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RESEARCH ARTICLE

Role of tyrosine autophosphorylation and methionine residues in BRI1 function in *Arabidopsis thaliana*

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Abstract

Background Brassinosteroids (BRs), a group of plant growth hormones, control biomass accumulation and biotic and abiotic stress tolerance, and therefore are highly relevant to agriculture. BRs bind to the BR receptor protein, brassinosteroid insensitive 1 (BRI1), which is classified as a serine/threonine (Ser/Thr) protein kinase. Recently, we reported that BRI1 acts as a dual-specificity kinase both in vitro and in vivo by undergoing autophosphorylation at tyrosine (Tyr) residues.

Objective In this study, we characterized the increased leaf growth and early flowering phenotypes of transgenic lines expressing the mutated recombinant protein, *BRI1(Y831F)-Flag*, compared with those expressing *BRI1-Flag*. *BRI1(Y831F)-Flag* transgenic plants showed a reduction in hypocotyl and petiole length compared with *BRI1-Flag* seedlings. Transcriptome analysis revealed differential expression of flowering time-associated genes (*AP1*, *AP2*, *AG*, *FLC*, and *SMZ*) between *BRI1(Y831F)-Flag* and *BRI1-Flag* transgenic seedlings. We also performed site-directed mutagenesis of the *BRI1* gene, and investigated the effect of methionine (Met) substitution in the extracellular domain (ECD) of BRI1 on plant growth and BR sensitivity by evaluating hypocotyl elongation and root growth inhibition.

Methods The *pBIB-Hyg*$^+$*-pBR-BRI1-Flag* construct(Li et al. 2002) was used as the template for SDM with QuickChange XL Site Directed Mutagenesis Kit (Stratagene, La Jolla, CA, USA) to make the SDM mutants. After PCR with SDM kit, add 1 µl of Dpn1 to PCR reaction. Incubate at 37 °C for 2 h to digest parental DNA and then transformed into XL10-gold competent cells. Transcriptome analysis was carried out at the University of Illinois (Urbana-Champaign, Illinois, USA). RNA was prepared and hybridized to the Affymetrix GeneChip Arabidopsis ATH1 Genome Array using the Gene Chip Express Kit (Ambion, Austin, TX, USA).

Results Tyrosine 831 autophosphorylation of BRI1 regulates Arabidopsis flowering time, and mutation of methionine residues in the extracellular domain of BRI1 affects hypocotyl and root length. *BRI1(M656Q)-Flag*, *BRI1(M657Q)-Flag*, and *BRI1(M661Q)-Flag* seedlings were insensitive to the BL treatment and showed no inhibition of root elongation. However, *BRI1(M665Q)-Flag* and *BRI1(M671Q)-Flag* seedlings were sensitive to the BL treatment, and exhibited root elongation inhibition. the early flowering phenotype of *BRI1(Y831F)-Flag* transgenic plants is consistent with the expression levels of key flowering-related genes, including those promoting flowering (*AP1*, *AP2*, and *AG*) and repressing flowering (*FLC* and *SMZ*).

Keywords Autophosphorylation · Brassinosteroids · BRI1 · Receptor kinases · Methionine residue

Introduction

In *Arabidopsis thaliana*, receptor-like kinases (RLKs) are grouped into more than 20 families, based on the structure of the leucine-rich repeat (LRR) containing extracellular domain (ECD). In the LRR-RLK subfamily, BRASSINOSTEROID INSENSITIVE 1 (BRI1) and its co-receptor BAK1 play an important role in brassinosteroid (BR) signal transduction, thus affecting plant growth and development (Clouse 2002; Gendron et al. 2007). Among the other LRR-RLK proteins, CLAVATA1 controls the meristem

Jae-Han Choi and Eun-Seok Oh contributed equally to this work.

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stem cell fate (Vert et al. 2005); ERECTA determines organ shape (DeYoung et al. 2006); HAESA is involved in organ abscission (Torii 2004); FLS2 binds to the bacterial flagellin peptide ligand (Cho et al. 2008); and AtSERK1 is involved in early somatic embryogenesis (Boller et al. 2009). Among the LRR-RLKs, BRI1 is one of the most thoroughly studied plant receptor kinases; BRI1, together with its co-receptor BAK1, functions in BR signaling (Gendron et al. 2007; Nodine et al. 2007). Both BRI1 and BAK1 are arginine-aspartate (RD) kinases. Interestingly, a recent study showed that plant receptor kinases act as dual-specificity kinases that can autophosphorylate serine (Ser), threonine (Thr), and tyrosine (Tyr) residues (Clouse 2011; Karlova et al. 2009; Oh et al. 2009a, b). BRs are important plant steroid hormones that control cell division, cell elongation, vascular differentiation, senescence timing, male sterility, and organ formation in plants (Oh et al. 2009a, b; Clouse et al. 1998; Altmann 1999; Nakaya et al. 2002). BRs bind to BRI1, which functions in conjunction with its co-receptor BAK1 in hormone perception and signal transduction (Gonzalez et al. 2010; Li et al. 2002).

In plants, Tyr phosphorylation has been investigated to a lesser extent than Ser and Thr phosphorylation. Nevertheless, experimental evidence demonstrates the functional importance of Tyr phosphorylation in plant metabolism, growth, and development (Karlova et al. 2009). Numerous phosphorylation sites have been identified in both BRI1 and BAK1 via ion trap liquid chromatography–tandem mass spectrometry (LC–MS/MS) and quadrupole time-of-flight (QTOF) LC/MS/MS analyses (Li et al. 2002; Oh et al. 2000; Wang et al. 2005), consistent with the classification of these proteins as Ser/Thr protein kinases. Interestingly, Oh et al. showed that the BR receptor, BRI1, can phosphorylate its Tyr residue both in vitro and in vivo. Transgenic Arabidopsis plants expressing the mutated *BRI1(Y831F)-Flag*, which is substituted tyrosine residue to phenylalanine with site directed mutagenesis, in the *bri1-5* mutant are bigger in leaf size, and total biomass than those expressing the wild-type *BRI1-Flag* (Oh et al. 2009a, b), suggesting that phosphorylation of Tyr-831 attenuates plant growth. To understand the basic knowledge for the increased growth of *BRI1(Y831F)-Flag* transformants, we evaluated their leaf morphology, photosynthetic parameters such as gas exchange rate, carbohydrate and free amino acid level in the leaves and transcriptomic analysis. On average, the relative growth rate of leaves was increased by 16% in *BRI1(Y831F)-Flag* transgenic lines in the *bri1-5* (Wang et al. 2008). RNA microarray analysis demonstrated that the expression of BR biosynthetic genes including secondary cell wall, vascular differentiation, and growth control was significantly changed compared to *BRI1-Flag* transgenic lines. Moreover, metabolite results revealed highly enriched accumulation of carbohydrates such as starch, sucrose, and amino acids, glycine and proline (Oh et al. 2011).

LRR-RLKs function as Ser/Thr kinases or as dual-specificity kinases in plants in contrast of receptor Tyr kinases function mainly in animal (Oh et al. 2018; Albrecht et al. 2008; Rudrabhatla et al. 2006). Karlova et al. reported the corresponding Tyr amino acid residue of SERK1 that serves as an autophosphorylation sites in vitro. However, it remains to be determined whether Tyr autophosphorylation or the hydroxyl group is essential for the kinase activity of SERK1, although we strongly suspect the latter.

In this study, we investigated the effect of the substitution of Tyr-831 phosphorylation site and methionine (Met) residues of BRI1 on plant growth, hypocotyl elongation, root growth inhibition, leaf growth, and flowering time in Arabidopsis. This study not only demonstrates the regulatory role of Tyr phosphorylation but also the function of Met residues, and possibly Met oxidation, in BRI1-mediated BR signaling.

Results and discussion

The mechanisms underlying the enhanced growth and flowering time phenotypes of *BRI1(Y831F)-Flag* transgenic plants compared with those expressing *BRI1-Flag* remain unclear. BRI1 and BR signal transduction closely related to plant growth (Karlova et al. 2009) and timing of flowering (Gonzalez et al. 2009), most likely because of the phosphorylation of Tyr-831, which is located in the juxtamembrane region of BRI1 cytoplasmic domain (CD). To clarify the biological relevance of Tyr-831 phosphorylation and the function of Met residues located in the ECD of BRI1, we characterized the phenotype of *BRI1 (Y831F)-Flag*, *BRI1(Y831D)-Flag*, *BRI1(Y831E)-Flag*, and *BRI1-Flag* transgenic plants. The results of phenotypic analysis did not elucidate how Tyr-831 phosphorylation regulates flowering time and plant growth, especially petiole length and leaf shape. Therefore, we performed microarray analysis of the *BRI1(Y831F)-Flag* and *BRI1-Flag* transgenic lines using Affymetrix ATH1 gene arrays(Wang et al. 2008). Several genes exhibited differential expression between *BRI1-Flag* and *BRI1(Y831F)-Flag* transformants, consistent with their divergent growth phenotypes, suggesting that BR signal transduction mediated by BRI1 depends on the phosphorylation status of Tyr-831. Studies show that the timing of flowering is regulated by light quality and quantity, temperature, and the action of gibberellins(Yu et al. 2008). The *BRI1(Y831F)-Flag* plants flowered much earlier than the other genotypes (Fig. 1A). Therefore, we investigated the effect of mutating the phosphorylation sites Ser-858 and Thr-872, located in the BRI1-cytoplasmic domain (CD), on the rescue of the *bri1-5* mutant using other site-directed mutagenesis (SDM)-derived mutants

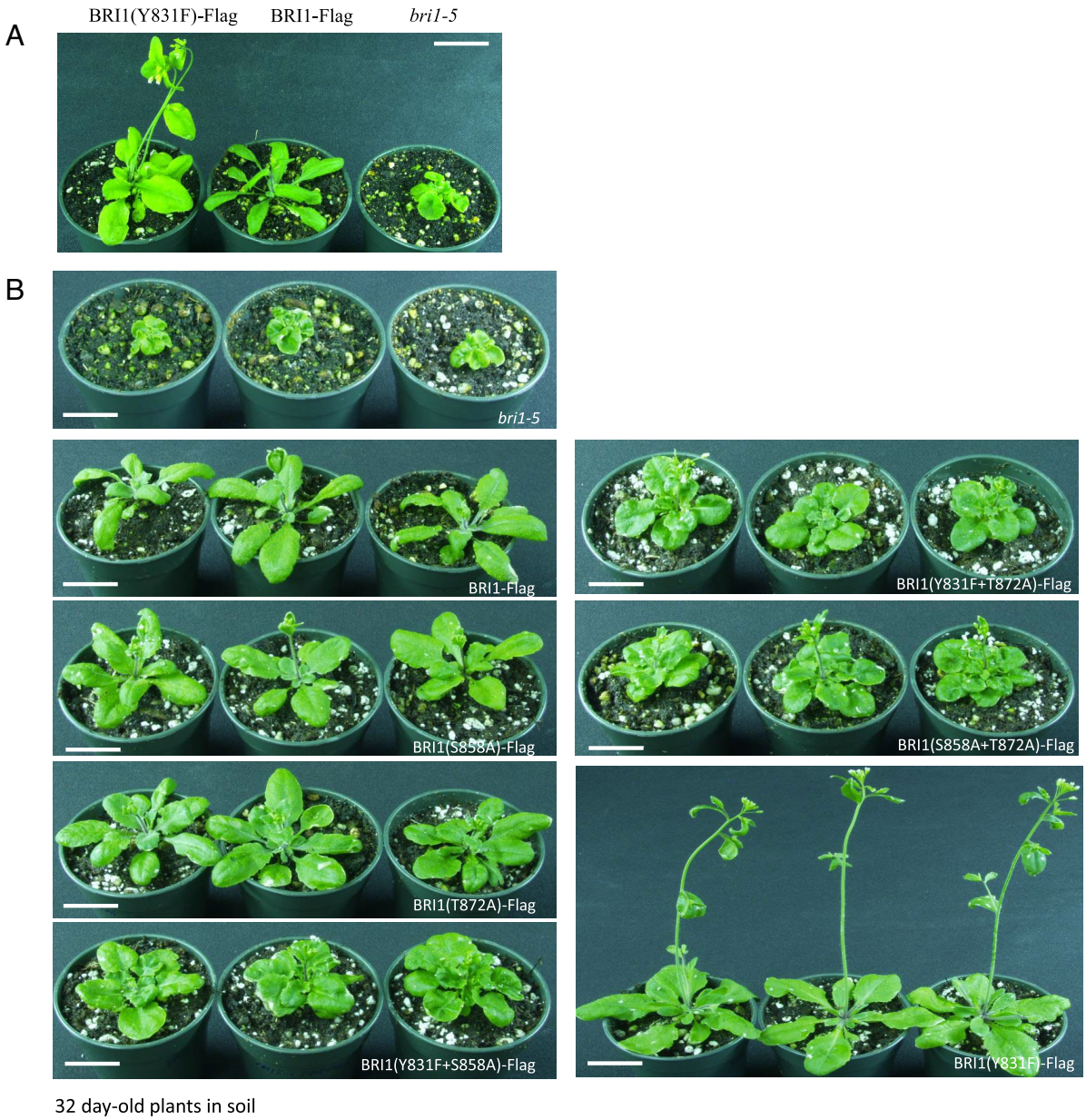

Fig. 1 Effect of the substitution of Tyrosine-831 residue, located in the juxtamembrane region of BRI1-CD, on the *bri1-5* mutant phenotype. **A** Rescue of leaf and petiole growth in vivo. Plants were grown for 32 days under long-day photoperiod (16 h light/8 h dark). **B** Effect of the substitution of Ser and Thr residues, located in BRI1-CD, on the growth and flowering time of the *bri1-5* mutant. Substitution of specific Thr or Ser residues with Ala and that of Tyr with Phe in the wild-type BRI1 is indicated. Three independent transgenic lines were generated for each construct in the *bri1-5* mutant background. All transgenic lines including *bri1-5* were grown in soil under the same conditions and for the same time period (32 days)

generated either previously, including *BRI1(S858A)-Flag* and *BRI1(T872A)-Flag* (Oh et al. 2000), or in this study, namely, *BRI1(Y831F + S858A)-Flag*, *BRI1(Y831F + T872A)-Flag*, and *BRI1(S858A + T872A)-Flag* (Fig. 1B). Three independent T2 transgenic lines of each construct were examined at 32 day post-germination under long-day photoperiod (16 h light/8 h dark). Interestingly, *BRI1(Y831F)-Flag* transgenic plants showed earlier flowering than other genotypes including *BRI1-Flag* (Fig. 1B). These results suggest that Tyr-831 plays a critical role in BR signaling in vivo. The *bri1-5* mutant, BRI1(C69Y), is impaired in BR signal transduction because of endoplasmic reticulum-mediated recycling and degradation of BRI1 in vivo (Komeda 2004; Noguchi et al. 1999). The characteristic dwarf phenotype can be rescued by the transformation of *bri1-5* plants with *BRI1-Flag* (Oh et al. 2000; Wang et al. 2005). Transformation of

bri1-5 plants with *BRI1(Y831F)-Flag*, *BRI1(Y831D)-Flag*, and *BRI1(Y831E)-Flag* partially rescued the hypocotyl and petiole growth phenotypes of soil-grown seedlings (Fig. 2). At the seedling stage, hypocotyl lengths of *BRI1(Y831F)-Flag* transgenic lines and the corresponding phosphomimic mutants, *BRI1(Y831D)-Flag* and *BRI1(Y831E)-Flag*, were highly similar but significantly shorter than those of *BRI1-Flag* transgenic seedlings (Fig. 2A, B). BRs possibly stimulate flowering by downregulating the major floral repressor gene, *FLOWERING LOCUS C* (*FLC*) (Hong et al. 2008). BRI1-dependent BR signaling promotes flowering time in wild-type Arabidopsis plants through repressing *FLC* expression, although the precise molecular mechanisms still unclear.

To understand the molecular basis of the early flowering phenotype of *BRI1(Y831F)-Flag* transgenic plants, we performed data mining of flowering-related genes from the existing microarray data (Wang et al. 2008). Comparison of the expression levels of flowering-related genes between *BRI1(Y831F)-Flag* and *BRI1-Flag* transgenic plants revealed that the expression of floral homeotic protein genes, *APETALA 1* (*AP1*) and *AP3*, which encode transcription factors that promote early floral meristem identity, was in synergy with that of *LEAFY*. Both *AP1* and *AP3* genes are essential for the vegetative-to-reproductive transition (Domagalska et al. 2007; Winter et al. 2015). In Arabidopsis, the *AGAMOUS* (*AG*) floral homeotic gene terminates meristem activity and promotes the development of stamens and carpels (Piwarzyk et al. 2007). Expression levels of *AG*, *AP1*, and *AP2* were higher in *BRI1(Y831F)-Flag* plants compared with *BRI1-Flag* transgenic plants, whereas those of *FLC* and *SCHLAFMUTZE* (*SMZ*) were significantly reduced (Fig. 3). The role of *FLC* in controlling the timing of flowering initiation is of special significance for vernalization (Gómez-Mena et al. 2005; Michaels et al. 1999), a period of low temperature exposure that stimulates flowering. *SMZ* encodes an *AP2* domain-containing transcription factor that can repress flowering (Sheldon et al. 1999). Therefore, the results shown in Fig. 3 suggest that the early flowering phenotype of *BRI1(Y831F)-Flag* transgenic plants is consistent with the expression levels of key flowering-related genes, including those promoting flowering (*AP1*, *AP2*, and *AG*) and repressing flowering (*FLC* and *SMZ*). Overall, *BRI1(Y831F)-Flag* transgenic plants flowered earlier than all the other genotypes (Fig. 1A, B), as described previously. Plants shown in Fig. 1A were grown under long days. *BRI1(Y831F)-Flag* plants clearly flowered earlier than *BRI1-Flag* transgenic plants because genes encoding the positive regulators of flowering (*AP1*, *AP2*, and *GA*) were up-regulated, whereas those encoding the negative regulators of flowering (*FLC* and *SMZ*) were down-regulated in the early flowering *BRI1(Y831F)-Flag*

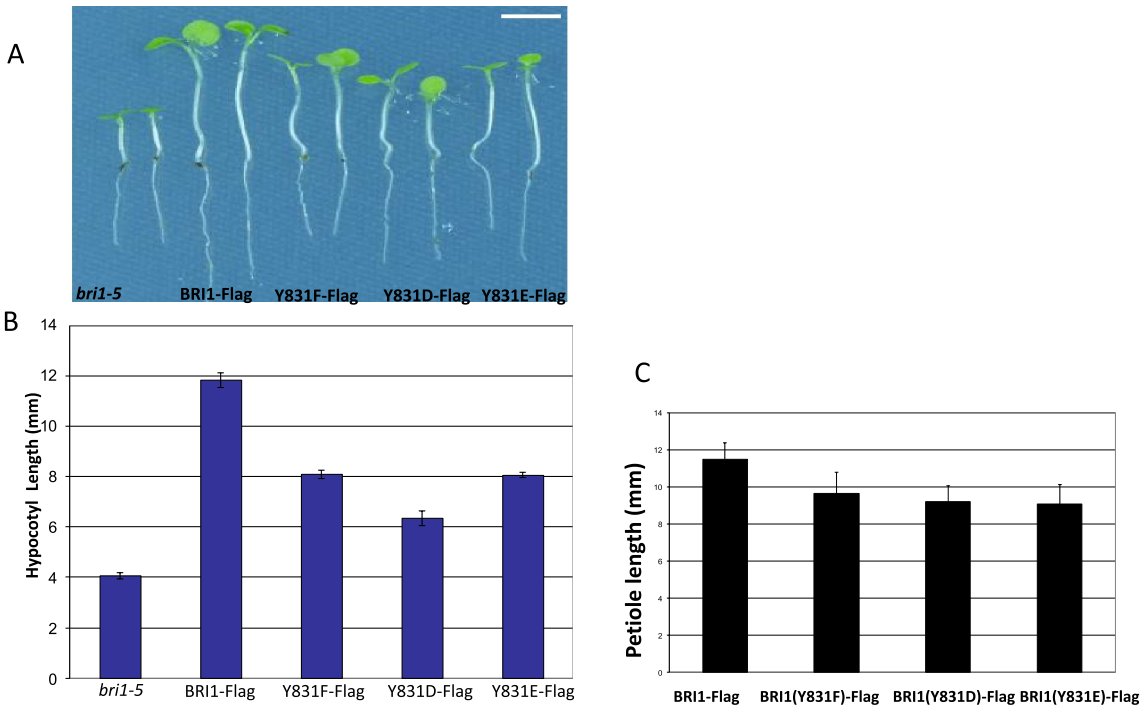

Fig. 2 Phenotypic analysis of *bri1-5*, *BRI1(Y831F)-Flag*, *BRI1(Y831D)-Flag* and *BRI1(Y831E)-Flag* transgenic plants grown in soil for 6 days under a long-day photoperiod. Photographs were taken 6 days after germination in soil (**A**), and hypocotyl and petiole length measurements were expressed as the mean and standard deviation of 20 individual seedlings (**B, C**) with three independent transgenic lines

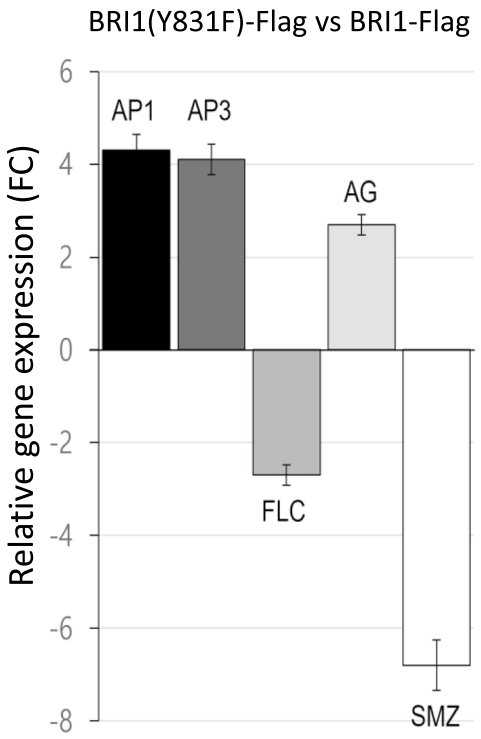

Fig. 3 Expression level of flowering-related genes in *BRI1(Y831F)-Flag* vs. *BRI1-Flag* transgenic plants. Total RNA was prepared and hybridized to the Gene Chip Arabidopsis ATH1 Genome Array. Floral homeotic genes, *AP1*, *AP3*; *AG*, *FLC*, *SMZ*

transgenic plants. Analysis of SDM-derived mutant plants showed that the phosphorylation of *BRI1*(Tyr-831) in BRI1 changes BR signaling. Phosphorylation of Tyr-831 delays flowering and inhibits leaf growth through the alteration of BR signaling, possibly via a conformational change in BRI1 or through as yet unknown phosphotyrosine-binding proteins(Yant et al. 2010).

Additionally, we identified Met residues surrounding the island domain, i.e., brassinolide (BL)-binding domain of the ECD in BRI1. Generally, oxidation of Met residues in tissue proteins can cause them to misfold or otherwise render them dysfunctional (Yaffe 2002; Vogt 1995; Griffiths et al. 2002). Slight changes in protein conformation influence the oxidation of specific Met residues (Yin et al. 2000). Met residues surround the Leucine-Rich Repeat 22 (LRR22) of the ECD in BRI1. Therefore, we focused on the mutation of Met residues in the ECD of BRI1. We speculated that Met residues surrounding the island domain of BRI1 are important for BR perception. Therefore, we generated transgenic plants in the *bri1-5* mutant background by SDM: *BRI1(M656Q)-Flag*, *BRI1(M656L)-Flag*, *BRI1(M657Q)-Flag*, *BRI1(M657L)-Flag*, *BRI1(M661Q)-Flag*, *BRI1(M661L)-Flag*, *BRI1(M665Q)-Flag*, *BRI1(M665L)-Flag*, and *BRI1(M671Q)-Flag*, and *BRI1(M671L)-Flag*. The SDM-derived transgenic plants were grown in soil and half-strength MS media, and various phenotypic features including plant growth pattern, leaf shape, hypocotyl length, and root length were evaluated. As a result, BRs inhibit root growth in *Arabidopsis thaliana*. The BR insensitive mutant, *bri1*, showed that insensitivity in the root growth in contrast of inhibition the elongation of wild-type roots in the presence of BR (Chu et al. 2004). This suggests that BRs inhibit root growth in Arabidopsis. First, we grew all genotypes including *bri1-5* and *BRI1-Flag* (controls) in soil. The *bri1-5* mutant phenotype was partially rescued in all SDM-derived mutants, except in *BRI1(M665Q)-Flag/bri1-5* and *BRI1(M661L)-Flag/bri1-5* plants.

However, the growth and shape of rosette leaves were fully rescued in the *bri1*-5: *BRI1(M665Q)-Flag* transgenic line compared with *bri1*-5: *BRI1-Flag* transgenic plants (Fig. 4A, B). Next, we examined the hypocotyl and root length of SDM-derived Met mutant plants. The hypocotyls of *BRI1-Flag* plants grown in 100 nM BL-containing 1/2 MS media showed normal growth; however, the dwarf phenotype of the *bri1*-5 mutant was not rescued in any of the Met mutants (Fig. 5A). Only the *BRI1(M665Q)-Flag* transgenic plants fully rescued the rosette growth of the *bri1*-5 mutant (Fig. 5A). We also tested the elongation of the primary root of five Met mutants grown in the presence of 100 nM epibrassinolide (BL). The results indicated that *BRI1(M656Q)-Flag*, *BRI1(M657Q)-Flag*, and *BRI1(M661Q)-Flag* seedlings were insensitive to the BL treatment and showed no inhibition of root elongation. However, *BRI1(M665Q)-Flag* and *BRI1(M671Q)-Flag* seedlings were sensitive to the BL treatment, and exhibited root elongation inhibition (Fig. 5B). Therefore, current results suggest that BR signal transduction was impaired in *BRI1(M656Q)-Flag*, *BRI1(M657Q)-Flag*, and *BRI1(M661Q)-Flag* in the *bri1-5* background. Although a little change in protein structure can be critical influence through Met residues oxidation (Yin et al. 2000), we could not provide any evidence for the oxidation of Met residues in the ECD of BRI1. However, it is possible that Met residues surrounding the LRR22 of BRI1 ECD are important for BR signaling.

Materials and methods

Plant materials

Brassinosteroid-insensitive dwarf mutants, *bri1-5* weak alleles was used as the background plants for BRI1 wild type and diverse site-directed mutagenesis complementation experiments. The resulting constructs were introduced into *Arabidopsis thaliana bri1-5* plants by *Agrobacterium*-mediated transformation using the floral dip method. Transgenic Arabidopsis plants expressing

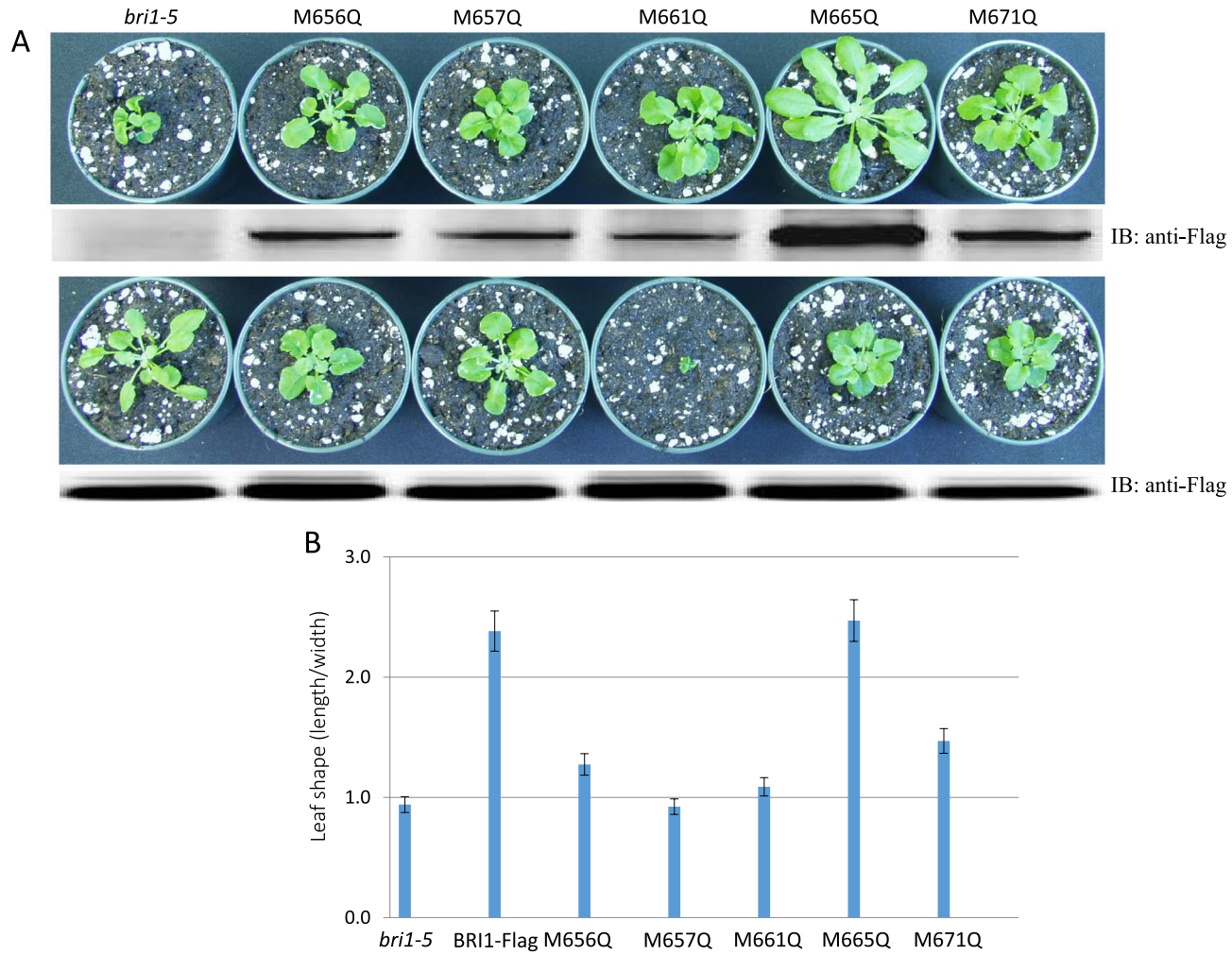

Fig. 4 Effect of the substitution of Met residues for plant growth and leaf shape in the ECD domain of BRI1 receptor kinase on the *bri1-5* mutant phenotype. **A** Growth of site-directed mutagenesis (SDM)-derived mutants, such as *BRI1(M656Q/L)-Flag*, *BRI1(M657Q/L)-Flag*, *BRI1(M661Q/L)-Flag*, *BRI1(M665Q/L)-Flag*, and *BRI1(M671Q/L)-Flag*. **B** Analysis of normal BR signaling according to substitution of methionine residues by comparative analysis of leaf shapes of ten different transgenic plants. Photographs were taken 21 days after germination in soil and leaf shape(length/width) measurements were expressed as the mean and standard deviation of ten individual plants with three independent transgenic lines

BRI1-Flag, *BRI1(Y831F)-Flag*, *BRI1(Y831D)-Flag*, *BRI1(Y831E)-Flag*, *BRI1(M656Q/L)-Flag*, *BRI1(M657Q/L)-Flag*, *BRI1(M661Q/L)-Flag*, *BRI1(M665Q/L)-Flag*, and *BRI1(M671Q/L)-Flag* in the *bri1-5* mutant background have been described previously (Oh et al. 2000).

After keep growth and harvest seeds (T_0), dried seed for 4–5days. To select T_1 transgenic plants, seeds were surface-sterilized with EtOH (100%) for 2 min and add enough volume of 50% bleach treatment for 15 min. After 15 min, discard the bleach and wash seeds throughly with a large amount of steril water for three times. After washing, add steril water and keep at 4 °C for 2 days, and then sown on medium containing 1/2 MS salts and vitamins (PhytoTechnology Laboratories), supplemented with 1.2% (w/v) phytoagar and 2% sucrose (pH 5.7). The transformed plants were selected on media containing hygromycin (20 µg/ml) and transferred to soil. Homozygous T_3 lines were determined according to the segregation ratio.

Experimental design

To conduct phenotypic analysis, plants of various genotypes were grown in sterilized square plastic plates (125 × 125 × 20 mm) at 23 °C under a long day (16 h/8 h light) photoperiod. For hypocotyl and root length analysis, surface-sterilized seeds were grown under a long day photoperiod for 7 days on half strength MS salts containing 1% sucrose (pH 5.7) and 1.2% phytoagar. The hypocotyl and root length of 20 individual seedlings from three independent transgenic lines was then measured. To investigate the

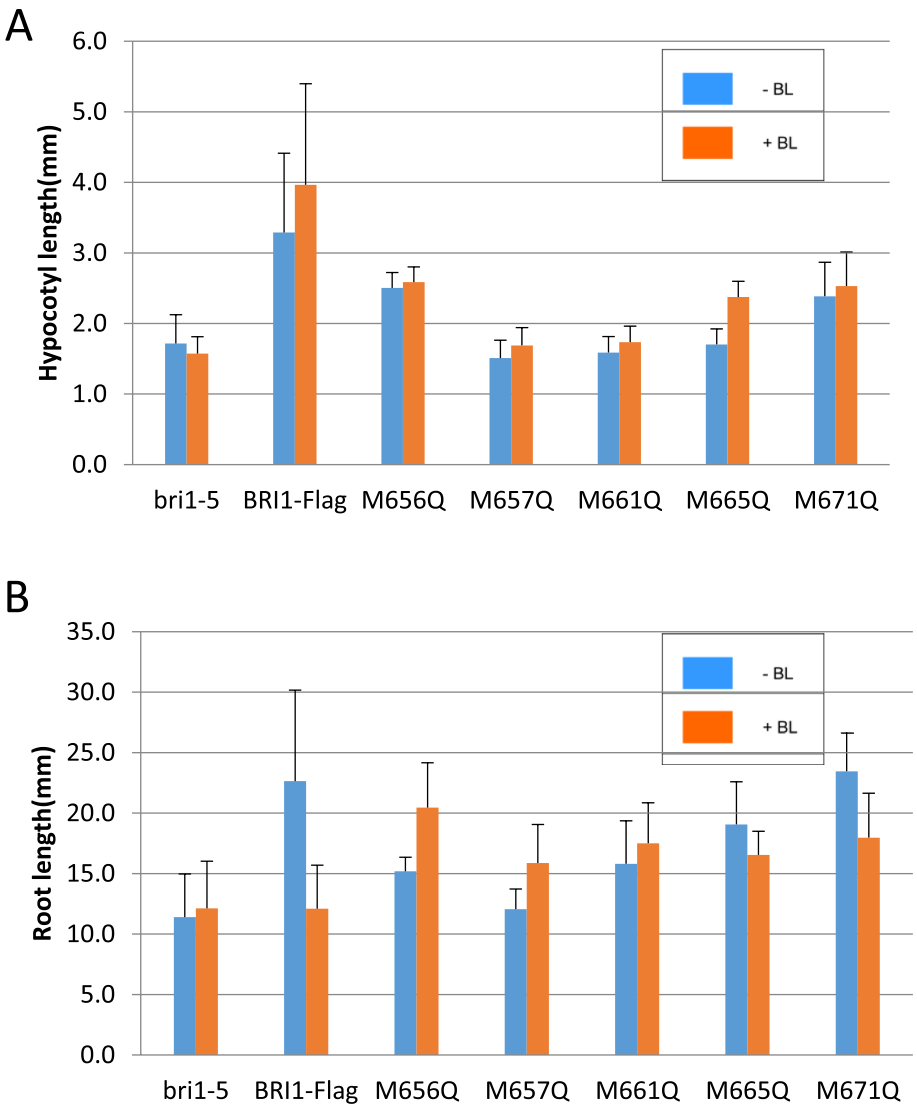

Fig. 5 Effect of Met substitution in the ECD of BRI1 on hypocotyl and root growth in the *bri1-5* mutant background. Quantification of hypocotyl length (**A**) and root length (**B**). Seedlings were grown on 1/2 MS medium containing 1.2% agar and supplemented with or without 1 µM epibrassinolide (BL). Plants were growth under long-day (16 h light/8 h dark) photoperiod, and hypocotyl and root lengths were measured after 7 days. Data represent mean ± SD of 20 individual seedlings with three independent transgenic lines

effect that substituting Met residues in the ECD domain of the BRI1 receptor kinase in the *bri1-5* mutant has on plant growth and leaf shape, *BRI1(M656Q/L)-Flag*, *BRI1(M657Q/L)-Flag*, *BRI1(M661Q/L)-Flag*, *BRI1(M665Q/L)-Flag*, and *BRI1(M671Q/L)-Flag* mutants were grown for 21 days post-germination in soil contained in round pots. Photographs were taken and leaf shape (length/width) was measured. Data were expressed as the mean and standard deviation of ten individual plants from three independent transgenic lines. Epibrassinolide (BL; Sigma-Aldrich, St, Louis, MO, USA) was dissolved in 100% EtOH (final concentration, 10^{-3} M) and sterilized by filtration with 0.45 µm pore size (Millipore, MA, USA). This was then added to the culture medium (final concentration, 10^{-7} M). The plates were placed vertically in a growth chamber at 23 °C, with a long day photoperiod (16 h light/8 h dark). After 7 days, hypocotyl and root length were measured in 20 individual seedlings from three independent transgenic lines.

SDM of BRI1-Flag

The *pBIB-Hyg*$^{+}$*-pBR-BRI1-Flag* construct (Li et al. 2002) was used as the template for SDM with QuickChange XL Site Directed Mutagenesis Kit (Stratagene, La Jolla, CA, USA) to make the SDM mutants. After PCR with SDM kit, add 1 µl of Dpn1 to PCR reaction. Incubate at 37 °C for 2 h to digest parental DNA and then transformed into XL10-gold competent cells. 10 constructs, each carrying a different variant of the *BRI1* gene sequence, were generated: *BRI1(Y831F)-Flag, BRI1(S858A)-Flag, BRI1(T872A)-Flag, BRI1(Y831F + S858A)-Flag, BRI1(Y831F + T872A)-Flag, BRI1(S858AF + T872A)-Flag, BRI1(Y831F)-Flag, BRI1(Y831D)-Flag, BRI1(Y831E)-Flag, BRI1(M656Q)-Flag, BRI1(M656L)-Flag, BRI1(M657Q)-Flag, BRI1(M657L)-Flag, BRI1(M661Q)-Flag, BRI1(M661L)-Flag, BRI1(M665Q)-Flag, BRI1(M665L)-Flag, BRI1(M671Q)-Flag, and BRI1(M671L)-Flag*. All constructs

were sequenced in both directions to verify the presence of specific mutations and the lack of additional mutations (Karlova et al. 2009). After generation and selection homozygous transgenic lines on the half strength MS agar plates containing hygromycine (20 µg/ml), all transgenic plants used for phenotypic analysis on the soil and half strength MS medium.

Total RNA isolation and microarray analysis

Transcriptome analysis was carried out at the University of Illinois (Urbana-Champaign, Illinois, USA). RNA was prepared and hybridized to the Affymetrix Gene Chip Arabidopsis ATH1 Genome Array using the Gene Chip Express Kit (Ambion, Austin, TX, USA). Plants were grown in soil for 25 days under the long-day photoperiod. Total RNA was isolated from shoots harvested at the rosette stage (before bolting) at the middle of the photoperiod, and cleaned using the RNeasy Plant Mini Kit (Qiagen, Manchester, UK). Total RNA was isolated from *bri1-5*, *BRI1-Flag*, and *BRI11(Y831F)-Flag* transgenic plants. Additional details are provided in Supplemental Text S1 (Wang et al. 2005).

Conclusion

Post-translational modifications, such as phosphorylation, are important for the activity and stability of proteins in higher plants, and underlie their responses to diverse stimuli including plant hormones and small peptide ligands, such as flg22, elf18, IDA, pep1, and CLV3. In the present study, we demonstrated that the phosphorylation of Tyr831, which is located in the juxtamembrane domain (JM) of BRI1, is essential for leaf growth and flowering time in Arabidopsis. *BRI1(Y831F)-Flag* transgenic seedlings showed a reduction in hypocotyl and petiole length compared with *BRI1-Flag* seedlings. Transcriptome analysis revealed differential expression of flowering time-associated genes (*AP1*, *AP2*, *AG*, *FLC*, and *SMZ*) between *BRI1(Y831F)-Flag* and *BRI1-Flag* transgenic seedlings. The essential role of *AP1, AP2*, and *AG* genes as positive regulators of flowering is well known in Arabidopsis. Expression levels of *AG*, *AP1*, and *AP2* were significantly higher in *BRI1(Y831F)-Flag* plants compared with *BRI1-Flag* transgenic plants, whereas those of *FLC* and *SMZ* (flowering repressor genes) was significantly downregulated in *BRI1(Y831F)-Flag/bri1-5* transgenic plants. Interestingly, *BRI1(Y831F)-Flag* transgenic plants showed earlier flowering time than all the other genotypes, including *BRI1-Flag*, because of the expression levels of key flowering-related genes, including those promoting flowering (*AP1*, *AP2*, and *GA*) and repressing flowering (*FLC* and *SMZ*). We also investigated the effects of substitution of Met residues located in the ECD of BRI1 on plant growth and BR sensitivity by evaluating hypocotyl elongation and root growth. Our results suggest that Met residues surrounding the LRR22 in BRI1-ECD potentially play an important role in BR signaling, thus affecting plant growth and root elongation.

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Author contributions Conceptualization, MH: Methodology, JH, ES, HS, and MH: Experiments, JH, ES, and MH: original draft preparation. All authors have read and agreed to the published version of the manuscript.

Declarations

Conflict of interest The authors declare no conflict of interest.

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