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Engineering *OsBAK1* gene as a molecular tool to improve rice architecture for high yield

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Summary

Generating a new variety of plant with erect-leaf is a critical strategy to improve rice grain yield, as plants with this trait can be dense-planted. The erect-leaf is a significant morphological trait partially regulated by Brassinosteroids (BRs) in rice plants. So far, only a few genes can be used for molecular breeding in rice. Here, we identified *OsBAK1* as a potential gene to alter rice architecture. Based on rice genome sequences, four closely related homologs of *Arabidopsis BAK1* (*AtBAK1*) gene were amplified. Phylogenetic analysis and suppression of a weak *Arabidopsis* mutant *bri1-5* indicated that *OsBAK1* (Os08g0174700) is the closest relative of *AtBAK1*. Genetic, physiological, and biochemical analyses all suggest that the function of *OsBAK1* is conserved with *AtBAK1*. Overexpression of a truncated intracellular domain of *OsBAK1*, but not the extracellular domain of *OsBAK1*, resulted in a dwarfed phenotype, similar to the rice BR-insensitive mutant plants. The expression of *OsBAK1* changed important agricultural traits of rice such as plant height, leaf erectness, grain morphologic features, and disease resistance responses. Our results suggested that a new rice variety with erect-leaf and normal reproduction can be generated simply by suppressing the expression level of *OsBAK1*. Therefore, *OsBAK1* is a potential molecular breeding tool for improving rice grain yield by modifying rice architecture.

Keywords: *OsBAK1,* Brassinosteroid signalling, erect-leaf, architecture modification, grain yield, rice *SERK* family.

Introduction

Rice is one of the most important food crops and feeds more than half of the world population. In most developing countries, population growth has led to the dramatically increase of rice demand. To meet this challenge, new varieties of rice with high yield need to be developed (Yang and Hwa, 2008). Plant architecture is a major factor on high yield (Reinhardt and Kuhlemeier, 2002; Li *et al.*, 2003; Wang *et al.*, 2005). Yield-related plant architecture includes tillering pattern, plant height, leaf angle, etc. 'The Green Revolution' is a successful example for developing high-yield rice and wheat with shorter and sturdier stems. The grain yield of rice peaked after the Green Revolution. Researchers have tried to increase yield by dense planting plants with erect-leaf (Sakamoto, 2006). Rice brassinosteroid (BR)-related mutants such as *d61, d2, brd2* bear erect leaves (Yamamuro *et al.*, 2000; Hong *et al.*, 2003, 2005). So the modification of rice architecture by manipulating BR biosynthesis or signalling transduction could be a feasible approach for improving rice grain yield.

BRs are a group of phytohormones critical for plant growth and development. Extensive molecular, genetic, and biochemical studies in *Arabidopsis* defined BR signalling from BR perception on the cell surface to gene expression in the nucleus (Friedrichsen and Chory, 2001; Gendron and Wang, 2007). In contrast, the knowledge about BR signalling in rice, the model plant of monocots, is still limited. BR signalling was initially revealed in rice when the BR-insensitive mutant *d61* was identified (Yamamuro *et al.*, 2000). Genetic analysis indicated that *d61* mutant was caused by the loss-of-function of *OsBRI1*, the BR receptor in rice.

Mutant alleles such as *d61-2* show dwarfed, erected leaves, and aberrant reproductive development. Nakamura *et al.* isolated two homologs of *OsBRI1*, *OsBRL1,* and *OsBRL3*, which were proposed to function in the roots as BR receptors (Nakamura *et al.*, 2006). *OsGSK1*, an ortholog of *Arabidopsis BIN2*, was cloned by T-DNA tagged screening, and the suppression of *OsGSK1* could enhance transgenic rice's tolerance to various abiotic stresses (Koh *et al.*, 2007). Our previous studies revealed that 14-3-3 proteins directly inhibit *OsBZR1* function by reducing its nuclear localization (Bai *et al.*, 2007).

Leaf angle can be regulated by BRs in rice. Our previous studies on OsLIC and OsBZR1 also confirmed this notion (Bai *et al.*, 2007; Wang and Li, 2008). Therefore, BR signalling components could be able to mediate plant architecture of rice. Researchers have attempted to modify rice architecture by regulating the expression of *OsBRI1*. A weak *OsBRI1* mutant, *d61-7*, conferred some agronomic important traits such as semi-dwarfed stature and erected leaves (Morinaka *et al.*, 2006). However, mutant plants bear small-size grains, so grain yield was not increased. Unfortunately, weaker *OsBRI1* could not be identified after screening for 1000 semi-dwarf mutants. To obtain a crop potentially with a high yield, a truncated OsBRI1 containing only a kinase domain and a juxtamembrane region was expressed in transgenic rice plants to partially suppress the expression of *OsBRI1*. Thus, developing a new elite variety of rice with high yield by regulating the expression of *OsBRI1* seems not practically feasible.

BAK1, the co-receptor of *BRI1*, may have potential for modifying architecture and improving variety in rice. However, no reports of *BAK1* in rice are available. *AtBAK1*, also called *AtSERK3*, is a member of the *Arabidopsis* Somatic Embryogenesis Receptor Kinase (SERK) gene family, so the rice homolog of *AtBAK1* was presumed to belong to the rice *SERK* family. Besides the function in BR signalling, *AtBAK1* also plays critical roles in regulating innate immunity and programmed cell death (Chinchilla *et al.*, 2007; He *et al.*, 2007; Heese *et al.*, 2007; Kemmerling *et al.*, 2007). In general, the *SERK* family regulates embryonic competence in plants, but *OsSERK1* may have roles in non-embryonic tissue (Ito *et al.*, 2005). In addition, *OsSERK1* (Song *et al.*, 2008) and *OsSERK2* (Hu *et al.*, 2005) collectively participate in disease resistance responses in rice.

To test whether *OsBAK1* can be used to modified rice morphology for high yield, we cloned all homologs of *AtBAK1* in rice. Our results clearly indicated that all rice *SERK* family members are involved in BR signal transduction. Among them, *OsSERK1* is the closest homolog of *AtBAK1*. Modifying the expression of *OsBAK1* alters height, leaf erectness, and grain morphology of rice plants. Transgenic rice plants expressing antisense *OsBAK1* (*OsBAK1*-AS) showed erected leaves and normal reproduction. Rice plants with these characteristics are thought to have potential to be used for dense planting. Our data demonstrated that *OsBAK1* is a potential target gene for rice molecular breeding.

Results

Rice *SERK*s are involved in BR signalling

A BLAST search of the whole genome sequence of rice with the *AtBAK1* cDNA sequence revealed four closely related homologs of *AtBAK1*, including *OsSERK1* (Os08g0174700), *OsSERK2* (Os04g0457800), *OsSERK3* (Os06g0225300), *OsSERK4* (Os02g0283800). Phylogenetic analysis of the *SERKs* from *Arabidopsis* and rice showed that *OsSERK1* and *OsSERK2* were likely co-orthologs of *AtSERK1* (At1g71830) and *AtSERK2* (At1g34210), with a gene cluster form by *AtBAK1* (*AtSERK3*, At4g33430), *AtSERK4* (At2g13790) and *AtSERK5* (At2g13800) being their sister groups (Supplement 1). *OsSERK1* and *OsSERK2* were the two closest rice relatives of *AtBAK1.* Protein sequences of *OsSERK1–4* showed some conserved features, including five LRRs, a Ser-Pro-Pro (SPP) motif, a hydrophobic transmembrane domain, and a cytoplasmic kinase domain (Supplement 2). Comparison of amino acid sequences among the rice *SERK* family indicated that OsSERK1, OsSERK2, OsSERK3, and OsSERK4 shared 77%, 76%, 57% and 51% sequence identity with AtBAK1, respectively. These data clearly suggested that *OsSERK1* is one of the closest relatives of *AtBAK1*.

In *Arabidopsis*, overexpression of *BAK1* can suppress weak alleles of *BRI1* mutants (Li *et al.*, 2002). To test whether *OsSERK1–4* exhibit similar biological functions with *AtBAK1,* we overexpressed these four rice genes in a weak *BRI1* mutant, *bri1-5*. The transgenic *Arabidopsis* lines ectopically expressing *OsSERK1–4* partially rescued the defective phenotypes of *bri1-5* (Figure 1). Compared to *bri1-5*, transgenic plants expressing *OsSERK1–4* showed larger statures, longer petioles and earlier flowering phenotypes (Figure 1a,b). Furthermore, the hyposensitivity of *bri1-5* to 24-epibrassinolide (24-epiBL, a bioactive brassinosteroid) was rescued (Figure 1c,d). Under dark-growing condition, the transgenic seedlings showed elongated hypocotyls relative to *bri1-5*. When treated with the BR

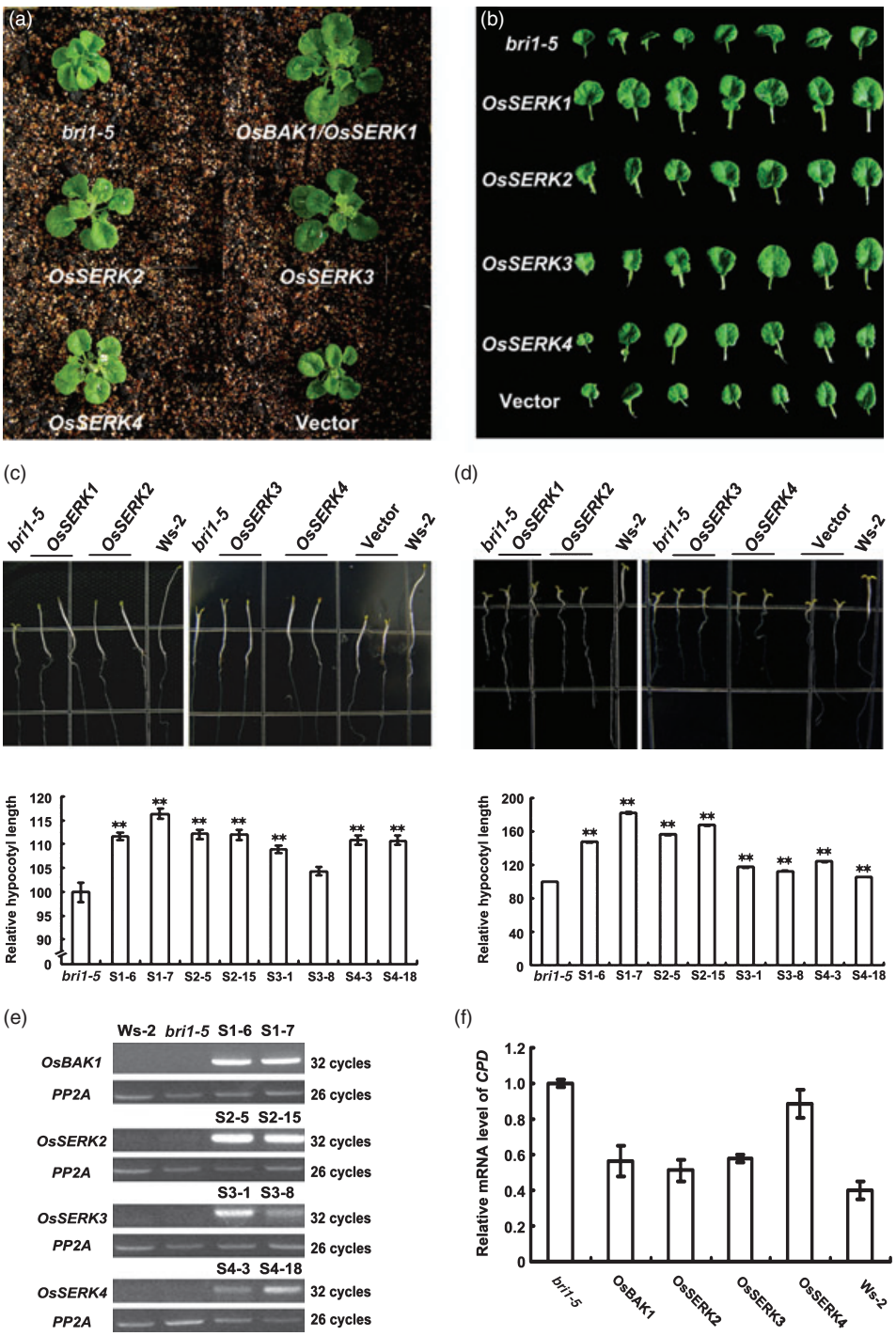

Figure 1 *OsBAK1* and its homologs rescue partially the *Arabidopsis* mutant *bri1-5*. (a) Phenotypes of *bri1-5* and transgenic *bri1-5,* respectively, expressing *OsSERK1/OsBAK1, OsSERK2, OsSERK3, OsSERK4*, vacant vector are shown, which were grown in soil for 3 weeks. (b) The phenotypes of longer petioles and larger leaves in transgenic *Arabidopsis* (a) are shown. (c) *bri1-5* seedlings expressing *OsSERK1/OsBAK1, OsSERK2, OsSERK3* and *OsSERK4* as well as controls (wild-type Ws-2, *bri1-5* itself and *bri1-5* expressing vacant vector) were grown on 1/2 MS medium in the dark at 25°C for 4 days. Graphs represent relative hypocotyl lengths (the percentages of *bri1-5* hypocotyl length). Each data is an average of 40 seedlings. Error bars represent SE. '**', significant differences from *bri1-5*. Transgenic *Arabidopsis* Lines: S1-6, *OsSERK1-6*; S1-7, *OsSERK1-7*; S2-5, *OsSERK2-5*; S2-15, *OsSERK2-15*; S3-1, *OsSERK3-1*; S3-8, *OsSERK3-8*; S4-3, *OsSERK4-3*; S4-18, *OsSERK4-18*. (d) Transgenic seedlings were grown on 1/2 MS medium with 2 μM BRZ. The conditions of seedlings growth and root length measurement are the same as in (c). (e) The expression of *OsSERKs* in transgenic plants analysed by RT-PCR. Expression of the *PP2A* gene is used as a loading control. (f) Real-time PCR analysed the expression of *CPD* gene in transgenic plants, *bri1-5* itself and wild-type Ws-2.

biosynthesis inhibitor brassinazole (BRZ), the hypocotyl length of the transgenic plants expressing *OsSERK1* or *OsSERK2* was increased by 50%–80%, in comparison with that of *bri1-5*; the transgenic plants expressing *OsSERK3* or *OsSERK4* was increased by 10%–20%. The expression of *CPD* has been widely used as a molecular marker to detect the effectiveness of the BR signalling pathway (Albrecht *et al.*, 2008). *BRI1* mutants usually show higher *CPD* expression levels than that of wild-type plants. Compared to *bri1-5*, the transgenic lines expressing *OsSERKs* showed significantly decreased expression of *CPD* (Figure 1f). These results indicated that *OsSERKs*, like *AtBAK1*, can partially rescue *bri1-5* mutant phenotypes. Our detailed analysis showed the degree of recovering from strong to weak was *OsSERK1*, *OsSERK2*, *OsSERK3* and *OsSERK4*, respectively. Functionally, *OsSERK1* is the closest homolog of *AtBAK1* in rice. Therefore, we named it as *OsBAK1*.

OsBAK1 interacts with OsBRI1 *in vivo*

OsBAK1-GFP fusion protein was principally detected on the plasma membrane (Figure 2a). Full length OsBAK1, the extracellular domain (OsBAK1-ECD), the intracellular domain (OsBAK1-ICD), and a truncated intracellular domain (OsBAK1-ICDΔ) were fused to the N-terminal half of the yellow fluorescence protein (YN), respectively (Figure 2b). The full length OsBRI1 protein was fused to the C-terminal half of the yellow fluorescence protein (YC). A YFP fluorescence signal appeared in the onion epidermis cells co-expressing OsBRI-YC and OsBAK1-YN, OsBRI-YC and OsBAK1-ECD-YN, OsBRI-YC and OsBAK1-ICD-YN or OsBRI-YC and OsBAK1-ICDΔ-YN (Figure 2c). In contrast, no fluorescence signal was generated in cells co-expressing OsBRI-YC and YN or OsBAK1-YN and YC, as controls. Thus, the whole protein of OsBAK1, the extracellular domain, the intracellular domain and the truncated intracellular domain all appeared to interact with OsBRI1.

Rice plants overexpressing *OsBAK1* are semi-dwarfed and hypersensitive to 24-epiBL

To assess the function of *OsBAK1* in rice, transgenic rice plants overexpressing *OsBAK1* (*OsBAK1*-OE) were generated. The expression level of *OsBAK1* in the *OsBAK1*-OE was about six times higher than that in wild type (Figure 3b). The *OsBAK1*-OE plants showed typical BR gain-of-function phenotypes, including enlarged lamina joint angle, stunted stature and hypersensitivity to 24-epiBL (Figure 3). The average height of the *OsBAK1*-OE plants was approximately

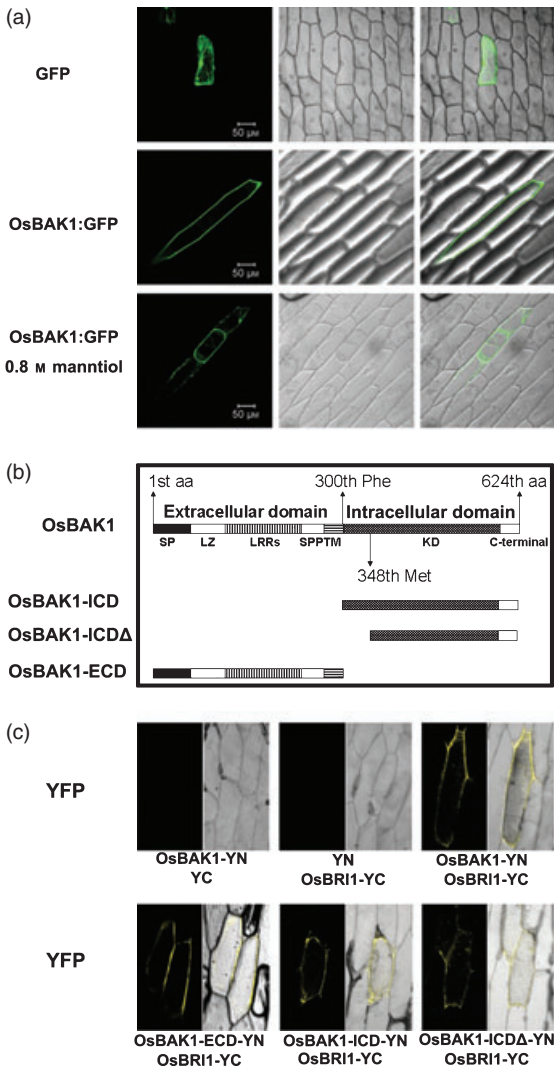

Figure 2 Interaction of OsBAK1 with OsBRI1 *in vivo*. (a) The subcellular localization of OsBAK1 protein in onion epidermis cells. The upper panel shows the 35S::GFP signal alone, which is distributed on the plasma membrane, cytoplasm and nucleus; the middle panel displays the signal from 35S::OsBAK-GFP which is primarily located in the plasma membrane; the lower panel shows 35S::OsBAK-GFP and plasma membrane retracting from the cell wall when onion epidermis cells were treated with 0.8 M mannitol and subsequently plasmolysed. Left line represents green fluorescence signal detected at 488 nm; middle line represents bright field; right line represents the merge of GFP signal and bright field. (b) The structure sketch map of OsBAK1 protein. OsBAK1 encoding 624 amino acid residues and including an extracellular domain (amino acid 1–299) and an intracellular domain (amino acid 300–624). The extracellular domain of OsBAK1 was named OsBAK1-ECD; the intracellular domain of OsBAK1 was named OsBAK1-ICD; a truncated intracellular domain of OsBAK1 (amino acid 348–624) was named OsBAK1- ICDΔ. (c) BiFC assay showed the interaction of OsBAK1 and OsBRI1 *in vivo*. The onion epidermis cells were cotransformated with OsBAK1-YN and YC, OsBRI1-YC and YN, OsBRI1-YC and OsBAK1-YN, OsBRI1-YC and OsBAK1-ECD-YN, OsBRI1-YC and OsBAK1-ICD-YN, and OsBRI1-YC and OsBAK1-ICDΔ-YN. Left line represents yellow fluorescence signal; right line represents the merge of YFP signal and bright field.

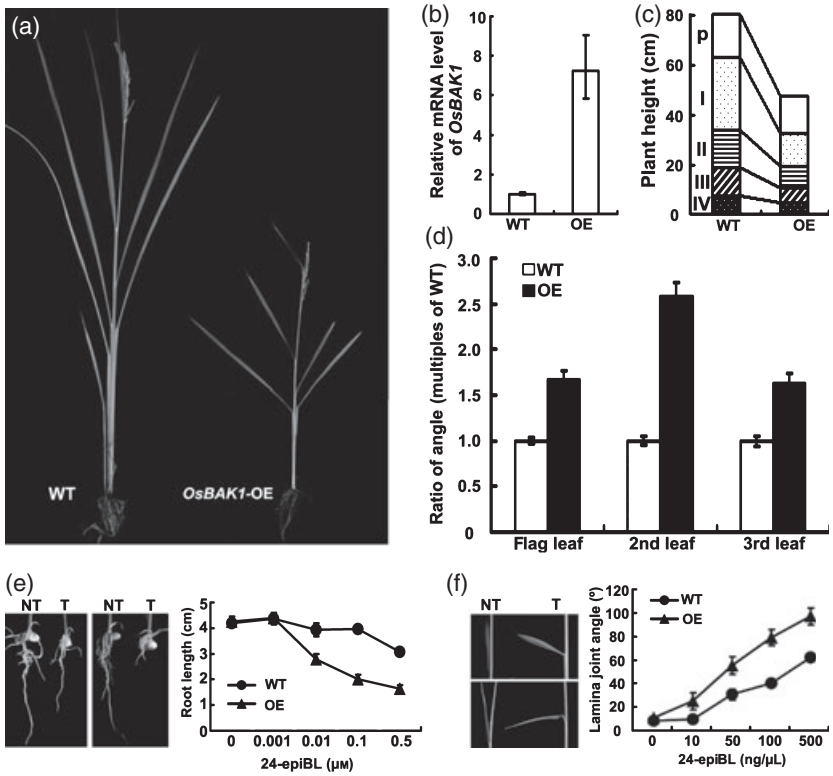

Figure 3 Phenotypes and sensitivities to 24-epiBL of transgenic rice plants overexpressing *OsBAK1* (*OsBAK1*-OE). (a) Mature plant phenotypes of wild-type (WT) Zhonghua 10 and *OsBAK1*-OE. (b) Relative expression level of *OsBAK1* in WT and transgenic plant detected by real-time PCR. (c) Plant height and comparison of internode elongation between WT and *OsBAK1*-OE. P, panicle; I, the first internode under panicle; II, the second internode under panicle; III, the third internode under panicle; IV, the fourth internode under panicle. (d) Relative lamina joint angles of flag leaf, the second and third leaf in heading stage. Data represented the averages of 20 plants. (e) Effect of 24-epiBL on root length. Phenotypes of root length in WT (left) and *OsBAK1*-OE (right) are shown. NT, no 24-epiBL treatment; T, 0.5 μM 24-epiBL treatment. Graph is the curves of root length in wild-type and *OsBAK1*-OE by 24-epiBL dose-dependent fashion. Data represented the averages of 24 plants. Error bars represent SE. (f) Effect of 24-epiBL on lamina joint angle. Phenotypes of lamina joint angle in WT (upper) and *OsBAK1*-OE (lower) are shown. NT, no 24-epiBL treatment; T, 1 μL 24-epiBL (500 ng/μL) treatment. Graph is the curves of lamina joint angle in WT and *OsBAK1*-OE by 24-epiBL dose-dependent fashion. Data represented the averages of 24 plants. Error bars represent SE. OE, transgenic rice lines of *OsBAK1*-OE.

60% that of wild type (Figure 3c). In the heading stage, the degree of lamina joint bending in the *OsBAK1*-OE plants was larger than that of wild-type plants, Zhonghua 10 (Figure 3d) Root elongation inhibition and lamina joint inclination assays were used to examine the sensitivity to 24-epiBL in rice (Figure 3e,f). The root growth curve of *OsBAK1*-OE plants descended faster than that of wild type when treated with 24-epiBL. For example, when the rice seedlings grown on 1/2 MS with 0.5 μM 24-epiBL, the average length of roots was inhibited by 60% in *OsBAK1*-OE plants but only by 30% in wild type. The lamina inclination curve of the *OsBAK1*-OE plants ascended faster than that of wild type by a 24-epiBL dose-dependent means. When the second lamina joints of seedlings were treated with 1 μL 24-epiBL (500 ng/μL) under a light condition, obtuse angles were observed in *OsBAK1*-OE plants but only acute angles in wild-type rice. Thus, the BR signal is enhanced in transgenic rice lines overexpressing *OsBAK1*.

Transgenic plants expressing an antisense RNA construct show a mild BR-insensitive phenotype and are hyposensitive to 24-epiBL

An antisense cDNA (a truncated open reading frame starting at base 376) of *OsBAK1* under the control of the maize ubiquitin1 promoter (Ubi::*OsBAK1*-AS) was transfected into rice. The transgenic plants (*OsBAK1*-AS) were regenerated; and seven independent lines were obtained. Real-time PCR showed the expression of *OsBAK1* reduced by 50% in the *OsBAK1*-AS plants as compared to that of wild-type plants (Figure 4b). In line 2 (AS-2), the expression of *OsBAK1* was reduced greatly and that of *OsSERK2* was reduced moderately, whereas that of *OsSERK3* and *OsSERK4* was reduced slightly (Figure 4c). Unlike the phenotypes of known BR signalling-deficient mutants (e.g. *d61),* the *OsBAK1*-AS transgenic rice plants showed similar height to wild-type plants (Figure 4e) but with erect leaves. We analysed angles

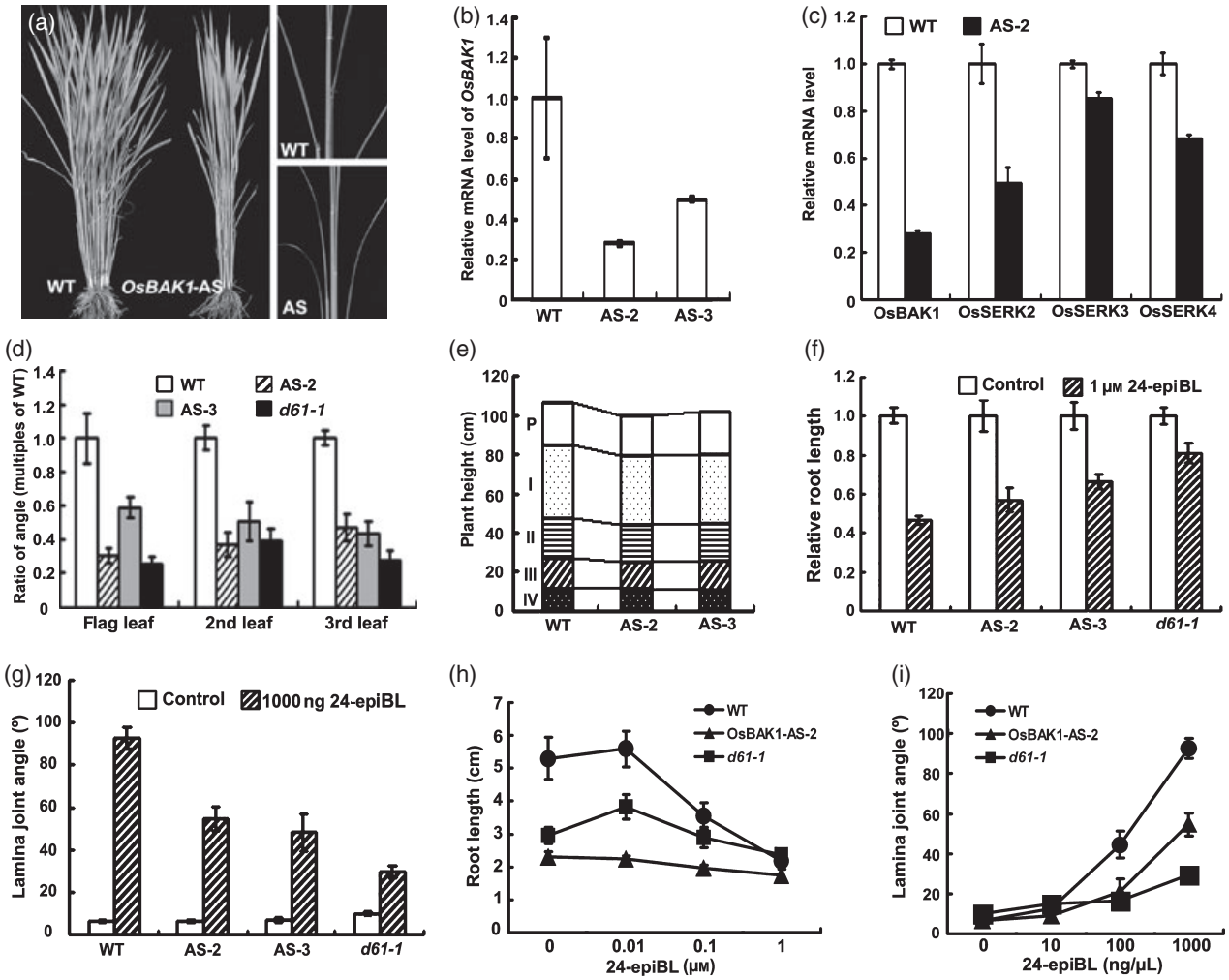

Figure 4 Phenotypes and sensitivities to 24-epiBL of transgenic rice plants suppressing *OsBAK1* by antisense RNA (*OsBAK1*-AS). (a) Mature plant phenotypes of wild-type Zhonghua 10 (WT) and *OsBAK1*-AS in left section. The phenotypes of lamina joint angle of WT and *OsBAK1*-AS in right section. (b) Relative expression level of *OsBAK1* in WT and two transgenic lines of AS-2 and AS-3 detected by real-time PCR. (c) Relative expression level of *OsBAK1* and its homologs (*OsSERK2*, *OsSERK3*, *OsSERK4*) in WT and the transgenic line of AS-2 detected by real-time PCR. (d) Relative lamina joint angles of flag leaf, the second leaf, and third leaf of *OsBAK1*-AS lines in heading stage. WT, wild-type Zhonghua 10; AS-2 and AS-3, two transgenic lines of *OsBAK1*-AS; *d61-1,* a weak rice BR-insensitive mutant. Data represented the averages of 20 plants. (e) Plant height and comparison of internode elongation between WT and *OsBAK1*-AS. P, panicle; I, the first internode under panicle; II, the second internode under panicle; III, the third internode under panicle; IV, the fourth internode under panicle. (f) The histogram of relative root length of transgenic plants under 1 μM 24-epiBL treatment. Plants in this figure are same as in (d). (g) The histogram of lamina joint angle of transgenic rice with 1 μL 24-epiBL (1000 ng/μL) treatment. Plants in this figure are same as in (d). (h) The curves of root length in transgenic plants by BL dose-dependent fashion. WT, wild-type Zhonghua 10; AS-2, a typical transgenic line of *OsBAK1*-AS; *d61-1,* a weak rice BR-insensitive mutant. (i) The curves of lamina joint angles of transgenic plants by BL dose-dependent fashion. Plants in this figure are same as in (h). (f–i) Data represented the averages of 24 plants. Error bars represent SE.

of flag leaves, as well as those of the second leaves and third leaves in *OsBAK1*-AS plants at heading stage (Figure 4d), together with wild-type Zhonghua 10 and the rice BR-insensitive mutant *d61-1*. In the flag leaves, the relative average value of angles in transgenic line AS-2 or AS-3 was about 31% or 59% of that in wild type. The angles of the second or third leaves in *OsBAK1*-AS plants showed the same trend as the flag leaves. Moreover, short sheaths were seen in *OsBAK1*-AS plants (data not shown). Although the *OsBAK1*-AS plants showed only a mild BR signalling-deficient phenotype, the sensitivity of *OsBAK1*-AS plants to 24-epiBL was decreased (Figure 4f–i). The length of roots in the transgenic lines AS-2 and AS-3 was inhibited by 44% and 34%, respectively, when treated with 1 μM 24-epiBL; whereas wild-type roots were inhibited by 54%, and *d61-1* roots by 20%. The 24-epiBL affecting root elongation was dosage dependent. Moderate changes occurred in AS-2, similar to that of *d61-1*. The second lamina joint of seedling

in trefoil stage did not bend under normal conditions. When the lamina joints were treated with 1 μL 24-epiBL (1000 ng/μL), the lamina joint bending angle reached approximately 93°, 55°, 48°and 30° in wild type, AS-2, AS-3 and *d61-1*, respectively. Similarly, the concentration curves of 24-epiBL in lamina joint bending showed reduced change in AS-2 compared to wild type. Thus, suppression of *OsBAK1* by expressing antisense RNA reduces the BR response in rice.

Overexpression of *OsBAK1* rescues the rice BR-insensitive mutant *d61-1*

To further confirm the function of *OsBAK1* in BR signalling, we performed a suppression experiment by introducing the full length cDNA of *OsBAK1* driven by a maize ubiquitin promoter into the weak rice BR-insensitive mutant *d61-1*. In transgenic *d61-1*, the expression of *OsBAK1* was increased to about nine times that of *d61-1* (Figure 5b). Compared to controls, either *d61-1* or *d61-1* transformed with an empty vector, *d61-1* expressing *OsBAK1* showed a suppressed erect-leaf phenotype, which was the best characterized BR response in rice (Figure 5a). Real-time PCR showed that the decreased expression patterns of *OsBRI1*, *OsBZR1* and *OsD2* occurred in the *d61-1* expressing *OsBAK1* (Figure 5c), suggesting a feedback-regulation.

BR signalling is inhibited by overexpressing of a truncated intracellular but not the extracellular domain of OsBAK1

As shown in Figure 2b, OsBAK1 is a putative transmembrane protein containing an extracellular domain (OsBAK1-ECD) and an intracellular domain (OsBAK1-ICD). OsBAK1-ECD (amino acid 1–299) shares several characteristic features of the SERK family, such as a signal peptide, a leucine zipper motif (LZ), five tandem LRRs, and an SPP motif. OsBAK1-ICD (amino acid 300–624) consists of a kinase domain and a carboxy-terminal region. OsBAK1-ICDΔ, a truncated intracellular domain (amino acid 348–624), contains only an incomplete kinase domain and a carboxy-terminal region.

We overexpressed OsBAK1-ECD or OsBAK1-ICDΔ in rice. The expression levels of *OsBAK1* in the transgenic line OsBAK1-ICDΔ-1 or OsBAK1-ICDΔ-5 were inhibited by 80%–90%, respectively (Figure 6c), which was greatly different from that in *OsBAK1*-AS transgenic rice lines (about 50%, see Figure 4b). The expression of *OsBAK1* in transgenic line OsBAK1-ECD-2 or OsBAK1-ECD-3 was induced by two to three times that of wild-type plants (Figure 6d). OsBAK1-ECD and OsBAK1-ICDΔ plants showed contrasting phenotypes (Figure 6). At seedling stage, OsBAK1-ICDΔ but not OsBAK1-ECD plants showed erect leaves (data not shown). At mature stage, OsBAK1-ICDΔ plants were dwarfed (Figure 6e). Their leaves became erect (Figure 6g). Meanwhile OsBAK1-ECD plants showed a phenotype similar to a wild-type plant (Figure 6f,h). Moreover either OsBAK1-ICDΔ or OsBAK1-ECD plants showed different sensitivities to 24-epiBL (Figure 7). When treated with 1 μM 24-epiBL, the average length of roots in OsBAK1-ICDΔ plants was reduced by 30%–40%, lower than the inhibition ratio for OsBAK1-ECD and wild type, which is about 50%. The root growth curve of OsBAK1-ICDΔ was close to that of *d61-1*. The root growth curve of OsBAK1-ECD plants was similar to that of wild type. A similar trend in lamina joint bending occurred. In addition, the expression patterns of *OsBRI1* and *OsBZR1* in the OsBAK1 transgenic rice lines match the phenotypes of the transgenic rice lines (Supplement 3). Compared to wild type, the expression levels of *OsBRI1* and *OsBZR1* were up-regulated in the transgenic lines *OsBAK1*-AS or

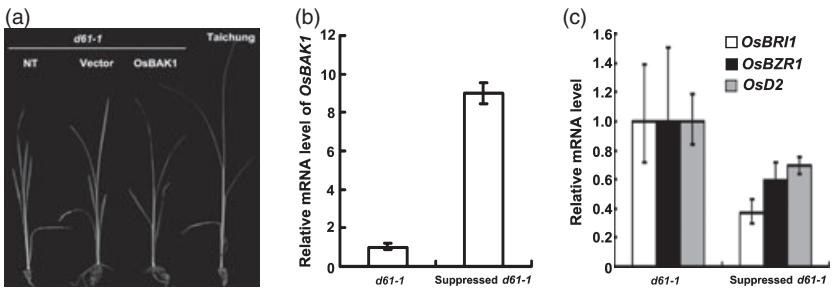

Figure 5 Suppression of the *d61-1* mutant by introducing *OsBAK1*. (a) Phenotypes of the *d61-1* mutant and transgenic lines, as well as Taichung (the background of *d61-1* mutant). NT, *d61-1* mutant; vector, *d61-1* mutant expressing vacant vector; OsBAK1, suppressed *d61-1* mutant by expressing *OsBAK1*. (b) The relative expression level of *OsBAK1* in the *d61-1* mutant and the suppressed *d61-1* mutant detected by real-time PCR. (c). The relative expression level of *OsBRI1*, *OsBZR1* and *OsD2* in the *d61-1* mutant and the suppressed *d61-1* mutant detected by real-time PCR.

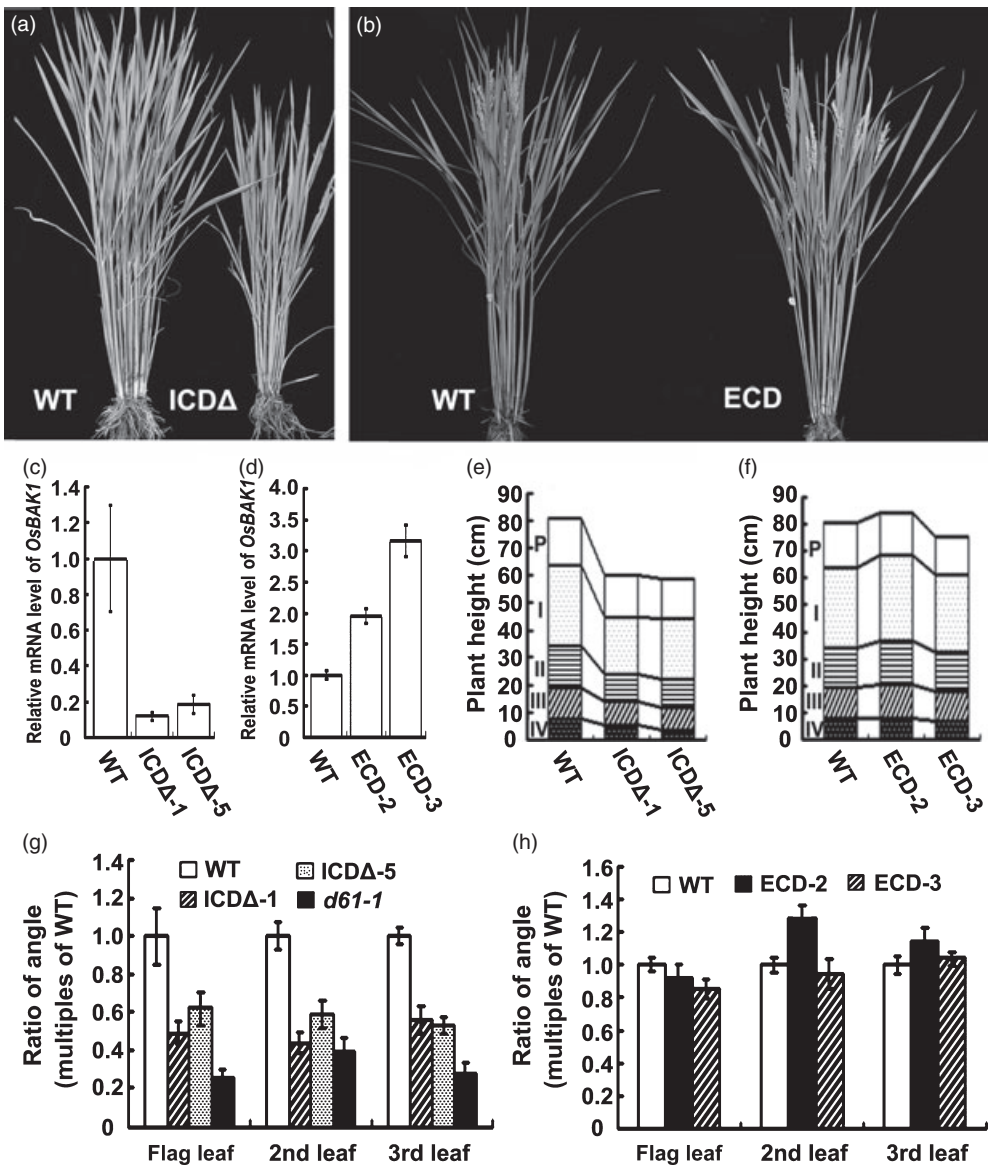

Figure 6 Overexpressing the truncated intracellular domain but not the extracellular domain of OsBAK1 results in dominant negative phenotypes in transgenic rice plants. (a and b) Phenotypes of OsBAK1-ICDΔ transgenic lines (a) and OsBAK1-ECD transgenic lines (b). (c and d). The relative expression level of *OsBAK1* in OsBAK1-ICDΔ transgenic lines (c) and OsBAK1-ECD transgenic lines (d) detected by real-time PCR. (e and f). Plant height at mature stage and comparison of internode elongation of OsBAK1-ICDΔ transgenic lines (e) and OsBAK1-ECD transgenic lines (f). P, panicle; I, the first internode under panicle; II, the second internode under panicle; III, the third internode under panicle; IV, the fourth internode under panicle. Data represented the averages of 20 plants. (g and h). The relative lamina joint angle of OsBAK1-ICDΔ transgenic lines (g) and the OsBAK1-ECD transgenic lines (h) in heading stage. Data represented the averages of 20 plants. ICDΔ-1 and ICDΔ-5, two random-selected transgenic lines of OsBAK1-ICDΔ; ECD-2 and ECD-3, two random-selected transgenic lines of OsBAK1-ECD. Zhonghua 10, wild type as control; *d61-1*, a weak rice BR-insensitive mutant.

OsBAK1-ICDΔ and down-regulated in the *OsBAK1*-OE plants. The expression showed no changes in the OsBAK1-ECD plants (Supplement 3). This result indicated that the BR pathway was blocked in *OsBAK1*-AS or OsBAK1-ICDΔ transgenic plants but activated in *OsBAK1*-OE lines. These results suggested that overexpression of OsBAK1-ICDΔ instead of OsBAK1-ECD could suppress BR signalling.

Modification endogenous expression levels of *OsBAK1* alters agricultural traits in rice

Both *OsBAK1*-OE and OsBAK1-ICDΔ plants showed a similar dwarfed phenotype. But the molecular mechanism leading the phenotype is different. Longitudinal sections of the upper second and third internodes stained with

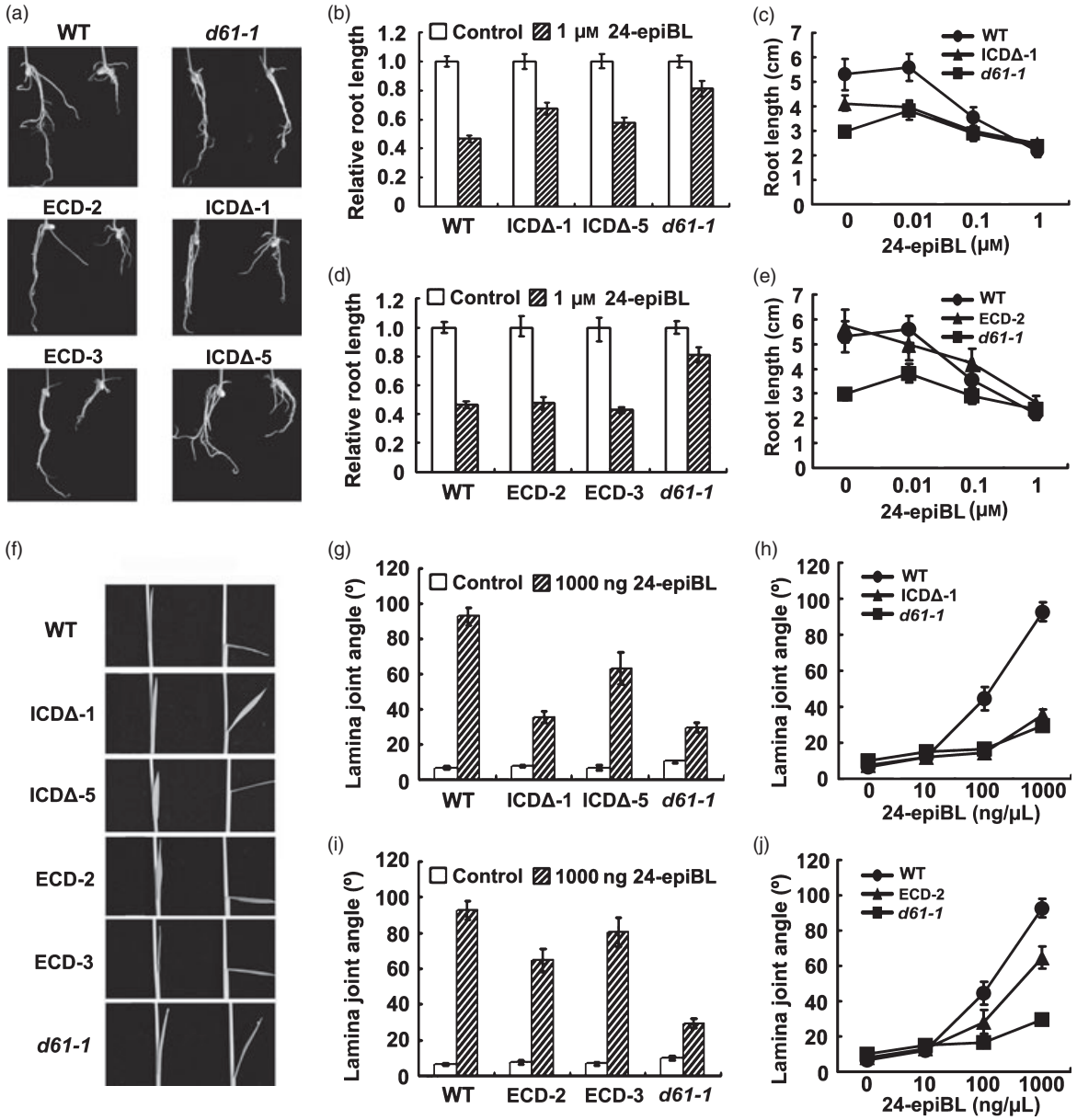

Figure 7 Overexpressing the truncated intracellular domain but not the extracellular domain of OsBAK1 results in the insensitivity to 24-epiBL of transgenic rice plants. (a–e). Root responses to 24-epiBL in OsBAK1-ICDΔ transgenic lines and OsBAK1-ECD transgenic lines. (a) The root phenotype of transgenic rice with (right) or without (left) 1 μM 24-epiBL treatment. (b) The histogram of relative root length of OsBAK1-ICDΔ transgenic lines with 1 μM 24-epiBL treatment. (c) The curve of root length of OsBAK1-ICDΔ transgenic line in BL dose-dependent fashion. (d) The histogram of relative root length of OsBAK1-ECD transgenic lines with 1 μM 24-epiBL treatment. (e) The curve of root length of OsBAK1-ECD transgenic line in BL dose-dependent fashion. (f–j) Lamina joint bending test in OsBAK1-ICDΔ transgenic lines and OsBAK1-ECD transgenic lines. (f) The second lamina joint of seedling with (right) or without (left) 1 μL 24 -epiBL (1000 ng/μL) treatment. (g) The histogram of lamina joint bending of OsBAK1-ICDΔ transgenic lines with 1 μL 24-epiBL (1000 ng/μL) treatment. (h) The curve of lamina joint bending of OsBAK1-ICDΔtransgenic line in BL dose-dependent fashion. (i) The histogram of lamina joint bending of OsBAK1-ECD transgenic lines with 1 μL 24-epiBL (1000 ng/μL) treatment. (j) The curve of lamina joint bending of OsBAK1-ECD transgenic line in BL dose-dependent fashion. Wild-type Zhonghua 10 was used as control. ICDΔ-1 and ICDΔ-5, two random-selected transgenic lines of OsBAK1-ICDΔ; ECD-2 and ECD-3, two random-selected transgenic lines of OsBAK1-ECD. *d61-1*, a weak rice BR-insensitive mutant. Data represented the averages of 24 plants. Error bars represent SE.

propidium iodide (PI) showed a similar trend of cell elongation (Figure 8). The cell length of the OsBAK1-ICDΔ plant was shortened, compared to that of wild-type plants, but was not obviously altered in *OsBAK1*-OE plants.

OsBAK1 expression level also can control grain shape and yield (Figure 9, Table 1). Statistical analysis of seed size in *OsBAK1* transgenic plants demonstrated the gradations in either length or width. The gradation of seed length

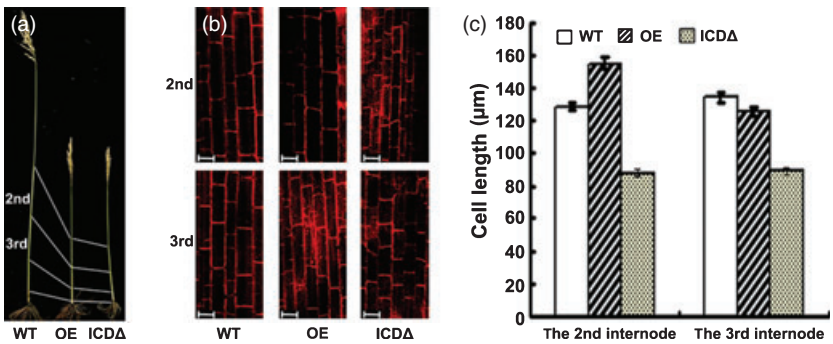

Figure 8 Analysis of dwarfed phenotype in transgenic lines *OsBAK1*-OE and OsBAK1-ICDΔ. (a) Plant height of wild-type Zhonghua 10, *OsBAK1*-OE (OE) and OsBAK1-ICDΔ (ICDΔ). (b) Cell morphology of the second internode (second) and the third internode (third) under panicle in mature stems of transgenic rice plants. Scale bars: 50 μm. (c) Quantitative analysis of cell length in the second internode and the third internode under the panicle. Error bars represent SE.

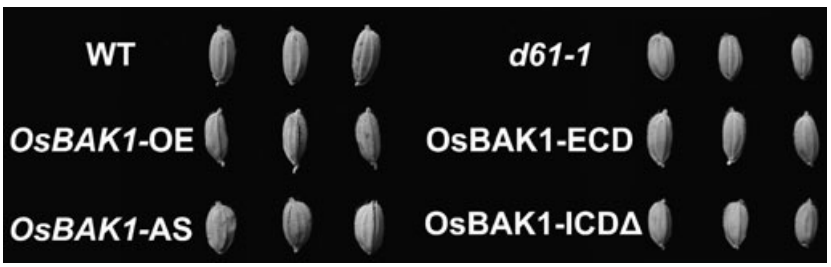

Figure 9 Grain morphology of the OsBAK1 transgenic rice plants. WT, Wild-type Zhonghua 10; *d61-1*, a weak rice BR-insensitive mutant; *OsBAK1*-OE, transgenic lines overexpressing *OsBAK1*; *OsBAK1*-AS, transgenic lines suppressing *OsBAK1* by antisense RNA; OsBAK1-ICDΔ, transgenic lines overexpressing the truncated intracellular domain of *OsBAK1*; OsBAK1-ECD, transgenic lines overexpressing the extracellular domain of *OsBAK1*.

from large to small was wild type (0.494 cm), OsBAK1-ECD (0.481 cm), *OsBAK1*-AS (0.457 cm), *OsBAK1*-OE (0.448 cm), OsBAK1-ICDΔ (0.414 cm), and *d61-1* mutant (0.397 cm) plants, respectively. In contrast, the gradation of seed width from large to small is the *OsBAK1*-AS (0.267 cm), wild type (0.254 cm), OsBAK1-ECD (0.240 cm), *d61-1* (0.230 cm), *OsBAK1*-OE (0.223 cm), and OsBAK1-ICDΔ (0.215 cm) plants, respectively. The seed size of OsBAK1-ICDΔ plant was the smallest; and that of *OsBAK1*-OE plant was the next largest, and that of OsBAK1-ECD plant was almost same as that of wild type. The seed shape of *OsBAK1*-AS plants becomes shorter and broader (Figure 9). Moreover, the seed weight of the *OsBAK1*-AS lines was 2.53 g per 100 seeds, close to that of wild-type or OsBAK1-ECD plants (2.64 g per 100 seeds). Corresponding to seed size, seed weight was decreased in *OsBAK1*-OE plants (2.23 g per 100 seeds) and OsBAK1-ICDΔ plants (1.90 g per 100 seeds); the latter seed weight did not obviously differ from that of *d61-1* (1.99 g per 100 seeds). The seed weight did not decrease, despite the change of grain shape in *OsBAK1*-AS plants, as compared to wild type. These findings, combined with the erect-leaf phenotype, suggest that the slightly reducing the expression levels of *OsBAK1* could be a novel strategy to improve rice yield.

Discussion

In this study, we have shown that *OsBAK1* and its homologs are involved in BR signalling. Overexpression of *OsBAK1* can partially rescue the *Arabidopsis* weak BR receptor mutant *bri1-5*. Our extensive studies, including *in vivo* physical interactions between *OsBAK1* and *OsBRI1*, gain-/loss-of-function genetic analysis, and suppressing defective phenotypes of *d61-1* by *OsBAK1*, all support that *OsBAK1* plays a conservative function in rice BR signalling. Our results also reveal that BR signalling was inhibited by the truncated intracellular domain but not by the extracellular domains of OsBAK1. The *OsBAK1* gene modifies the rice architecture by regulating plant height, lamina joint bending, and grain size. Therefore, manipulating the expression level of *OsBAK1* is a novel strategy for rice molecular breeding.

Table 1 Morphological characteristics of seeds

	WT	*OsBAK1*-OE	*OsBAK1*-AS	OsBAK1-ICDΔ	OsBAK1-ECD	*d61-1*
Width of seed (cm)*	0.254 ± 0.003	0.223 ± 0.010	0.267 ± 0.003	0.215 ± 0.003	0.240 ± 0.003	0.230 ± 0.004
Length of seed (cm)*	0.494 ± 0.004	0.448 ± 0.006	0.457 ± 0.004	0.414 ± 0.006	0.481 ± 0.005	0.397 ± 0.005
Weight of 100 seeds (g)†	2.642 ± 0.003	2.225 ± 0.005	2.527 ± 0.024	1.896 ± 0.014	2.643 ± 0.027	1.985 ± 0.105

*Data represent the mean ± SE of 20 seeds in each kind of plant.
†Data represent the mean ± SE of 3 × 100 seeds.

OsSERK1/OsBAK1 plays a conserved function with *AtBAK1*

BAK1, also named as *AtSERK3*, is the co-receptor of *BRI1*. *SERKs* are distributed widely in dicots, monocots and gymnosperms (Lin *et al.*, 2007). *AtSERKs* consist of five receptor kinases, *AtSERK1∼5,* which are co-receptors to regulate extracellular multiple signal transduction (Albrecht *et al.*, 2008). The four closely related rice homologs of *AtBAK1* also belong to a *SERK* family, named as *OsSERK1–4*. They have conserved protein structures with AtSERKs (Supplement 2). Moreover all of OsSERKs have an activation loop in the kinase domain and a phosphorylation site of Thr residue within the activation loop, which is necessary for *AtSERK1* phosphorylation (Shah *et al.*, 2001) and *AtBAK1* activation (Wang *et al.*, 2008b). The conserved structure of both *OsSERKs* and *AtSERKs* suggested conserved function of both. Our results revealed that *OsSERKs*, like *AtBAK1/AtSERK3*, are involved in BR signalling. In addition, AtBAK1 showed the highest sequence identity with OsSERK1 (77%) and OsSERK2 (76%) in rice, whereas AtBAK1 has a higher homology with other AtSERKs than either OsSERKs (Supplement 2), like the relationship of AtBZR1 and OsBZR1 (Bai *et al.*, 2007). Phylogenetic tree showed *OsSERK1* and *OsSERK2* are co-orthologs of *AtSERK1* and *AtSERK2*, rather than *AtBAK1* (*AtSERK3*) (Supplement 1). Therefore it was suggested that the components of BR signalling generated gene duplication and redifferentiation of gene function after separation of dicots and monocots during evolution.

Consistent with the gradation of their sequence similarity to *AtBAK1, OsSERK1* showed the strongest *bri1-5* suppression, followed by *OsSERK2*, *OsSERK3,* and *OsSERK4*. So *OsSERK1/OsBAK1* is the closest relative of *AtBAK1* in rice on the basis of phylogenetic and functional analysis. Our results further confirmed that *OsSERK1/OsBAK1* plays a conserved function with *AtBAK1* in rice. First, OsBAK1 is mainly localized on the plasma membrane and interacts with OsBRI1 *in vivo* (Figure 2), which is similar to AtBAK1. Second, BR signalling is enhanced in transgenic rice by either overexpression of *OsBAK1* (Figure 3) or overexpression of *AtBAK1* in rice plants (Wang *et al.*, 2007). Third, suppressing *OsBAK1* in rice by antisense RNA resulted in a mild BR-insensitive phenotype and a hyposensitivity to 24-epi-BL in rice (Figure 4). In addition, *OsBRI1* and *OsBZR1*, two components of the rice BR signalling pathway, were down-regulated or up-regulated in transgenic rice plants corresponding to the overexpression or suppression of *OsBAK1* (Supplement 3). Finally, overexpression of *OsBAK1* can rescue the rice *OsBRI1* mutant *d61-1* (Figure 5). These data support that *OsBAK1* plays a conserved function with *AtBAK1* in BR signalling.

Expression of OsBAK1-ICDΔ but not OsBAK1-ECD causes the dominant negative phenotypes in transgenic rice plants

In *Arabidopsis*, BR signalling transduction starts with two transmembrane receptors, BRI1 and BAK1. Binding BRs to BRI1 activates BRI1, the activated BRI1 can associate with BAK1 and transphosphorylate the residues in the activation loop and activate BAK1. The activated BAK1 then can phosphorylate BRI1 on its justamembrance and C-terminus domain, which are prerequisites for the formation of a functional BRI1/BAK1 complex and initiate the downstream signalling pathway. (Wang *et al.*, 2008b). Undoubtedly, the intracellular domain of BAK1 is important for the activation of BR signalling. Unlike BRI1, the extracellular domain has only five LRRs and no BR binding domain. So the extracellular domain of BAK1 may not bind BRs, and its function remains to be determined.

The OsBAK1-ECD plants showed no changes in morphology or physiologic features or molecular level, whereas the OsBAK1-ICDΔ plants showed severe BR-insensitive phenotypes, including dwarfism, erect leaves and sterility. Both the decreased BR sensitivity (Figure 7) and the up-regulation of *OsBRI1* and *OsBZR1* (Supplement 3) suggests that BR signalling is blocked in the OsBAK1-ICDΔ plants. Therefore, the intracellular

domain of OsBAK1 is pivotal to BR signalling. We speculate that OsBAK1-ICDΔ, as inactivated OsBAK1-ICD, competes with endogenous OsBAK1 for its interaction with OsBRI1. Once the dysfunctional OsBAK1-ICDΔ interacts with OsBRI1, it can interfere with the function of OsBRI1 and block the BR signalling pathway. Overexpression of OsBAK1-ECD has no similar effect in rice. The most logical explanation is that OsBAK1-ECD does not interact with the extracellular domain of OsBRI1 *in vivo*. Therefore, overexpression of OsBAK1-ECD cannot create a dominant negative phenotype. But our truncated experiments indicated that the extracellular domain of OsBAK1 can interact with OsBRI1 (Figure 2). Another explanation could be that the truncated version would be degraded in the endoplasmic reticulum (ER), as an ER quality control (ERQC) system may be initiated in plant cells (Jin *et al.*, 2007; Hong *et al.*, 2008). Although the extracellular domain of BAK1 is overexpressed in the OsBAK1-ECD plant at the transcriptional level, these imperfect proteins of OsBAK1-ECD may be retained and degraded in the ER and have no chance to be transmitted to the cell membrane and suppress BR signalling.

The modification of *OsBAK1* as a potential molecular breeding tool

Among ideal rice plant architectures for high yield, dwarfism and erect leaves are two most valuable traits modulated by BRs (Wang and Li, 2008). Our results showed that the regulation of *OsBAK1* expression levels can alter plant architecture in rice. The regulation of plant height by *OsBAK1* appears to be complicated. Although *OsBAK1*-OE plants and OsBAK1-ICDΔ plants showed similar dwarfed phenotype, the molecular mechanism causing the dwarfism, however, is different. The cells are short in OsBAK1-ICDΔ plants but remain the same size in *OsBAK1*-OE plants (Figure 8). *OsBAK1*-OE plants are not deficient in cell elongation, and the dwarfism probably results from insufficiency of cell division. Our previous studies also supported that enhanced BR signalling can result in a rice dwarf phenotype derived from the deficiency of cell division (Wang *et al.*, 2007, 2008a). Dwarfed OsBAK1-ICDΔ plants resulted from the deficiency in cell elongation, as in other rice BR-deficient or -insensitive mutants (Yamamuro *et al.*, 2000; Hong *et al.*, 2002, 2003, 2005).

Studies of BR-related mutants suggested that the combination of erect leaves and dense planting can improve rice grain yield (Morinaka *et al.*, 2006; Sakamoto *et al.*, 2006). To increase crop yield in breeding, BR signalling components are probably potential targets for genetic modification. *OsBRI1* is the receptor of BRs and the most crucial factor in BR signalling. The suppression of *OsBRI1* in rice resulted in not only leaf erectness, but also malformed leaves, flowers, and grains. In contrast, OsBAK1, as the co-receptor of OsBRI1, has a milder effect than OsBRI1 in the rice developmental process. The expression of *OsBAK1* in the transgenic rice lines was regulated. Except for the OsBAK1-ECD plants, three other kinds of transgenic rice lines, including *OsBAK1*-OE, *OsBAK1*-AS and OsBAK1-ICDΔ plants, have shown a BR-related phenotype. *OsBAK1*-OE plants showed a dwarfed phenotype, inclined leaves and appreciably small grains, whereas OsBAK1-ICDΔ plants bore a more severe BR-insensitive phenotype, with the smallest seeds and lowest grain yield of all OsBAK1 transgenic plants. They are difficult to improve grain yield. In contrast, the *OsBAK1*-AS plants, a desired weak BR-insensitive mutant with erect leaves and normal reproduction, may be a potential line with increased production. The normal fertility and grain weight ensured the yield per plant. The erect leaves can improve light capture for photosynthesis and produce a high leaf area index in dense plantings. Because of these advantages, the *OsBAK1*-AS transgenic rice line could be a new elite transgenic rice to improve grain yield by dense planting.

In summary, we identified the closest rice relative of *AtBAK*, *OsBAK1*, in this study. Functional analysis of *OsBAK1* completed the molecular mechanism of BR signalling in rice and demonstrates that *BAK1* is conserved as the co-receptor of *BRI1* in BR signalling. The identification of *OsBAK1* will open the door to explore the multiple signal regulation network of hormone responses and plant development in rice. Moreover, the regulation of *OsBAK1* expression can produce the ideal erect-leaf rice without decreased fertility. Therefore, *OsBAK1* could be used for a new strategy to improve rice plant architecture for high yield.

Experimental procedures

Plant materials and growth conditions

Wild-type *Arabidopsis thaliana* (Ws-2), *bri1-5* mutant plants and transgenic plants derived from *bri1-5* were grown in a growth chamber at 25°C day/22°C night cycles under 16-h-light/8-h-dark photoperiods. Rice plants (*Oryza sativa*) include two kinds of ecotype: Zhonghua10 and Taichung, a *OsBRI1* mutant of *d61-1*

derived from Taichung, as well as transgenic plants derived from Zhonghua10 or *d61-1*. They were grown in the field or in the greenhouse at 28°C day/25°C night cycles.

Gene cloning and *Arabidopsis* transformation

*OsSERK*s, including *OsSERK1–4*, were amplified by PCR with use of Pyrobest™ DNA polymerase (Takara, Japan) as follows: preheating at 94°C for 2 min, then 38 cycles of denaturation at 94°C for 10 s, annealing at 58–61°C for 30 s and extension at 72°C for 2 min, followed by a final extension at 72°C for 10 min. The PCR products of *OsSERK1/OsBAK1, OsSERK2, OsSERK3* and *OsSERK4* were cloned into *Sma*I/*Kpn*I, *Xba*I/*Kpn*I, *Kpn*I/*Sac*I, and *Kpn*I/*Sac*I sites of the pBIB-BASTA vector, respectively (Becker *et al.*, 1992). These constructs were confirmed by sequencing and then were transformed into a weak BR-insensitive *Arabidopsis* mutant, *bri1-5*, by floral dipping *Agrobacterium*-mediated transformation (Clough and Bent, 1998).

Arabidopsis hypocotyl assay

All transgenic seeds derived from pure transgenic lines were surface sterilized and placed on half-strength MS medium, together with controls. Seeds were kept at 4°C for 2 days and then generated at 25°C in dark for 2 days. Synchronously germinated seedlings were selected to culture on 1/2 MS with or without BR biosynthetic inhibitor BRZ at 25°C in dark. The hypocotyl lengths of dark-grown seedlings were measured 4 days later. Each experiment was performed in duplicate.

Vector construction and rice transformation

The whole open reading frame (ORF) of *OsBAK1*, the extracellular domain of *OsBAK1* (encoding amino acid 1–299) and the truncated intracellular domain of *OsBAK1* (encoding amino acid 348–624) were cloned into the pUN1301 expression vector in sense orientation at the *Sma*I/*Kpn*I sites, the *Bam*HI/*Kpn*I sites and the *Bgl*II/*Kpn*I sites. A truncated ORF of *OsBAK1* starting at base 376 was cloned into the pUN1301 expression vector in antisense orientation at the *Kpn*I/*Sac*I sites. The pUN1301 expression vector contains a high efficiency ubiquitin promoter and two markers of transgenic plant screening, GUS (β-glucuronidase) and hygromycin. These constructs were individually transformed into embryonic calli *O. sativa* cv Zhonghua 10 by *Agrobacterium*-EHA105 mediation as described previously (Ge *et al.*, 2004).

Yield-related rice architecture parameters

The rice plants examined were grown in field in normal rice growing seasons. At the heading stage, angles of flag leaves and angles of the second or third leaves under flag leaves were measured in all transgenic lines and controls. Twenty plants were used for each sample. Relative values (the mean angle of transgenic line /the mean angle of wild type) were used. At mature stage, plant height, including lengths of panicles and internodes, was measured in all transgenic lines and controls. The sample still included 20 plants. The parameters related to seeds, such as seed width, seed length and 100-seed weight, were measured. A sample included 20 above seeds in measuring width or length of seeds. The measurement of 100-seed weight was repeated three times.

Root elongation inhibition assay in rice

Rice seeds were surface sterilized by a combination of 75% ethanol for 5 min and 0.1% $HgCl_2$ for 10 min. After rinsing the seeds several times, seeds were soaked in distilled water for 2 days in the dark at 30°C. Synchronously germinated seeds were selected and cultivated on half-strength MS medium supplemented with or without concentrations of 24-epiBL at 28°C for 8 days. The lengths of the primary roots were measured, and the seedlings were photographed; 24 plants were measured for each treatment. This experiment was performed in duplicate. Our previous study was followed for root elongation inhibition assay (Duan *et al.*, 2006).

Lamina joint inclination assay in rice

The rice lamina joint inclination bioassay was performed as described (Bai *et al.*, 2007). Germinated seeds with uniform coleoptile length were sowed in soil and cultured in the greenhouse at 28°C for 3 days. One microlitre of ethanol containing 0, 10, 100, 500 or 1000 ng of 24-epiBL and 0.1% Triton X-100 were placed at the top of the lamina of wild-type and transgenic plants. After incubation for 3 days, the angles between the lamina and leaf sheath were photographed and measured; 24 plants were measured for each treatment. This experiment was performed in duplicate.

Suppression of the *d61-1* mutant

An entire ORF of *OsBAK1* driven by the ubiquitin promoter in the binary vector pUN1301 was introduced into the weak BR-insensitive mutant *d61-1* by *Agrobacterium*-mediated transformation. Transgenic plants, *d61-1* and Taichung were cultured in a greenhouse at 28°C for a month. To evaluating the *d61-1* mutant overexpressing *OsBAK1*, the expression of marker genes in the BR pathway was detected by real-time PCR.

Laser scanning confocal microscopy

Transgenic rice and wild-type Zhonghua 10 rice were grown in fields until maturity. To investigate transgenic rice dwarfism, cell size in the second or third internodes of stems under panicles by PI staining was observed by laser scanning confocal microscopy at 514 nm excitation wavelength (Wang *et al.*, 2007). Images were captured and processed by use of LSM5 Images Browser (Zeiss, Oberkochen, Germany). Individual data on cell size represent >100 cells from nine internodes from three different plants.

Quantitative real-time PCR and semiquantitative RT-PCR

Total RNA from rice seedlings or *Arabidopsis* seedlings was isolated using Trizol Reagent (Invitrogen, California, USA), and then reverse transcribed by use of Superscript reverse transcriptase-II (Invitrogen, California, USA) as per the manufacturer's instruction. Real-time PCR was used to identify transgenic plants and detect expression patterns of marker genes in transgenic plants. The cDNA samples were diluted 50 times. Triplicate quantitative assays were performed with 5 μL of each cDNA dilution by use of the SYBR Green Master mix (TOYOBO, Shanghai, China) in an Stratagene MX3000P™ system, following the manufacturer's protocol (TOYOBO, Shanghai, China). The relative quantification method (Δ–ΔC_t) was used. Real-time PCR primers were designed with PrimerExpress 2.0. Every experiment was performed in duplicate. In *Arabidopsis*, the amplification of *PP2A* was used as an internal control to normalize data, and in rice, *Actin* was used. The ectopic expression of *OsSERK1–4* was detected in transgenic plants derived from *bri1-5* by semiquantitative RT-PCR. The amplification of *PP2A* was used as an internal control.

Subcellular localization of OsBAK1

The subcellular localization of OsBAK1 was as described (Wang *et al.*, 2008a). The ORF cDNA of *OsBAK1* without the termination code was amplified and subcloned to the N terminus of green fluorescent protein (GFP) in the pBI221 vector. The 35S::OsBAK1-GFP construct vector was bombarded into onion epidermis cells, together with the pBI221 vector, by the Bio-Rad biolistic system (Hercules, CA, USA). After onion epidermis cells were cultured for 1 day, the GFP signals in epidermis cells were visualized at 488 nm excitation wavelength by use of a laser scanning confocal microscope (LSM 510; Zeiss, Oberkochen, Germany).

Bimolecular fluorescence complementation assay

Bimolecular fluorescence complementation assay (Walter *et al.*, 2004; Han *et al.*, 2008) was used to confirm whether OsBAK1, OsBAK1-ECD, OsBAK1-ICD, or OsBAK1-ICDΔ interacted with OsBRI1. The cDNA without the termination code of OsBAK1, OsBAK1-ECD, OsBAK1-ICD, or OsBAK1-ICDΔ was fused to pUC-SPYNE (YN) in-frame. In the same way, the cDNA without the termination code of OsBRI1 was fused to pUC-SPYCE (YC). The OsBRI1-YC construct vector and the OsBAK1-YN, OsBAK1-ECD-YN, OsBAK1-ICD-YN, or OsBAK1-ICDΔ-YN construct vector were co-transformed into onion epidermis cells by use of the Bio-Rad biolistic system (Hercules, CA, USA). Similarly, OsBRI1-YC construct vector and pUC-SPYNE, pUC-SPYCE and OsBAK1-YN construct vector all were bombarded into onion epidermis cells as controls. After 24 h, YFP signals were observed on laser scanning confocal microscopy (LSM 510, Zeiss, Oberkochen, Germany).

Statistical analysis

In this study, all statistical analyses involved use of Excel software and ImageJ software. Significant differences were determined by Student *t* test. Error bars represent SE.

All primer sequences are listed in Supplement 4.

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References

Albrecht, C., Russinova, E., Kemmerling, B., Kwaaitaal, M. and de Vries, S.C. (2008) Arabidopsis somatic embryogenesis receptor kinase proteins serve brassinosteroid-dependent and -independent signaling pathways. *Plant Physiol.*, **148**, 611–619.

Bai, M.Y., Zhang, L.Y., Gampala, S.S., Zhu, S.W., Song, W.Y., Chong, K. and Wang, Z.Y. (2007) Functions of OsBZR1 and 14-3-3 proteins in brassinosteroid signaling in rice. *Proc. Natl Acad. Sci. USA*, **104**, 13839–13844.

Becker, D., Kemper, E., Schell, J. and Masterson, R. (1992) New plant binary vectors with selectable markers located proximal to the left T-DNA border. *Plant Mol. Biol.*, **20**, 1195–1197.

Chinchilla, D., Zipfel, C., Robatzek, S., Kemmerling, B., Nurnberger, T., Jones, J.D., Felix, G. and Boller, T. (2007) A flagellin-induced complex of the receptor FLS2 and BAK1 initiates plant defence. *Nature*, **448**, 497–500.

Clough, S.J. and Bent, A.F. (1998) Floral dip: a simplified method for Agrobacterium-mediated transformation of Arabidopsis thaliana. *Plant J.*, **16**, 735–743.

Duan, K., Li, L., Hu, P., Xu, S.P., Xu, Z.H. and Xue, H.W. (2006) A brassinolide-suppressed rice MADS-box transcription factor, OsMDP1, has a negative regulatory role in BR signaling. *Plant J.*, **47**, 519–531.

Friedrichsen, D. and Chory, J. (2001) Steroid signaling in plants: from the cell surface to the nucleus. *BioEssays*, **23**, 1028–1036.

Ge, L., Chen, H., Jiang, J.F., Zhao, Y., Xu, M.L., Xu, Y.Y., Tan, K.H., Xu, Z.H. and Chong, K. (2004) Overexpression of OsRAA1 causes pleiotropic phenotypes in transgenic rice plants, including altered leaf, flower, and root development and root response to gravity. *Plant Physiol.*, **135**, 1502–1513.

Gendron, J.M. and Wang, Z.Y. (2007) Multiple mechanisms modulate brassinosteroid signaling. *Curr. Opin. Plant Biol.*, **10**, 436–441.

Han, Y., Cao, H., Jiang, J., Xu, Y., Du, J., Wang, X., Yuan, M., Wang, Z., Xu, Z. and Chong, K. (2008) Rice root architecture associated1 binds the proteasome subunit RPT4 and is degraded in a D-box and proteasome-dependent manner. *Plant Physiol.*, **148**, 843–855.

He, K., Gou, X., Yuan, T., Lin, H., Asami, T., Yoshida, S., Russell, S.D. and Li, J. (2007) BAK1 and BKK1 regulate brassinosteroid-

dependent growth and brassinosteroid-independent cell-death pathways. *Curr. Biol.*, **17**, 1109–1115.

Heese, A., Hann, D.R., Gimenez-Ibanez, S., Jones, A.M., He, K., Li, J., Schroeder, J.I., Peck, S.C. and Rathjen, J.P. (2007) The receptor-like kinase SERK3/BAK1 is a central regulator of innate immunity in plants. *Proc. Natl Acad. Sci. USA*, **104**, 12217–12222.

Hong, Z., Ueguchi-Tanaka, M., Shimizu-Sato, S., Inukai, Y., Fujioka, S., Shimada, Y., Takatsuto, S., Agetsuma, M., Yoshida, S., Watanabe, Y., Uozu, S., Kitano, H., Ashikari, M. and Matsuoka, M. (2002) Loss-of-function of a rice brassinosteroid biosynthetic enzyme, C-6 oxidase, prevents the organized arrangement and polar elongation of cells in the leaves and stem. *Plant J.*, **32**, 495–508.

Hong, Z., Ueguchi-Tanaka, M., Umemura, K., Uozu, S., Fujioka, S., Takatsuto, S., Yoshida, S., Ashikari, M., Kitano, H. and Matsuoka, M. (2003) A rice brassinosteroid-deficient mutant, ebisu dwarf (d2), is caused by a loss of function of a new member of cytochrome P450. *Plant Cell*, **15**, 2900–2910.

Hong, Z., Ueguchi-Tanaka, M., Fujioka, S., Takatsuto, S., Yoshida, S., Hasegawa, Y., Ashikari, M., Kitano, H. and Matsuoka, M. (2005) The rice brassinosteroid-deficient dwarf2 mutant, defective in the rice homolog of Arabidopsis DIMINUTO/DWARF1, is rescued by the endogenously accumulated alternative bioactive brassinosteroid, dolichosterone. *Plant Cell*, **17**, 2243–2254.

Hong, Z., Jin, H., Tzfira, T. and Li, .J (2008) Multiple mechanism-mediated retention of a defective brassinosteroid receptor in the endoplasmic reticulum of Arabidopsis. *Plant Cell*, **20**, 3418–3429.

Hu, H., Xiong, L. and Yang, Y. (2005) Rice SERK1 gene positively regulates somatic embryogenesis of cultured cell and host defense response against fungal infection. *Planta*, **222**, 107–117.

Ito, Y., Takaya, K. and Kurata, N. (2005) Expression of SERK family receptor-like protein kinase genes in rice. *Biochim. Biophys. Acta*, **1730**, 253–258.

Jin, H., Yan, Z., Nam, K.H. and Li, J. (2007) Allele-specific suppression of a defective brassinosteroid receptor reveals a physiological role of UGGT in ER quality control. *Mol. Cell*, **26**, 821–830.

Kemmerling, B., Schwedt, A., Rodriguez, P., Mazzotta, S., Frank, M., Qamar, S.A., Mengiste, T., Betsuyaku, S., Parker, J.E., Mussig, C., Thomma, B.P., Albrecht, C, de Vries, S.C., Hirt, H and Nurnberger, T. (2007) The BRI1-associated kinase 1, BAK1, has a brassinolide-independent role in plant cell-death control. *Curr. Biol.*, **17**, 1116–1122.

Koh, S., Lee, S.C., Kim, M.K., Koh, J.H., Lee, S., An, G., Choe, S. and Kim, S.R. (2007) T-DNA tagged knockout mutation of rice OsGSK1, an orthologue of Arabidopsis BIN2, with enhanced tolerance to various abiotic stresses. *Plant Mol. Biol.*, **65**, 453–466.

Li, J., Wen, J., Lease, K.A., Doke, J.T., Tax, F.E. and Walker, J.C. (2002) BAK1, an Arabidopsis LRR receptor-like protein kinase, interacts with BRI1 and modulates brassinosteroid signaling. *Cell*, **110**, 213–222.

Li, X., Qian, Q., Fu, Z., Wang, Y., Xiong, G., Zeng, D., Wang, X., Liu, X., Teng, S., Hiroshi, F., Yuan, M., Luo, D., Han, B. and Li, J. (2003) Control of tillering in rice. *Nature*, **422**, 618–621.

Lin, Q.G., Cui, B.M. and Peng, M. (2007) Advanced study on SERK genes family. *Yi Chuan*, **29**, 681–687.

Morinaka, Y., Sakamoto, T., Inukai, Y., Agetsuma, M., Kitano, H., Ashikari, M. and Matsuoka, M. (2006) Morphological alteration caused by brassinosteroid insensitivity increases the biomass and grain production of rice. *Plant Physiol.*, **141**, 924–931.

Nakamura, A., Fujioka, S., Sunohara, H., Kamiya, N., Hong, Z., Inukai, Y., Miura, K., Takatsuto, S., Yoshida, S., Ueguchi-Tanaka, M., Hasegawa, Y., Kitano, H. and Matsuoka, M. (2006) The role of OsBRI1 and its homologous genes, OsBRL1 and OsBRL3, in rice. *Plant Physiol.*, **140**, 580–590.

Reinhardt, D. and Kuhlemeier, C. (2002) Plant architecture. *EMBO Rep.*, **3**, 846–851.

Sakamoto, T. (2006) Phytohormones and rice crop yield: strategies and opportunities for genetic improvement. *Transgenic Res.*, **15**, 399–404.

Sakamoto, T., Morinaka, Y., Ohnishi, T., Sunohara, H., Fujioka, S., Ueguchi-Tanaka, M., Mizutani, M., Sakata, K., Takatsuto, S., Yoshida, S., Tanaka, H., Kitano, H. and Matsuoka, M. (2006) Erect leaves caused by brassinosteroid deficiency increase biomass production and grain yield in rice. *Nat. Biotechnol.*, **24**, 105–109.

Shah, K., Vervoort, J. and de Vries, S.C. (2001) Role of threonines in the Arabidopsis thaliana somatic embryogenesis receptor kinase 1 activation loop in phosphorylation. *J. Biol. Chem.*, **276**, 41263–41269.

Song, D., Li, G., Song, F. and Zheng, Z. (2008) Molecular characterization and expression analysis of OsBISERK1, a gene encoding a leucine-rich repeat receptor-like kinase, during disease resistance responses in rice. *Mol. Biol. Rep.*, **35**, 275–283.

Walter, M., Chaban, C., Schutze, K., Batistic, O., Weckermann, K., Nake, C., Blazevic, D., Grefen, C., Schumacher, K., Oecking, C., Harter, K. and Kudla, J. (2004) Visualization of protein interactions in living plant cells using bimolecular fluorescence complementation. *Plant J.*, **40**, 428–438.

Wang, Y. and Li, J. (2008) Molecular basis of plant architecture. *Annu. Rev. Plant Biol.*, **59**, 253–279.

Wang, X., Goshe, M.B., Soderblom, E.J., Phinney, B.S., Kuchar, J.A., Li, J., Asami, T., Yoshida, S., Huber, S.C. and Clouse, S.D. (2005) Identification and functional analysis of in vivo phosphorylation sites of the Arabidopsis BRASSINOSTEROID-INSENSITIVE1 receptor kinase. *Plant Cell*, **17**, 1685–1703.

Wang, L., Xu, Y.Y., Li, J., Powell, R.A., Xu, Z.H. and Chong, K. (2007) Transgenic rice plants ectopically expressing AtBAK1 are semi-dwarfed and hypersensitive to 24-epibrassinolide. *J. Plant Physiol.*, **164**, 655–664.

Wang, L., Xu, Y., Zhang, C., Ma, Q., Joo, S.H., Kim, S.K., Xu, Z and Chong, K. (2008a) OsLIC, a Novel CCCH-type zinc finger protein with transcription activation, mediates rice architecture via Brassinosteroids signaling. *PLoS ONE*, **3**, e3521.

Wang, X., Kota, U., He, K., Blackburn, K., Li, J., Goshe, M.B., Huber, S.C. and Clouse, S.D. (2008b) Sequential transphosphorylation of the BRI1/BAK1 receptor kinase complex impacts early events in brassinosteroid signaling. *Dev. Cell*, **15**, 220–235.

Yamamuro, C., Ihara, Y., Wu, X., Noguchi, T., Fujioka, S., Takatsuto, S., Ashikari, M., Kitano, H. and Matsuoka, M. (2000) Loss of function of a rice brassinosteroid insensitive1 homolog

prevents internode elongation and bending of the lamina joint. *Plant Cell*, **12**, 1591–1605.

Yang, X.C. and Hwa, C.M. (2008) Genetic modification of plant architecture and variety improvement in rice. *Heredity*, **101**, 396–404.

Supporting information

Additional Supporting Information may be found in the online version of this article:

Supplement 1 Phylogenetic Relationship of SERK family in Arabidopsis and rice.

Supplement 2 Alignment of amino acid sequence of SERK family in *Arabidopsis* and rice.

Supplement 3 The expression of *OsBRI1* and *OsBZR1* in the OsBAK1 transgenic rice detected by real-time PCR.

Supplement 4 Primer Sequences.

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