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Transgenic rice plants ectopically expressing *AtBAK1* are semi-dwarfed and hypersensitive to 24-epibrassinolide

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Semi-dwarf;
Signaling

Summary

Brassinosteroids (BRs) are endogenous plant hormones essential for plant growth and development. Brassinosteroid insensitive1 (BRI1)-assocaiated receptor kinase (BAK1) is one of the key components in the BR signal transduction pathway due to its direct association with the BR receptor, BRI1. Although BRI1 and its orthologs have been identified from both dicotyledonous and monocotyledonous plants, less is known about BAK1 and its orthologs in higher plants other than *Arabidopsis*. This article provides the first piece of evidence that AtBAK1 can greatly affect growth and development of rice plants when ectopically expressed, suggesting that rice may share similar BR perception mechanism via BRI1/BAK1 complex. Interestingly, transgenic rice plants displayed semi-dwarfism and shortened primary roots. Physiological analysis and cell morphology assay demonstrated that the observed phenotypes in transgenic plants were presumably caused by hypersensitivity to endogenous levels of BRs, different from BR insensitive and deficient rice mutants. Consistently, several known BR inducible genes were also upregulated in transgenic rice plants, further suggesting that BAK1 was able to affect BR signaling in rice.

Abbreviations: 24-EBL, 24-epibrassinolide; BRs, brassinosteroids; GFP, green fluorescent protein; GUS, β-glucuronidase; RT-PCR, reverse-transcription polymerase chain reaction; PI, propidium iodide

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On the other hand, the transgenic plants generated by overproducing *AtBAK1* may potentially have agricultural applications because the dwarfed phenotype is generally resistant to lodging, while the fertility remains unaffected.

Introduction

Brassinosteroids (BRs) are widely distributed phytohormones essential for regulating plant growth and development. BR-deficient or insensitive mutant plants exhibit multiple defective phenotypes, such as dwarfism, reduced fertility, delayed senescence, and de-etiolation in darkness (Clouse et al., 1996; Szekeres et al., 1996). Exogenous application of a low concentration of BRs can promote cell elongation, enhance disease resistance, and improve stress tolerance (Krishna, 2003; Nakashita et al., 2003; Zurek et al., 1994). Treatment with a high concentration of BRs, on the other hand, can greatly inhibit plant growth (Clouse and Sasse, 1998). Recent studies indicated that plant steroids were perceived by a cell surface receptor kinase complex including at least brassinosteroid insensitive1 (BRI1) and BRI1-assocaiated receptor kinase (BAK1). *bri1* was first identified by genetic screens for mutants with reduced or abolished root growth inhibition in medium containing a high concentration of brassinolide (BL), the most active BR (Clouse et al., 1996). Then BRI1 was cloned by a map-based cloning strategy, and it encodes a plasma membrane-localized leucine-rich repeat receptor-like kinase (LRR-RLK, Li and Chory, 1997). Previous reports suggested that the extracellular domain of BRI1 was involved in the perception of BRs, while the intracellular domain of BRI1 was responsible for the initiation of the BR signaling cascade by phosphorylation (He et al., 2000; Wang et al., 2001). Biochemical and genetic evidence also revealed that two *Arabidopsis* BRI1-like LRR-RLKs, BRL1 and BRL3, play partially redundant roles with BRI1 (Cano-Delgado et al., 2004; Zhou et al., 2004).

Another LRR-RLK, BAK1, distinct from BRI1, BRL1 or BRL3, was identified from *Arabidopsis* in two ways: by a gain-of-function genetic screen for *bri1-5* suppressors (Li et al., 2002), and through a yeast two-hybrid investigation for proteins interacting with the BRI1 kinase domain (Nam and Li, 2002). It encodes an LRR type II RLK with five extracellular LRRs, a transmembrane domain and an intracellular kinase domain. Genetic, biochemical, and cellular analyses demonstrated that BAK1 interacts with BRI1 both in vitro and in vivo (Li et al., 2002; Nam and Li, 2002; Russinova et al., 2004). More recently, BRI1/BAK1dimerization was found to be directly regulated by endogenous levels of BL (Wang et al., 2005). Moreover, phosphorylation of BAK1 Thr residues is also dependent upon endogenous BL levels (Wang et al., 2005). These results demonstrate that BRI1/BAK1 dimerization plays a critical role in BR signal perception. Whether the BAK1 extracellular domain is directly involved in ligand binding has not been directly tested. In a *bak1* null mutant, binding of H^3-labled BL by BRI1 was not apparently affected, which may suggest that BAK1 is not directly involved in BL binding (Kinoshita et al., 2005). So, BAK1 is most likely the immediate downstream component of BRI1 (Kinoshita et al., 2005; Li et al., 2002; Wang et al., 2005). Although BRI1 and its orthologs have been identified from both dicotyledonous and monocotyledonous plants, less is known about BAK1 and its orthologs in higher plants other than *Arabidopsis*.

To determine whether rice plants utilize a BR perception mechanism resembling that of *Arabidopsis*, transgenic rice plants harboring *AtBAK1* under the control of a strong maize ubiquitin promoter were generated. This research provides the first piece of evidence that ectopically expressed AtBAK1 can greatly affect growth and development of rice plants, which hints that both rice and *Arabidopsis* possess a similar BR perception mechanism via the BRI1/BAK1 complex. Furthermore, the transgenic plants generated by overproducing AtBAK1 may potentially have agricultural applications.

Materials and methods

Plant materials and growth conditions

All rice plants used were *Oryza sativa* cv *Zhonghua 10*. For physiological experiments, the seeds of T_1 and wild type were germinated at 30 °C in darkness for 2 d, then transferred to agar plates containing 0.5 × Murashige and Skoog (MS) media supplemented with various concentrations of 24-epibrassinolide (24-EBL). The plates were then moved to a growth chamber with 16 h photoperiod and 25 °C temperature for 10 d. The seedlings finally were transplanted to well-fertilized fields.

Statistic of the parameters related with crop yield

Three representative transgenic rice lines and wild type (*Oryza sativa* cv *Zhonghua 10*) were taken for yield comparison under the conditions shown above. Five panicles were taken from each material. The parameters related to yield, such as spikelet number, seed number in each panicle, and seed weight were measured.

Vector construction and rice transformation

A DNA fragment containing *BAK1-GFP* was removed from pKAN-35S-BAK1-GFP and re-inserted into the *Kpn*I and *Bam*HI sites of pUN1301 vector, a rice transformation construct vector (Ge et al., 2004). The resulting vector was transferred into *Agrobacterium tumefaciens* EHA105 after sequencing analysis identification. Rice embryonic calli induced from germinated seeds were used for *Agrobacterium*-mediated rice plant transformation as described previously (Ge et al., 2004; Xu et al., 2005).

Histochemical β-glucuronidase (GUS) enzyme assay

GUS staining was performed as described previously (Jefferson, 1989). The lamina of transgenic seedlings were incubated in a solution containing 50 mM Na_3PO_4 buffer (pH 7.0), 5 mM K_3 Fe $(CN)_6$, 5 mM K_4 Fe $(CN)_6$, 0.1% Triton X-100, and 1 mM X-Gluc, and incubated at 37 °C for 4 h.

DNA gel blot analysis

DNA gel blot was carried out as described previously (Sambrook et al., 1989). The probe was labeled with [^{32}P] dATP and [^{32}P] dCTP (China Isotope, Beijing) and synthesized by polymerase chain reaction (PCR) with the following primers 5′-GCATGATACGTCCTGTAGAAACCC-3′ and 5′-CAAAGCCAGTAAAGTAGAACGGT-3′. After pre-hybridization of about 4 h, the heat-denatured probe was added, followed by 20 h hybridization at 65 °C. The membrane was washed twice with 2x SSC, 0.1% SDS at 65 °C for 10 min each time, then one time with 1x SSC, 0.1% SDS at 65 °C for 10 min. The membrane was exposed to an X-ray film (Kodak, Rochester, NY) for 3–7 d at −70 °C.

RNA gel blot analysis

RNA gel blot analysis was carried out as described previously (Sambrook et al., 1989). Total RNA of 30 μg was loaded on each lane. The [^{32}P] dATP labeled *AtBAK1* cDNA probe was used for hybridization. After pre-hybridization of approximately 4 h, the heat-denatured probes were added. The membrane was incubated with the probe for 20 h at 68 °C. The membrane was then washed twice in 2x SSC, 0.1% SDS at 68 °C for 10 min each time, followed by a final wash in 1x SSC plus 0.1% SDS for 30 min at 37 °C. The membrane was exposed to X-ray film (Kodak, Rochester, NY) for 3–7 d at −70 °C.

Confocol laser scanning microscopy

Seminal roots were taken from seedlings of both transgenic plants and wild type plants, after germination in darkness for 4 d. Green fluorescent protein (GFP) images of the transgenic plants were captured with a fluorescence microscope (Axioplan 2 Imaging, Carl Zeiss, Germany). The wavelength of the ejection light was 488 nm. In order to exclude fluorescence from cell wall, META systems were used. Images were processed by LSM5 Imager Browser and Adobe Photoshop 6.0 software. Observation of Root Cells by PI Staining was similar to the GFP observation. The wavelength of the ejection light was 514 nm.

Semi quantitative reverse-transcription polymerase chain reaction (RT-PCR)

Total RNA was extracted and used to make first-strand cDNA as described previously (Ge et al., 2004). As the manufacturer's specifications described, 2 μg of total plant RNA was reverse transcribed using Superscript II Reverse Transcriptase (Gibco BRL life Technologies, UK). First-strand cDNA was generated using an oligo$(dT)_{18}$ primer. 5 μL of first-strand cDNA was diluted to 20 μL. The diluted first-strand cDNA of 2 μL was used as template in a subsequent PCR reaction. Gene-specific primers were used to amplify AY026332 (5′-GAAACGATGGAGATGGTGC3′, 5′-CATGGCATCCTTGAACCTC-3′), AJ278436 (5′-GTGTCGGAGAAGCAGTGGTA-3′, 5′-CTAAGCAAATACGGCTGACA-3′), AJ238318 (5′-ACTTCATTGCTTTTGCCGTAT-3′, 5′-ATACCCCACCAAACCCAAA-3′), Tubulin (5′-TCAGATGCCCAGTGACAGGA-3′, 5′-TTGGTGATCTCGGCAACAGA-3′). PCR components consisted of 10 μL 2x GC buffer, 1 μL 10 mM dNTP, 0.5 μL LA Taq polymerase (TAKARA company, Japan), 1 μL of each primer, and the first-strand cDNA template in 20 μL reaction volume.

PCR was produced in triplicate. Specific PCR conditions: initial denaturation (2 min, 94 °C), followed by 28 cycles of denaturation (45 s, 94 °C), annealing (45 s, 50–58 °C), and primer extension (45 s, 72 °C). After 28 cycles, 10 min extension at 72 °C was performed.

Results

Generation and morphological analyses of transgenic rice plants ectopically expressing *AtBAK1*

To test whether rice plants utilize a BR perception mechanism similar to that of *Arabidopsis*, a binary vector was constructed for rice transformation. In the vector, a *BAK1-GFP* fusion fragment was driven under a maize ubiquitin promoter, which can effectively drive the expression of foreign genes in monocot plants (Cornejo et al., 1993; Ge et al., 2004). Nine independent T_0 transgenic lines were obtained via *Agrobacteria*-mediated transformation after hygromycin screening. Three-fourths of the germination transgenic lines were regarded as a putative single T-DNA insertion. Among the original nine lines, five lines containing a putative single T-DNA insertion were further confirmed by DNA gel blot analysis (Fig. 1J). Phenotypic analyses clearly indicated that all five lines exhibited semi-dwarfism, shortened roots, and excessive adventitious roots (Figs. 1A, F, G and H). The phenotypes were unlikely to have been caused by insertion mutagenesis because different transgenic lines obviously resulted from different insertional events (Fig. 1J). To determine whether exogenous transgene silence occurred, RNA gel blot and confocol analyses were carried out to analyze *AtBAK1* expression at both transcriptional and translational levels. As shown in Fig. 1K, *AtBAK1* transcript was detected in all five transgenic lines, but not in control. Further, confocol analysis demonstrated that BAK1-GFP was only localized on the plasma membrane of transgenic plants, but not that of the non-transgenic plants (Fig. 1L). Thus, the altered morphology of transgenic plants was directly associated with the ectopic expression of *AtBAK1*. To examine whether the dwarfism of T_1 plants was limited to the seedling stage, growth was monitored and analyzed during the entire plant developmental stages. The fact that the dwarfed phenotype could be seen within all stages of plant growth and development implied that the transgene affects many important cellular processes during the growth of transgenic plants (Figs. 1B–D). Although the growth curves of the transgenic and WT plants were statistically different (Fig. 1I), the growth rates were similar, suggesting that unlike to rice BR deficient and insensitive mutants (Hong et al., 2003; Yamamuro et al., 2000), internodes of transgenic plants have a normal elongation pattern without specific internode inhibition. Moreover, fertility and crop yield of these plants remained unaffected (Fig. 1E and Table 1).

Transgenic plants do not exhibit a de-etiolated phenotype

To further distinguish the morphology of transgenic plants harboring the *BAK1* overexpression construct from BR deficient and insensitive mutants, transgenic seedlings were grown in darkness. If the BR signal transduction or biosynthetic pathway was blocked, the resulting mutant rice plants would display a de-etiolated phenotypes when grown in dark (Yamamuro et al., 2000). Unlike BR deficient and insensitive rice mutants, dark-grown transgenic rice seedlings did not exhibit de-etiolated phenotypes (Fig. 2A), indicating that both BR signaling and biosynthetic pathways in transgenic plants were not blocked. Also, the leaf angles in the transgenic plants were obviously enlarged, which was regarded as a classical BR response in rice. The leaf angle of transgenic plants was about 54.5° from the growing stem, while that of WT plants was approximately 14.9° (Fig. 2B). Taken together, these results indicated that the BR signal transduction pathway in these transgenic plants may have been activated, rather than inactivated.

Transgenic plants displayed a hypersensitive response to 24-EBL

If BR signal transduction was activated in transgenic plants harboring the *AtBAK1* construct, hypersensitivity of transgenic plants to BRs would be expected. Treatment with 100 nM 24-EBL could dramatically inhibit root elongation in WT rice plants during seedling growth, while not in *d61-1*, BR-insensitive mutants (Yamamuro et al., 2000). A root elongation inhibition assay was used to determine whether the transgenic plants were hypersensitive to 24-EBL. As shown in Figs. 3A and B, *AtBAK1* transgenic seedlings were hypersensitive to 24-EBL compared to WT seedlings under both dark and light conditions. The relative root length of the transgenic seedlings was only about 43.2% that of control plants in medium containing 100 nM 24-EBL in 16 h light/8 h dark photoperiod, whereas the relative root length of WT plants was about 80.5% that of the control plants. When the 24-EBL was increased to

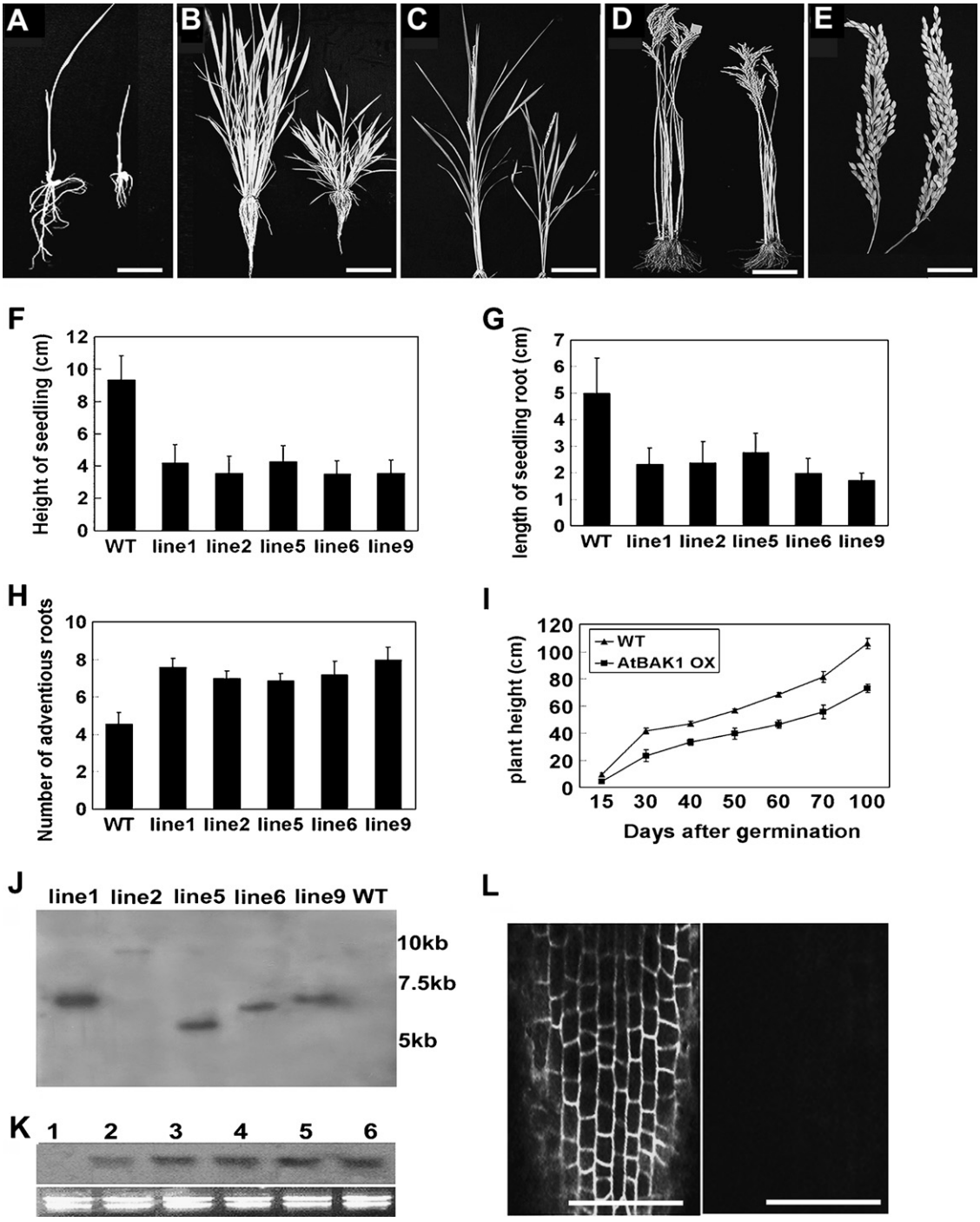

Figure 1. Phenotypes and detection of transgenic plants expressing *AtBAK1*. (A) Seedlings grown 14 d on 1% agar under 16 h light/8 h dark photoperiod. Size bar = 1 cm. (B) Tilling stage. Size bar = 5 cm. (C) Flowering stage. Size bar = 10 cm. (D) Mature plant stage. Size bar = 10 cm. (E) Comparison of panicle morphology in T_1 progenies and wild types (WT), size bar = 2 cm. From (A) to (E), WT and transgenic plants *AtBAK1* OX (*AtBAK1* ectopic expression) shown on the left and right side, respectively. (F) T_1 progenies significantly dwarfed in seedling stage. (G) T_1 progenies showed dramatically shortened roots compared to WT. (H) T_1 progenies exhibited more adventitious roots than WT. (I) Growth curve of transgenic plants compared with WT. Days after germination (DAG). From *G* to *H*, Error bars represent SE from at least 20 plants. (J) DNA gel blot analysis to show T-DNA insertional sites of the three representative lines were different. A single T-DNA insertion was identified in all three lines. (K) Expression of *AtBAK1* in five independent transgenic lines by RNA gel blot analysis. Lane 1, WT; Lane 2–6, 5 independent transgenic lines respectively. Thirty microgram total RNA was loaded on each lane. rRNA was loading control. (L) Subcellular localization of BAK1-GFP in the root tips of the transgenic plants. Seedlings grown 3 d were used for observation. Upper-panels show transgenic confocol images. Lower-panels show WT confocol images as control. Size bars = 50 μm.

Table 1. Yield parameters among wild type and three representative transgenic *AtBAK1* ectopic expression lines

	Number of primary spikelets	Number of seeds in each panicle	Weigh of each 100 seeds (g)
WT	13.16±0.89	159.33±8.33	2.48±0.067
Line 5	14±0.67	160.83±13.5	2.56±0.07
Line 6	12.83±0.56	150.33±7.78	2.53±0.054
Line 9	13.67±0.67	155.33±6.78	2.59±0.053

Mean±SD from six independent panicles. Lines 5, 6 and 9 were picked as three representative lines in the statistics.

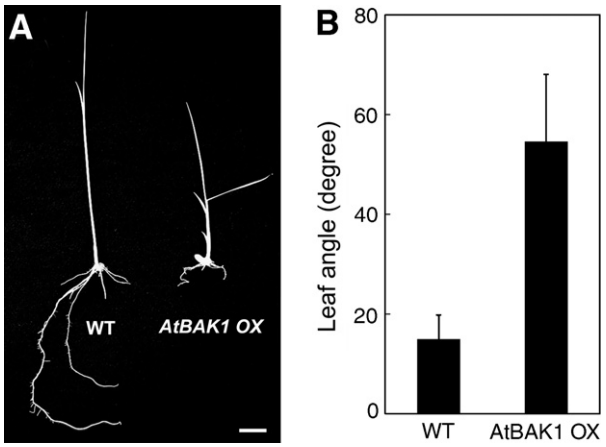

Figure 2. Morphology of transgenic seedlings in darkness. (A) Transgenic seedlings did not display deetiolated phenotype, but displayed an enlarged leaf angle in dark. (B) Statistics of leaf angle in transgenic plants. The leaf angle of seedlings was about 54.5° in transgenic plants, WT were only about 14.9°. Error bars represent SE from 15 plants.

1 μM, the relative root growth of the *AtBAK1* transgenic seedlings was only 27.5% that of the control plants, and the relative root growth of WT plants was about 65.1% that of the controls. These results clearly demonstrated that *AtBAK1* transgenic plants were hypersensitive to 24-EBL.

24-EBL inducible genes were up-regulated in transgenic plants

The effects of BRs on plant growth and development are mediated via the modulation of gene expression. Many BL up-regulated genes have been identified in rice (Yang et al., 2004). To confirm whether the BR signal transduction pathway was activated in the *AtBAK1* transgenic rice plants at the level of gene regulation, expression patterns of three rice BR upregulated genes were analyzed. Previous reports indicated that upon 24-EBL treatments, rice genes *AY026332*, *AJ238318*, and *AJ278436* were up-regulated to 4.91, 2.56, and 7.67 folds more than the levels of untreated plants, respectively. As indicated in Fig. 4, all three selected genes showed similar induction expression patterns in the three representative transgenic lines even without the 24-EBL treatments. This further identified the activation of the BR signal transduction pathway in the transgenic plants.

Phenotypes of *AtBAK1* transgenic rice could be phenocopied by wild type plants treated with 24-EBL

To further confirm that the transgenic phenotypes were caused by BR-activation, WT rice plants were treated with 24-EBL concentrations ranging from 10 to 10 μM. As expected, WT plants exhibited distinct phenotypes in a dose dependent manner (Fig. 5A). As shown in Figs. 5B and C, when treated with 100 nM 24-EBL, the roots were significantly shortened and shoot growth was inhibited. Dwarfed seedlings and shortened primary roots were observed in WT plants treated with either 1 or 10 μM 24-EBL. These results illustrate that WT plants can phenocopy the phenotypes of transgenic plants harboring *BAK1* in an intermediate concentration of 24-EBL.

Primary root growth was not inhibited in BR-deficient and BR-insensitive rice mutants

The primary roots of rice *d2-1*, a BR-deficient mutant, and *d61-1*, a BR-insensitive mutant, were compared. The primary roots of *d2-1* and *d61-1* were similar to control plants, whereas the primary root length of *AtBAK1* transgenic plants was just half that of WT (Fig. 6). These data further confirmed that the phenotypes observed in *AtBAK1* transgenic rice plants were not attributable to BL deficiency or inhibitory effects of BR signaling transduction. Rather, they were most likely a result

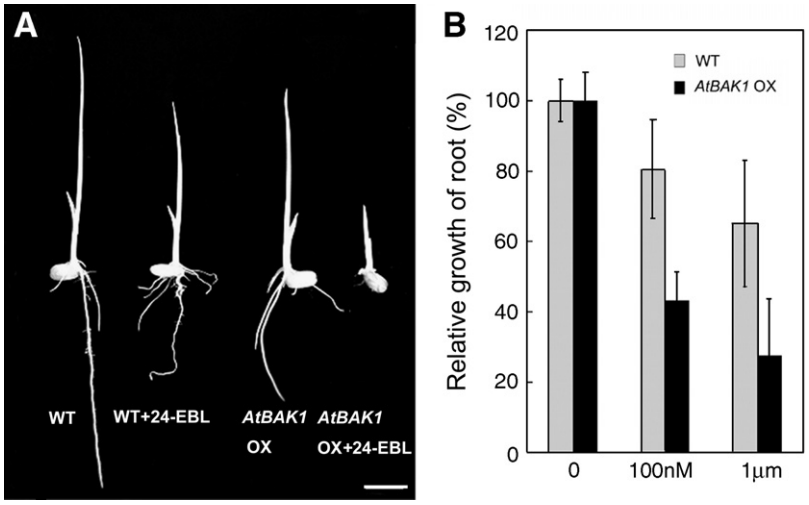

Figure 3. Assay of sensitivity to 24-EBL. (A) WT; WT+100 nM 24-EBL; *AtBAK1* OX; *AtBAK1* OX+100 nM 24-EBL grown in dark from left to right, respectively. Bar = 1 cm. (B) 16 h light/8 h dark photoperiod. Transgenic plants of *AtBAK1* OX were hypersensitive to 24-EBL. Measurements taken 5 d after germination and were averaged from 15 individual plants.

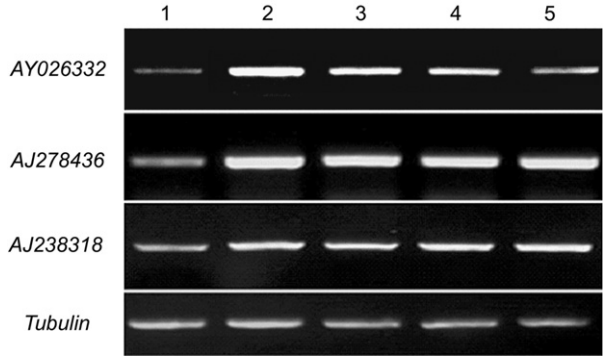

Figure 4. Expression patterns of three 24-EBL up-regulated marker genes in transgenic plants. Lane 1: WT, lane 2: WT treated with 24-EBL for 6 h, Lanes 3–5: three independent transgenic lines, respectively. Twenty-eight cycles were used for RT-PCR.

of hypersensitivity toward endogenous BR levels caused by the overexpression of *AtBAK1*.

The shortened primary root was mainly caused by reduced cell division in transformants

To investigate the mechanistic details of shortened primary roots at the cellular level, primary roots from 3-d-old plants were observed by confocol with propidium iodide (PI) staining. In maturation and cell elongation zones, the cells from both the transgenic and WT plants treated with 100 nM 24-EBL did not show significant differences in cell size (data not shown). However, in the cell division zone, transgenic plant cell morphology was altered (Fig. 7C), which is similar to WT plants treated with 100 nM 24-EBL, compared with control plants (Fig. 7A). The cell numbers in longitudinal direction were counted as indicated in Fig. 7B. In the same longitudinal distance, total number of cells in the transgenic plants was less than WT control plants (Fig. 7D), suggesting that the shortened primary roots were not caused by cell expansion but perhaps cell division. Similar results were observed in leaf sheath via longitudinal section (data not shown). Conversely, in both BR-deficient (*d2-1*) and BR-insensitive mutants (*d61-1*), cell elongation accounted for the morphology (Hong et al., 2003; Yamamuro et al., 2000). This evidence demonstrated that morphology of transformants harboring *BAK1* was naturally different from both BR-deficient and BR-insensitive mutants.

Discussion

Although transgenic rice plants overexpressing *AtBAK1* displayed semi-dwarfism and shortened root phenotypes, these transgenic plants were distinct from rice BR-deficient or insensitive mutants in three aspects: First, unlike both BR deficient and insensitive mutants, transgenic plants ectopically expressing *AtBAK1* did not display de-etiolated phenotypes in dark. Second, they were hypersensitive to 24-EBL in a root elongation inhibition assay, which was obviously different from rice BR-insensitive mutants. Third, three upregulated BL genes exhibited upregulation in transgenic

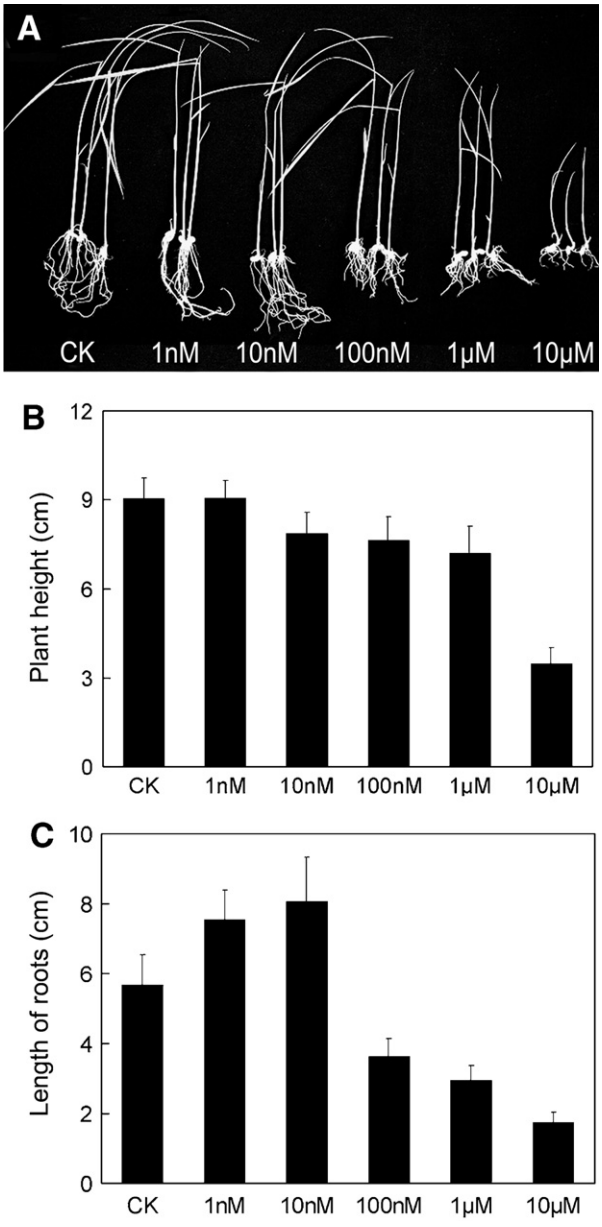

Figure 5. Phenotypes of transgenic plants can be mimicked by WT plants treated with 24-EBL in dose-dependant manner. (A) WT plants exhibit variant phenotype in a dose-dependant manner. (B) Hypocotyls are shorter when a higher concentration of 24-EBL was used. Error bars represent SD from 20 plants. (C) Roots are also shorter when a more 24-EBL was used. Error bars represent SE in at least 20 plants.

plants. This indicated that BR signal transduction in transgenic plants was actually activated, rather than attenuated. Morphologies of transgenic plants ectopically expressing *AtBAK1* could be phenocopied by WT plants treated with 24-EBL in a dose-dependant manner and the fact that primary roots were not shortened in BR-deficient and insensitive rice mutants further confirmed the possibility.

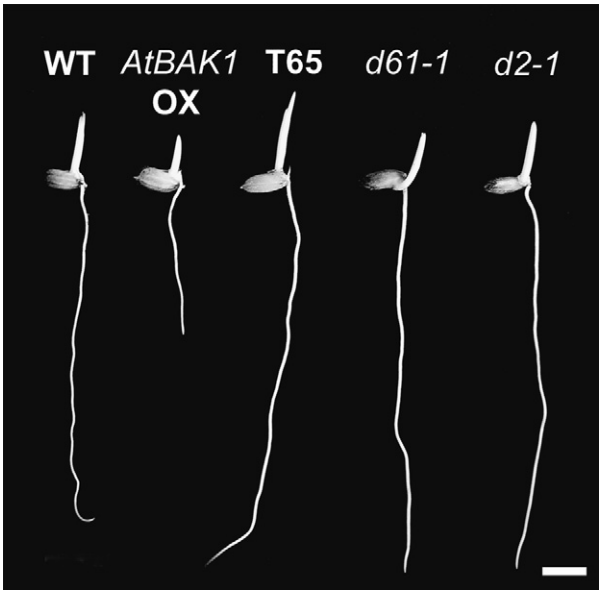

Figure 6. Comparison of primary roots between BR-related mutants and transgenic plants. Primary roots of WT (*Oryza sativa* cv. *Zhonghua 10*), transgenic plant, T65 (*Oryza sativa* cv. *Japonica*), d61-1, the weak allele of d61, a BR-insensitive mutant, and d2-1, a BR-deficient mutant, were photographed 2 d after germination. Bar = 1 cm.

Thus, it was clearly demonstrated that overexpression of *AtBAK1* in rice affected BR signal transduction. A BLASTx search with the *AtBAK1* sequence detected at least eight *AtBAK1* orthologs in the sequenced rice genome (data not shown), which combined with the influence of *BAK1* ectopic expression on rice BR signaling, further implied that rice plants use a mechanism similar to the BRI1/BAK1 complex to initiate BR signaling. Overall, these results support the hypothesis that the mechanism of BR perception via BRI1/BAK1 complex might be similar in rice to that in *Arabidopsis*.

Interestingly, the semi-dwarfism and shortened primary root phenotypes were observed in transgenic plants overexpressing *AtBAK1*. These phehotypes might be caused by overproduction of *AtBAK1* directed by the maize ubiquitin promoter. The ubiquitin (Ubi-1) promoter has been proven to be highly active in monocots, especially in rapidly dividing cells and protoplasts (Cornejo et al., 1993). Expression levels of the reporter gene driven by Ubi-1 were over 10-fold higher than those directed by the CaMV 35S promoter in dividing cells of monocots (Christensen and Quail, 1996). In rice transformation, the Ubi-1 promoter was expressed in most of the tissues and directed dramatically higher transgene expression levels than other promoters (Cornejo et al., 1993). When the ubiquitin promoter was used to direct *AtBAK1*

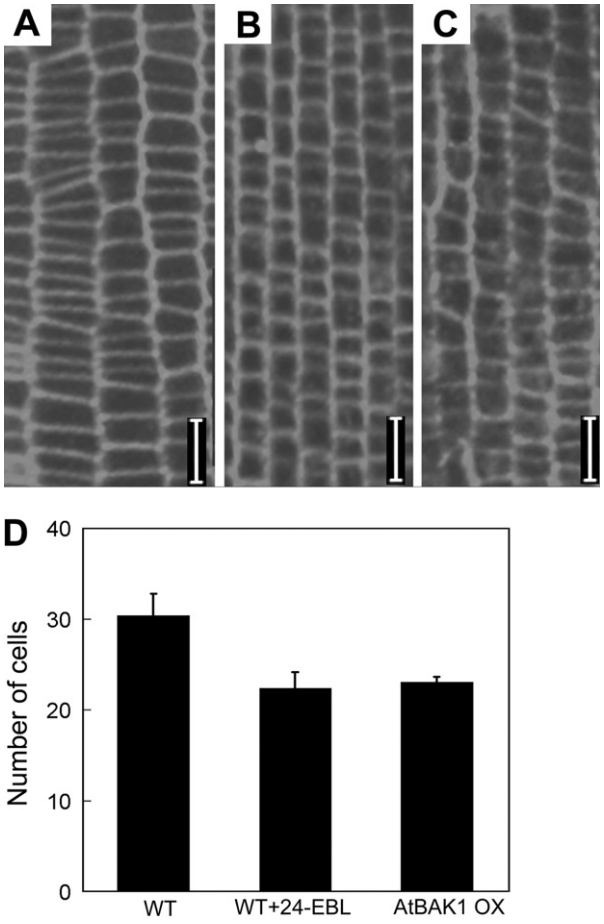

Figure 7. Confocol assay of cell size in the division zone of primary roots. (A) Division zone of WT primary root. (B) Division zone of WT primary root treated with 24-EBL. (C) Division zone of transgenic primary root *AtBAK1* OX; in a, b and c, roots were stained by PI 3 min, and were observed with confocol instrument, Bars = 20 μm. (D) Statistic of cell number in 200 μm longitudinal distance of division zones.

ectopic expression in rice, it probably created an overdose of *AtBAK1* which disturbed the balance of endogenous OsBRI1 with rice BAK1 (OsBAK1). Unlike rice BR-deficient and insensitive mutants, cell expansion was not inhibited in transgenic plants overexpressing *AtBAK1*. Instead, semi-dwarfism and shortened primary roots were mainly due to reduced cell division (Fig. 7D). Taken together, we propose that ectopic expression of *BAK1* in rice disturbed the delicate balance of endogenous OsBRI1 with rice BAK1, which subsequently may reduced cell division in transgenic plants and resulting in the phenotypes of semi-dwarfism and shortened primary roots.

Gibberellins acids (GA) and BRs are two plant hormones essential to rice plant architecture. However, many loss-of-function mutants related to GA and BR biosynthetic and signal transduction pathways usually produce small seeds and reduced fertility together with dwarfism. Very recently, Morinaka and his collegues demonstrated that *d61-7*, the weakest allele caused by OsBRI1 mutation, could produce much more biomass than wild type plants at high planting density, but they failed to observe more yields because of the small grain size of *d61-7* (Morinaka et al., 2006). They further constructed rice transgenic plants containing partial suppression of endogenous OsBRI1, and succeeded to find enhanced yields at high density (Morinaka et al., 2006). Two redundant genes, *CYP90B2/OsDWARF4* and *CYP724B1/D11,* both play function in C-22 hydroxylation, the rate-limiting step of BR biosynthesis. Sakamoto and his collegues verified that mutation of *OsDWARF4* alone caused only limited defects in BR biosynthesis and could improve rice yields without the negative environmental effects of fertilizers under dense planting conditions (Sakamoto et al., 2006). These results first demonstrated that overexpression of *BAK1* in rice directed by a strong promoter, the maize ubiquitin promoter (Ge et al., 2004), could obtain semi-dwarfed rice transformants without affecting fertility. Further, it was found that grain yields of rice transformants harboring an ectopic *BAK1* expression construct were almost the same as WT plants (Table 1). In all, the evidence suggested that regulation of BR signaling in rice may have potential applications in rice yield.

In conclusion, this report provides the first piece of evidence that BAK1 can greatly affect growth and development of rice plants when ectopically expressed in rice. This, combined with the discovery of at least eight *AtBAK1* orthologs in the sequenced rice genome by BLASTx analysis with sequence of *AtBAK1*, suggested that the mechanism of BR perception via the BRI1/BAK1 complex in rice might be similar to that in *Arabidopsis*. Finally, the transgenic plants generated by overproducing *AtBAK1* could have future agricultural applications.

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