A, McSteen P (2014) Transport of boron by the tassell-less1 aquaporin is critical for vegetative and reproductive development in maize. Plant Cell 26(7): 2978-2995.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

Fabregas N, Li N, Boeren S, Nash TE, Goshe MB, Clouse SD, Vries S, Cano-Delgado AI (2013) The brassinosteroid insensitive1-like3 signalosome complex regulates arabidopsis root development. Plant Cell 25(9): 3377-3388.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

Fridman Y, Elkouby L, Holland N, Vragovic K, Elbaum R, Savaldi-Goldstein S (2014) Root growth is modulated by differential hormonal sensitivity in neighboring cells. Gene Dev 28(8): 912-920.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

Fujioka S, Inoue T, Takatsuto S, Yanagisawa T, Yokota T, Sakurai A (1995) Identification of a new brassinosteroid, cathasterone, in cultured-cells of catharanthus-roseus as a biosynthetic precursor of teasterone. Biosci. Biotechnol. Biochem 59(8): 1543-1547.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

Fujioka S, Li JM, Choi YH, Seto H, Takatsuto S, Noguchi T, Watanabe T, Kuriyama H, Yokota T, Chory J et al (1997) The Arabidopsis deetiolated2 mutant is blocked early in brassinosteroid biosynthesis. Plant Cell 9: 1951-1962.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

Fujiwara T, Hirai MY, Chino M, Komeda Y, Naito S (1992) Effects of sulfur nutrition on expression of the soybean seed storage protein genes in transgenic petunia. Plant Physiol 99: 263-268.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

Gendron JM, Haque A, Gendron N, Chang T, Asami T, Wang ZY (2008) Chemical genetic dissection of brassinosteroid-ethylene interaction. Mol. Plant 1(2): 368-379.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

Goda H, Sawa S, Asami T, Fujioka S, Shimada Y, Yoshida S (2004) Comprehensive comparison of auxin-regulated and brassinosteroid-regulated genes in Arabidopsis. Plant Physiol 134(4): 1555-1573.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

Goda H, Shimada Y, Asami T, Fujioka S, Yoshida S (2002) Microarray analysis of brassinosteroid-regulated genes in Arabidopsis. Plant Physiol 130(3): 1319-1334.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

Goldbach HE (1997) A critical review on current hypotheses concerning the role of boron in higher plants: suggestions for further research and methodological requirements. Trace and Microprobe Techniq 15: 51-91.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

Gonzalez-Garcia MP, Vilarrasa-Blasi J, Zhiponova M, Divol F, Mora-Garcia S, Russinova E, Cano-Delgado AI (2011) Brassinosteroids control meristem size by promoting cell cycle progression in Arabidopsis roots. Development 138: 849-859.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

Hacham Y, Holland N, Butterfield C, Ubeda-Tomas S, Bennett MJ, Chory J, Savaldi-Goldstein S (2011) Brassinosteroid perception in the epidermis controls root meristem size. Development 138: 839-848.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

Hardtke CS, Dorcey E, Osmont KS, Sibout R (2007) Phytohormone collaboration: zooming in on auxin-brassinosteroid interactions. Trends Cell Biol 17(10): 485-492.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

Hua Y, Zhou T, Ding G, Yang Q, Shi L, Xu F (2016) Physiological, genomic and transcriptional diversity in responses to boron deficiency in rapeseed genotypes. J. Exp. Bot 67(19): 5769-5784.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

Jaillais Y, Vert G (2016) Brassinosteroid signaling and BRI1 dynamics went underground. Curr. Opin. Plant Biol 33: 92-100.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Jia Z, Giehl RFH, Meyer RC, Altmann T, Nicolaus von Wirén (2019) Natural variation of BSK3 tunes brassinosteroid signaling to regulate root foraging under low nitrogen. Nat Commun 10(1): 2378.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Kato Y, Miwa K, Takano J, Wada M, Fujiwara T (2009) Highly boron deficiency-tolerant plants generated by enhanced expression of NIP5;1, a boric acid channel. Plant Cell Physiol 50(1): 58-66.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Kim TW, Guan SH, Sun Y, Deng ZP, Tang WQ, Shang JX, Sun Y, Burlingame AL, Wang ZY (2009) Brassinosteroid signal transduction from cell-surface receptor kinases to nuclear transcription factors. Nat. Cell. Biol 11: 1254-1260.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Kim TW, Hwang JY, Kim YS, Joo SH, Chang S, Lee J, Takatsuto S, Kima SK (2005) Arabidopsis CYP85A2, a cytochrome P450, mediates the Baeyer-Villiger oxidation of castasterone to brassinolide in brassinosteroid biosynthesis. Plant Cell 17(8): 2397-2412.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Kobayashi M, Matoh T, Azuma JI (1996) Two chains of rhamnogalacturonan II are cross-linked by borate-diol ester bonds in higher plant cell walls. Plant Physiol 110: 1017-1020.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Li J, Chory J (1997) A putative leucine-rich repeat receptor kinase involved in brassinosteroid signal transduction. Cell 90: 929-938.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Li J, Wen J, Lease KA, Doke JT, Tax FE, Walker JC (2002) BAK1, an arabidopsis LRR receptor-like protein kinase, interacts with BRI1 and modulates brassinosteroid signaling. Cell 110: 213-222.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Miwa K, Fujiwara T (2010) Boron transport in plants: co-ordinated regulation of transporters. Ann Bot 105(7): 1103-1108.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Miwa K, Takano J, Fujiwara T (2006) Improvement of seed yields under boron-limiting conditions through overexpression of BOR1, a boron transporter for xylem loading, in Arabidopsis thaliana. Plant J 46(6): 1084-1091.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Müller J, Toev T, Heisters M, Teller J, Moore K, Hause G, Dinesh DC, Burstenbinder K, Abel S (2015) Iron-dependent callose deposition adjusts root meristem maintenance to phosphate availability. Dev Cell 33(2): 216-230.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Mussig C, Shin GH, Altmann T (2003) Brassinosteroids promote root growth in Arabidopsis. Plant Physiol 133: 1261-1271.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Nakagawa Y, Hanaoka H, Kobayashi M, Miyoshi K, Miwa K, Fujiwara T (2007) Cell-type specificity of the expression of OsBOR1, a rice efflux boron transporter gene, is regulated in response to boron availability for efficient boron uptake and xylem loading. Plant Cell 19(8): 2624-2635.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Nam KH, Li J (2002) BRI1/BAK1, a receptor kinase pair mediating brassinosteroid signaling. Cell 110: 203-212.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Nemhauser JL, Hong F, Chory J (2006) Different plant hormones regulate similar processes through largely nonoverlapping transcriptional responses. Cell 126: 467-475.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Nemhauser JL, Mockler TC, Chory J (2004) Interdependency of brassinosteroid and auxin signaling in Arabidopsis. PLoS Biol 2(9): e258.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Ohnishi T, Godza B, Watanabe B, Fujioka S, Hategan L, Ide K, Shibata K, Yokota T, Szekeres M, Mizutani M (2012) CYP90A1/CPD, a

brassinosteroid biosynthetic cytochrome P450 of Arabidopsis, catalyzes C-3 oxidation. J. Biol. Chem 287(37): 31551- 31560.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

O'Neill M, Eberhard S, Albersheim P, Darvill A (2001) Requirement of borate cross-linking of cell wall rhamnogalacturonan ii for Arabidopsis growth. Science 294: 846-849.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

Poza-Viejo L, Abreu I, González-García MP, Allauca P, Bonilla I, Bolanos L, Reguera M (2018) Boron deficiency inhibits root growth by controlling meristem activity under cytokinin regulation. Plant Sci S0168945217310580.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

Roddick JG, Rijkenberg AL, Ikekawa N (1993) Developmental effects of 24-epibrassinolide in excised roots of tomato grown in vitro. Physiol. Plantarum 87: 453-458.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

Shao JF, Yamaji N, Liu XW, Yokosho K, Shen RF, Ma JF (2018) Preferential distribution of boron to developing tissues is mediated by the intrinsic protein OsNIP3. Plant Physiol 176(2): 1739-1750.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

Shimada Y, Goda H, Nakamura A, Takatsuto S, Yoshida FS (2003) Organ-specific expression of brassinosteroid-biosynthetic genes and distribution of endogenous brassinosteroids in Arabidopsis. Plant Physiol 131(1): 287-297.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

Shorrocks V (1997) The occurrence and correction of boron deficiency. Plant Soil 193: 121-148.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

Singh AP, Fridman Y, Friedlander-Shani L, Tarkowska D, Strnad M, Savaldi-Goldstein S (2014) Activity of the brassinosteroid transcription factors BRASSINAZOLE RESISTANT1 and BRASSINOSTEROID INSENSITIVE1-ETHYL METHANESULFONATE-SUPPRESSOR1/BRASSINAZOLE RESISTANT2 blocks developmental reprogramming in response to low phosphate availability. Plant Physiol 166: 678-688.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

Singh AP, Fridman Y, Holland N, Ackerman-Lavert M, Zananiri R, Jaillais Y, Savaldi-Goldstein S (2018) Interdependent nutrient availability and steroid hormone signals facilitate root growth plasticity. Dev. Cell 46(1): 59-72.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

Sun Y, Fan XY, Cao DM, Tang WQ, He K, Zhu JY, He JX, Bai MY, Zhu SW, Oh E et al (2010) Integration of brassinosteroid signal transduction with the transcription network for plant growth regulation in Arabidopsis. Dev. Cell 19: 765-777.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

Szekeres M, Németh K, Koncz-Kálmán Z, Mathur J, Kauschmann A, Altmann T, Redei GP, Nagy F, Schell J, Koncz C (1996) Brassinosteroids rescue the deficiency of CYP90, a cytochrome P450, controlling cell elongation and de-etiolation in Arabidopsis. Cell 85(2): 171-182.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

Takano J, Wada M, Ludewig U, Schaaf G, Von Wirén N, Fujiwara T (2006) The Arabidopsis major intrinsic protein NIP5; 1 is essential for efficient boron uptake and plant development under boron limitation. Plant Cell 18(6): 1498-1509.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

Tanaka K, Asami T, Yoshida S, Nakamura Y, Matsuo T, Okamoto S (2005) Brassinosteroid homeostasis in Arabidopsis is ensured by feedback expressions of multiple genes involved in its metabolism. Plant Physiol 138: 1117-1125.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

Tanaka M, Takano J, Chiba Y, Lombardo F, Ogasawara Y, Onouchi H, Naito S, Fujiwara T (2011) Boron-dependent degradation of NIP5;1 mRNA for acclimation to excess boron conditions in Arabidopsis. Plant Cell 23(9): 3547-3559.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

Wang XL, Chory J (2006) Brassinosteroids regulate dissociation of BKI1, a negative regulator of BRI1 signaling, from the plasma membrane. Science 313: 1118-1122.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

Wang ZY, Nakano T, Gendron J, He J, Chen M, Vafeados D, Yang Y, Fujioka S, Yoshida S, Asami T et al (2002) Nuclear localized BZR1 mediates brassinosteroid-induced growth and feedback suppression of brassinosteroid biosynthesis. Dev. Cell 2: 505-513.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Warrington K (1923) The effect of boric acid and borax on the broad bean and certain other plants. Ann. Bot 37: 629-672.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Wei Z, Li J (2016) Brassinosteroids regulate root growth, development and symbiosis. Mol. Plant 9: 86-100.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Wu X, Riaz M, Yan L, Du C, Liu Y, Jiang C (2017) Boron deficiency in trifoliate orange induces changes in pectin composition and architecture of components in root cell walls. Front Plant Sci 8: 1882.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Xu W, Huang J, Li B, L J, Wang Y (2008) Is kinase activity essential for biological functions of BRI1? Cell Res 18: 472-478.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Yang CJ, Zhang C, Lu YN, Jin JQ, Wang XL (2011) The mechanisms of brassinosteroids' action: from signal transduction to plant development. Mol. Plant 4: 588-600.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Yin YH, Wang ZY, Mora-Garcia S, Li JM, Yoshida S, Asami T, Chory J (2002) BES1 accumulates in the nucleus in response to brassinosteroids to regulate gene expression and promote stem elongation. Cell 109: 181-191.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Yokota T, Ogino Y, Takahashi N, Saimoto H, Fujioka S, Sakurai A (1990) Brassinolide is biosynthesized from castasterone in Catharanthus roseus crown gall cells. Agric. Biol. Chem 54(4): 1107-1108.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Yu XF, Li L, Zola J, Aluru M, Ye HX, Foudree A, Guo HQ, Anderson S, Aluru S, Liu P et al (2011). A brassinosteroid transcriptional network revealed by genome-wide identification of BES1 target genes in Arabidopsis thaliana. Plant J 65: 634-646.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Zhang S, Wei Y, Lu Y, Wang X (2009) Mechanisms of brassinosteroids interacting with multiple hormones. Plant Signal. Behav 4: 1117-1120.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)