Supporting Information

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SI Materials and Methods

Plant Growth Conditions and Chemical Treatment. Rice (*Oryza sativa* L.) plants were grown in a paddy field under natural conditions or on 1/2 MS (Murashige and Skoog) in a growth chamber (28 °C, 12-h light/12-h dark). Unless specified, Nipponbare was used as WT for analyses. For hormone treatment and gene-expression analysis, 7-d-old plants were immersed in liquid 1/2 MS medium containing different kinds of phytohormones for 2 h, including BL (brassinolide, 10 μM), GA_3 (gibberellin, 10 μM), IAA (10 μM), 6-BA (6-benzylaminopurine, 10 μM), ACC (1-aminocyclopropane-1-carboxylic acid, 10 μM), JA (jasmonic acid, 10 μM), IBA (10 μM), NAA (10 μM), and CHX (50 μM). Twenty plants for each treatment were collected for RNA isolation. For auxin and NPA response, de-hulled seeds were directly sowed on the 1/2 MS agar medium supplemented with various concentrations of IAA and NPA. After 1-wk growth, the seedling roots were washed with water and analyzed using WinRHIZO system. *Arabidopsis* were grown on 1/2 MS or soil in a growth chamber at 22 °C (14-h light/10-h dark). Columbia-0 (Col-0) ecotype was used as WT for analyses.

Gene Cloning and Expression Analysis. Site-finding PCR were performed according to previous reports (1, 2). Full-length cDNA of *BG1* was amplified by Invitrogen RACE system. Total RNAs were isolated using the TRIzol reagent (Life Technologies). After Rnase-free DNase (Promaga) treatment, 2 μg RNA was reversely transcribed using oligo (dT) primer and AMV reverse transcriptase (Promega). Quantitative PCR experiments were performed using SSoFast EvaGreen Supermix (Bio-Rad) with Bio-Rad CFX96 real-time PCR machine. The rice *ACTIN1* gene was used as internal control. Primers used are listed in Tables S3–S5. Values are means ± SD of three biological repeats. GUS staining was performed as previously reported (3).

Vector Construction and Plant Transformation. *BG1* full-length coding sequences were cloned into *pCAMBIA2300-Actin* to generate the *pCAMBIA2300-Actin:BG1* overexpression construct. A 3.6-kb genomic fragment containing the 2-kb native promoter and the entire *BG1* coding region as well as 667-bp 3′ downstream sequence was cloned into *pCAMBIA2300* vector to generate *pCAMBIA2300-PRO$_{BG1}$:BG1* overexpression construct. To generate *BG1-RNAi* construct, a 440-bp gene-specific fragment of *BG1* coding sequence was amplified and cloned into *pCAMBIA2300-Actin* vector as described previously (4). For promoter-GUS assay, a 2-kb genomic fragment upstream of the *BG1* translation start codon was amplified and cloned into the *pCAMBIA2391Z*. Upon sequence confirmation, all resulting constructs were transformed into the rice genome by *Agrobacterium tumefaciens*-mediated transformation methods (5). The primer sequences for vector construction are listed in Table S6.

Histological Analysis and RNA in Situ Hybridization. Cross-sections of spikelet hulls were analyzed by light microscopy (BX51; Olympus). Spikelet hulls were fixed with FAA (1:1:18) glacial acetic acid: 40% (vol/vol) formaldehyde: 70% (vol/vol) ethanol, and then dehydrated in a graded ethanol series. Fixed tissues were embedded in Paraplast Plus chips (Sigma), and cut into 10-μm-thick sections using a microtome and then affixed to poly-L-lysine–coated slides (Sigma), followed by dewaxed in xylene, gradually rehydrated and dried before toluidine blue staining for light microscopy. Cell number and cell area in the outer parenchyma layer of the spikelet hulls were measured by Olympus stream software. The sample pretreatment for scanning electron microscopy observation (S-3000N; Hitachi) was performed as described previously (6). For RNA in situ hybridization, a gene-specific region of *BG1* cDNA was cloned into the *pGEM-T* vector (Promega) and then transcribed to generate digoxigenin-labeled RNA probes (Roche). RNA in situ hybridization with sense and antisense probes was performed as described by Luo et al. (7). Primers used are listed in Table S7.

Subcellular Localization of *BG1*. The *BG1* cDNA sequence was cloned in frame with the *eGFP* under the control of CaMV 35S promoter. For confocal microscopy, the localization patterns of BG1-eGFP was examined by imaging root of 1-wk-old seedlings of *BG1-eGFP* transgenic lines and rice protoplasts using a confocal laser scanning microscope (Leica TCS SP5). eGFP was excited at 488-nm wavelength, and the emission filters were 500–530 nm. Preparation and transfection of rice protoplasts were performed according to the method described previously with minor modifications (8). Microsomal fractionations were isolated from 2-wk-old rice plants and two-phase membrane partitioning experiments were conducted as previously described (9, 10). Equal amount of proteins of the total microsomal and the PM-enriched upper phase were analyzed by SDS/PAGE and immunoblotting with anti-GFP (Roche), anti-PIP2 (Agrisera), anti-PEPC (Agrisera), and anti-BIP (Santa Cruz) antibody.

Protein Sequence and Phylogenetic Analysis. For phylogenetic analysis of BG1 homologs in plants, a total of 22 protein sequences were obtained through Plant Comparative Genomics website (Phytozome) and National Center for Biotechnology Information BLAST search. All of the sequences were aligned using a ClustalW program. Phylogenetic tree was constructed using MEGA 6.0 based on the neighbor-joining method. Topological robustness was assessed by bootstrap analysis with 1,000 replicates (11).

IAA Measurement and Auxin Transport Assay. The young panicles with about 10-cm length were collected for IAA content analysis. IAA extraction and measurement were performed according to previous reports (12). The polar auxin transport assays were performed using 5-d-old dark-grown coleoptiles, as described previously with minor modifications (13, 14). Briefly, 2.0-cm length apical coleoptile segments (0.2 cm away from the tip was cut off) were preincubated in 1/2 MS medium with shaking at 50 rpm for 2 h. The apical ends of coleoptile segment were then inserted horizontally into 50 μL 1/2 MS agar medium containing 500 nM ^{3}H-IAA and 500 nM free IAA in a 1.5-mL microcentrifuge tube in the dark at room temperature for 3 h. NPA and ^{3}H-BA were added as the IAA transport control. After 3-h transport assay, 0.5-cm segments from the apex were excised and washed three times in 1/2 MS liquid medium. The segments were then grouped in sets of five and incubated in 2.0 mL of universal scintillation fluid for 18 h. The radioactivity was counted by a liquid scintillation counter (1450 MicroBeta TriLux, Perkin-Elmer). Acropetal auxin transport was also measured as a negative control based on the orientation of the coleoptile segments submerged in the agar blocks.

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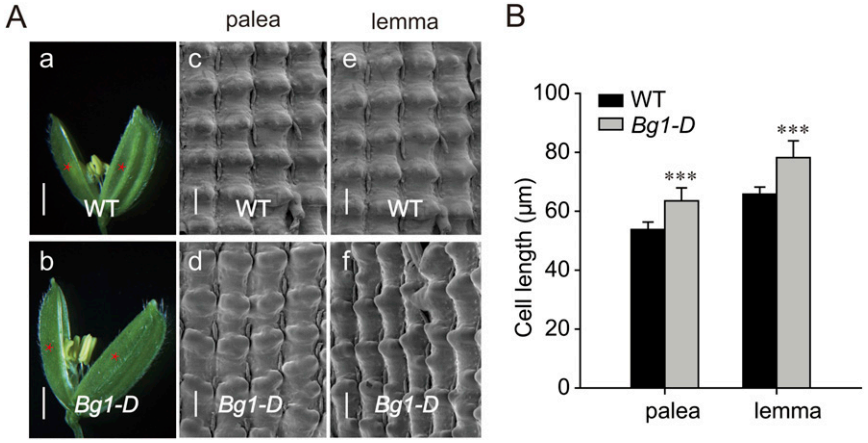


Fig. S1. Scanning electron microscopy observation of spikelet hull of WT and *Bg1-D*. (*A*) Grain and cell morphology of WT and *Bg1-D* were shown. The asterisk indicates the position for observation. (Scale bar, 2 mm in *a* and *b*, and 50 μm in *c*–*f*). (*B*) Statistical data of cell length of palea and lemma in WT and *Bg1-D* plants. Data are given as mean ± SD ($n = 15$). ***$P < 0.001$ (*t* test).

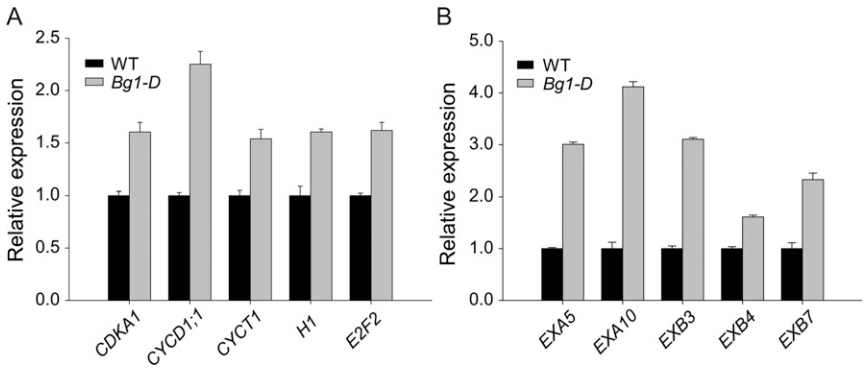


Fig. S2. Expression of genes involved in cell cycle and cell expansion in WT and *Bg1-D*. (*A*) Expression of genes involved in cell cycle G1 to S phase in WT and *Bg1-D*. (*B*) Expression of genes involved in cell expansion in WT and *Bg1-D*. Error bar denotes means ± SD.

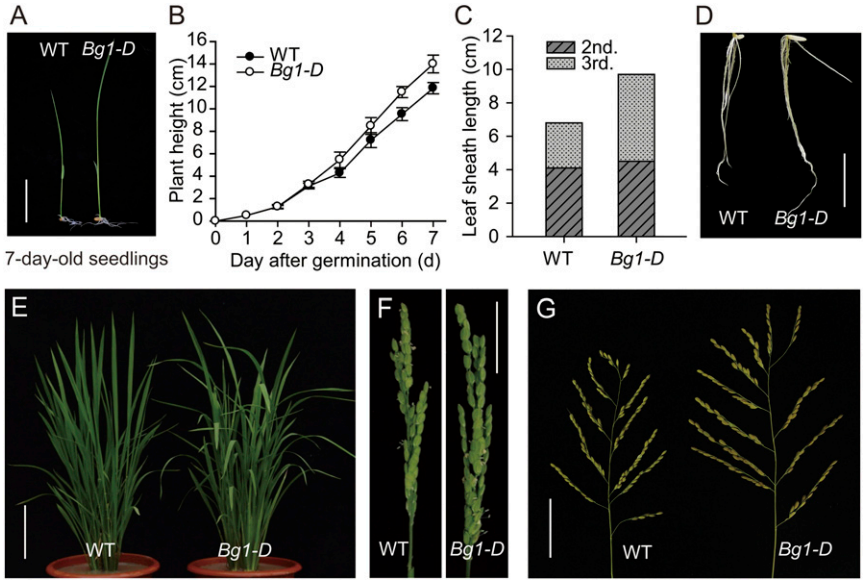


Fig. S3. Phenotypes of *Bg1-D* seedling and panicle. (*A*) One-week-old seedlings of WT and *Bg1-D* plants. (Scale bar, 5 cm.) (*B–D*) Comparison of plant height (*B*), leaf sheath length (*C*), and root (*D*) between WT and *Bg1-D* in 1-wk-old seedlings. Error bars indicate SD ($n = 20$). (*E*) Growth phenotype of 2-mo-old plants of WT and *Bg1-D*. (*F* and *G*) The young panicles (*F*) and harvested panicles (*G*) of WT and *Bg1-D*. [Scale bars: 2 cm (*D*), 20 cm (*E*), 5 cm (*F*), and 5 cm (*G*).]

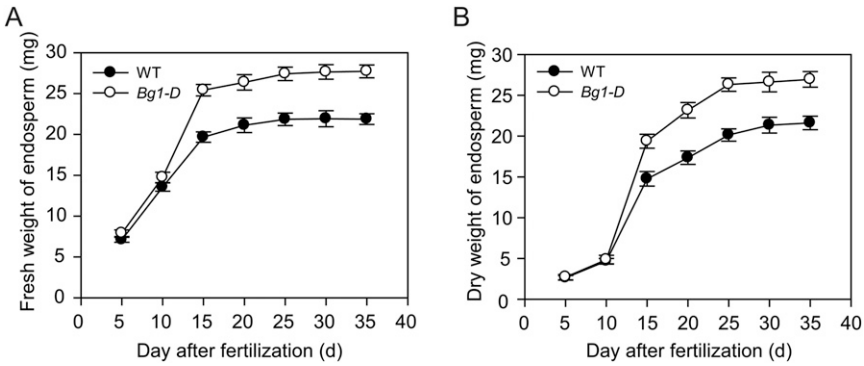
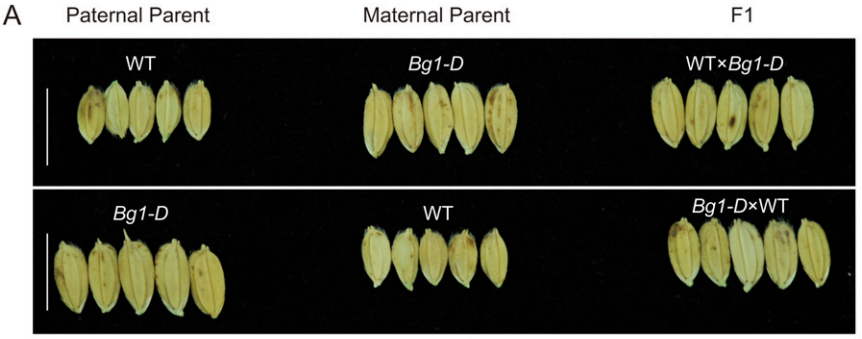


Fig. S4. Comparison of endosperm weight between WT and *Bg1-D*. (*A*) Comparison of fresh weight between WT and *Bg1-D* after fertilization. (*B*) Comparison of dry weight between WT and *Bg1-D* after fertilization. Means ± SD were given ($n = 20$).



B

Genotype	Number of plants	Plant height (cm)	Grain length (mm)	Grain width (mm)	1,000-grain weight (g)
WT	20	94.30 ± 3.80	7.35 ± 0.18	3.23 ± 0.08	21.90 ± 0.30
Bg1-D	20	110.50 ± 5.30	8.47 ± 0.25	3.78 ± 0.17	29.30 ± 0.40
Bg1 (+)	53	105.10 ± 8.50	8.16 ± 0.51	3.64 ± 0.35	26.89 ± 0.55
Bg1 (-)	16	95.20 ± 5.40	7.43 ± 0.15	3.34 ± 0.13	22.34 ± 0.50

Fig. S5. Confirmation of *Bg1-D* as a dominant mutant. (*A*) F_1 progenies of reciprocal crosses between *Bg1-D* and WT have big grain phenotype. (Scale bar, 1 cm.) (*B*) Segregation of *Bg1-D* phenotype in F_2 population from the cross between WT and the *Bg1-D* mutant.

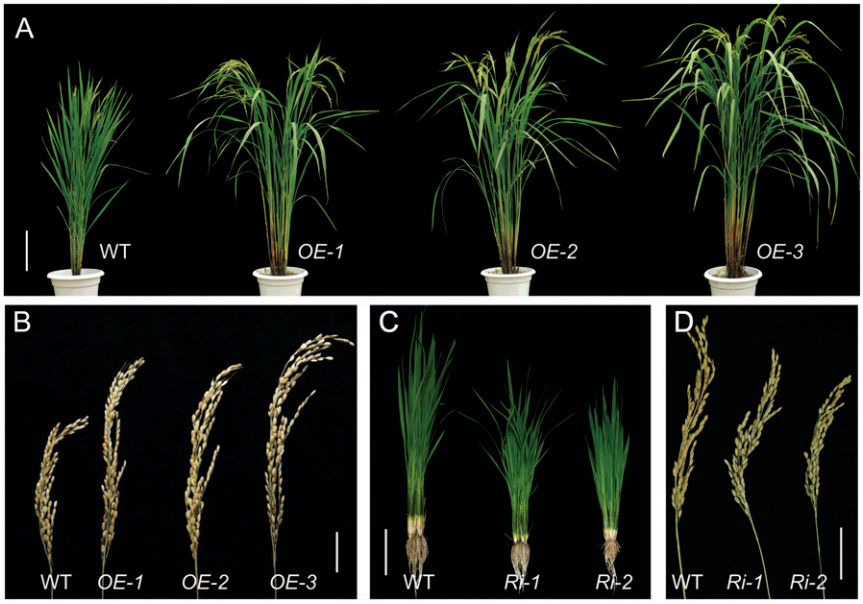


Fig. S6. Phenotypes of *BG1*-overexpression and *BG1-RNAi* plants. (*A*) Gross morphology of WT and *BG1*-overexpression plants (*OEs*). (Scale bar, 20 cm.) (*B*) Panicle morphology of the plants. (Scale bar, 5 cm.) (*C*) Gross morphology of WT and *BG1-RNAi* plants (*Ris*) at the 60-d-old stage. (Scale bar, 10 cm.) (*D*) Panicle morphology of the plants in *C*. (Scale bar, 5 cm.)

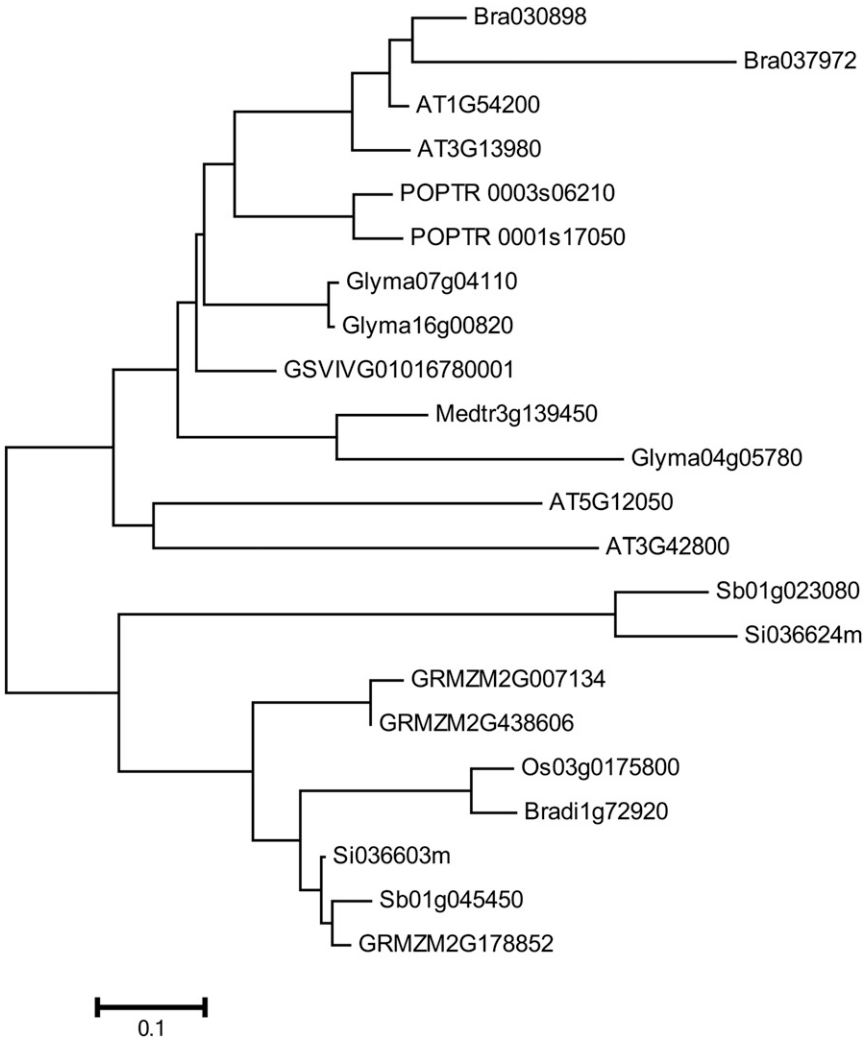


Fig. S7. Phylogenetic analysis of BG1. MEGA6 Neighbor-Joining tree was inferred from the amino acid sequences of the BG1 homologs among higher plants. Bootstrap values are based on 1,000 replications and are indicated in their respective nodes. The scale bar indicates genetic distance based on branch length.

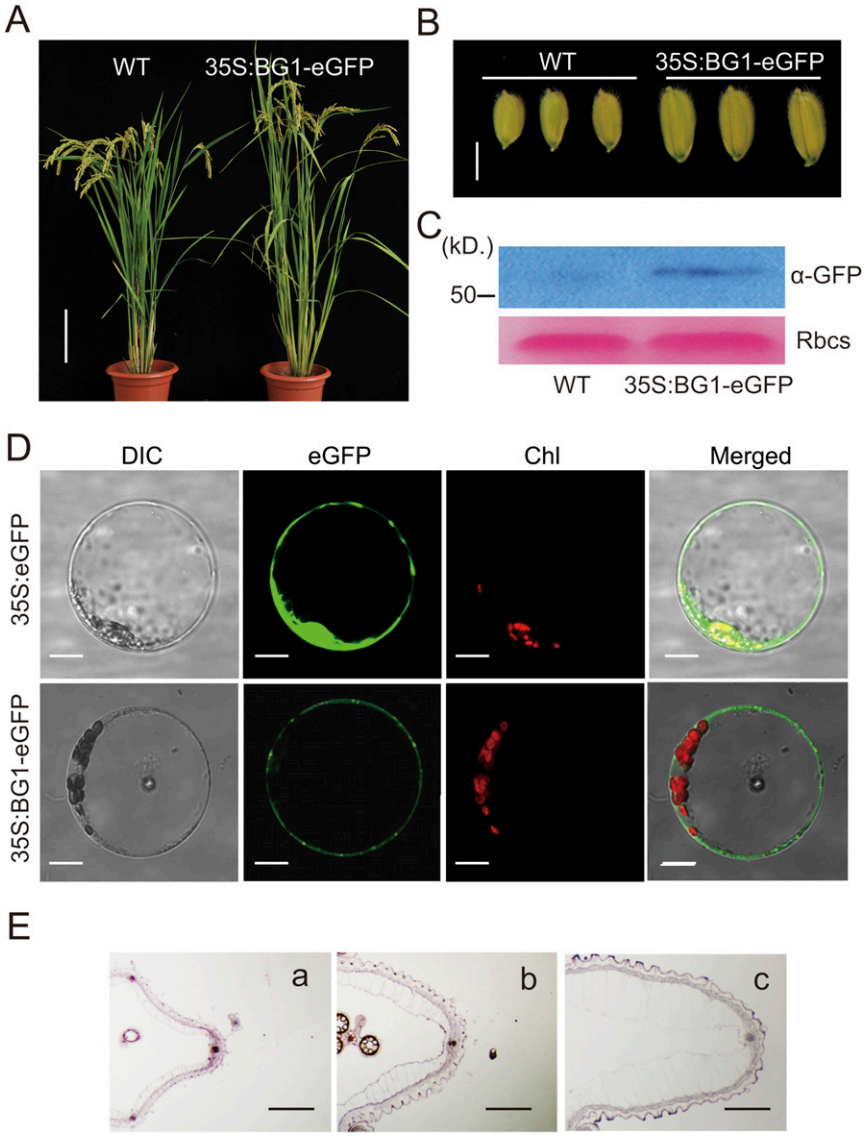


Fig. S8. Verification of BG1 subcellular localization and RNA in situ hybridization. (*A*) Phenotype of *35S:BG1-eGFP* transgenic plants. (Scale bar, 20 cm.) (*B*) Grain phenotype of the plants. (Scale bar, 0.5 cm.) (*C*) Detection of the GFP fusion protein by immunoblotting. (*D*) Confocal micrographs of rice protoplasts transformed with *35S:eGFP* (*Upper*) and *35S:BG1-eGFP* (*Lower*) plasmids. (Scale bar, 10 μm.) (*E*) In situ localization of *BG1* mRNA in the developing husks. Husks from different lengths [(*E*, *a*) 5 cm, (*E*, *b*) 10 cm, and (*E*, *c*) 20 cm] of panicles were used for the analysis. (Scale bars, *E*, *a*–*c*: 200 μm.)

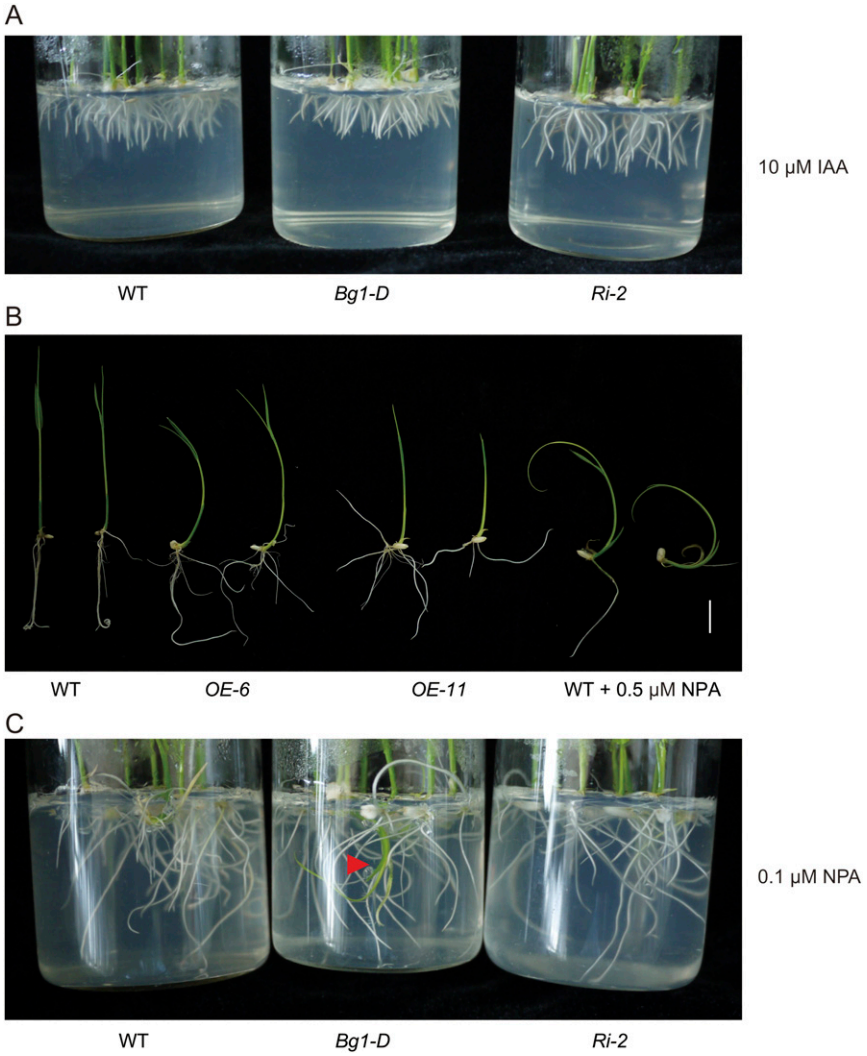


Fig. S9. Plant responses to IAA, NPA, and gravity. (*A*) Root phenotype of WT, *Bg1-D*, and *Ri-2* under 10-μM IAA treatment. (*B*) Seedling phenotypes of WT and two *BG1*-overexpression plants. The plants were grown for 4 d on agar medium and taken out for photograph. WT seedlings grown on 0.5 μM NPA were shown for comparison. (Scale bar, 1 cm.) (*C*) Growth of WT, *Bg1-D*, and *Ri-2* under 0.1 μM NPA treatment. Arrowhead indicates one seedling shoot grown downward into the medium. Zhonghua 11 was used as WT in *B*.

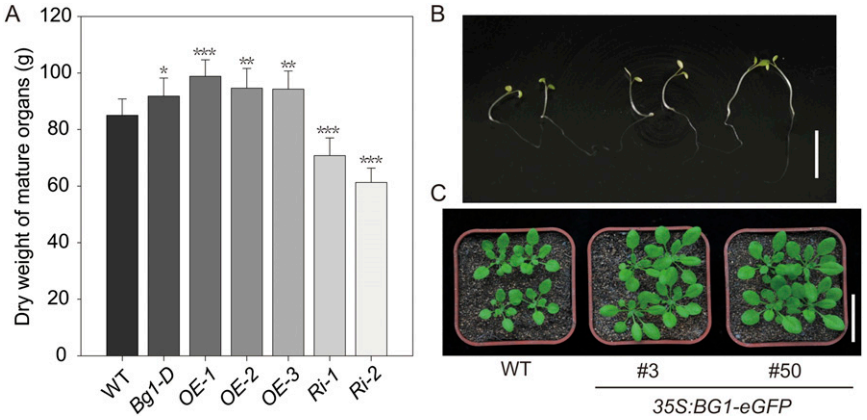


Fig. S10. Enhanced plant biomass by overexpression of *BG1* in rice and *Arabidopsis*. (*A*) Overexpression of *BG1* leads to increased plant biomass in rice. Means ± SD were given ($n = 10$). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ (*t* test). (*B* and *C*) Overexpression of *BG1* leads to increased plant growth in *Arabidopsis*. (Scale bars, 1 cm in *B* and 5 cm in *C*).

Table S1. The agronomic traits of WT and *Bg1-D* plants

Traits	WT	*Bg1-D*
Plant height (cm)	94.3 ± 3.8	110.5 ± 5.3***
Tiller number	12.0 ± 2.0	11.0 ± 3.0
Panicle length (cm)	19.6 ± 0.9	23.2 ± 1.2***
Grain no. per panicle	105.9 ± 11.7	109.3 ± 10.8
Length of flag leaf (cm)	34.6 ± 3.9	42.4 ± 4.8***
Width of flag leaf (cm)	1.3 ± 0.1	1.6 ± 0.1***

Agronomic traits were analyzed in 2010, Beijing, China. Results represent means ± SD (n = 30). Asterisks indicate the significance of differences between WT and *Bg1-D* plants. ***P < 0.001 (t test).

Table S2. Yield test in a paddy field

Traits	WT	OE-5	OE-7
Panicles per plot	3450.3 ± 59.4	3223.6 ± 41.5	3159.5 ± 34.1
Effective grains per panicle	87.4 ± 4.6	83.7 ± 3.5	82.2 ± 4.3
Effective 1,000-grain weight (g)	22.3 ± 0.4	27.3 ± 0.6	29.2 ± 0.5
Theoretical yield per plot (kg)	6.7 ± 0.7	7.4 ± 0.5	7.6 ± 0.6
Actual yield per plot (kg)	5.3 ± 0.5	6.1 ± 0.4**	6.4 ± 0.5***

The planting density was 20 cm × 20 cm, the area per plot was 15 m^2. Data were calculated from plants in block region with three replications under natural condition in 2012, Beijing, China. Results represent means ± SD, **P < 0.01, ***P < 0.001 (t test).

Table S3. Primers used in this study: Quantitative real-time PCR

Gene	Forward primer (5′-3′)	Reverse primer (5′-3′)
BG1	GATGGAGAGCGACGAGGAC	GCAATGGCGGCGAAGTTC
Actin	TGGCATCTCTCAGCACATTCC	TGCACAATGGATGGGTCAGA
CDKA1	GGTTTGGACCTTCTCTCTAAAATGC	AGAGCCTGTCTAGCTGTGATCCTT
CYCD1;1	CCGTCGCTCGCATTCGTA	TCATGTGCCTGGTGGTGA
CYCT1	GCTTCTTTCAGAGGGTCAT	GCATTTCAAGTACACGAGGT
H1	GCAAGGCACCTGCAGCTT	AGGCAGCCTTTGTACAGATCCT
E2F2	TGTTGGTGGCTGCCGATAT	CGCCAGGTGCACCCTTT
OsEXPA5	AAGGCTGTGGCTTGATTGACA	TTAGGCCCAATTTTGCTATTTTG
OsEXPA10	TGACCAACTACAACGTGGTCCC	GCCAGTGTATGTTTTGCCGAAG
OsEXPB3	CTTTGAGTGGTTGGAGTGGTGG	GCAGCCTTCTTGGAGATGGAA
OsEXPB4	GTCGGTCTGTGTTGCGATTTG	CCTCCATTTCCCACACAGCTT
OsEXPB7	ACGGTGATCATCACGGACAT	TCGAAGTGGTACAGCGACACT

Table S4. Primers used in this study: For site-finding PCR

Primer name	Sequence (5′-3′)
SF1	CACGACACGCTACTCAACACACCACCTCGCACAGCGTCCTANNNNNNGCATG
SFP1	CACGACACGCTACTCAACAC
SFP2	ACTCAACACACCACCTCGCACAGC
GSP1	ACAATGCTGAGGGATTCAAATTCTACCCA
GSP2	TACTGAATTAACGCCGAATTGAATTC

Table S5. Primers used in this study: For RACE PCR

Primer name	Sequence (5′-3′)
GeneRacer 5′ primer	CGACTGGAGCACGAGGACACTGA
GeneRacer RNA Oligo primer	CGACUGGAGCACGAGGACACUGACAUGGACUGAAGGAGUAGAAA
BG1-1R	CTTGGCCGCCGCAGCCACCGCCTCCA
BG1-2R	AGAACCCGCCGTAGCTAGAACACT
BG1-3R	GCGAAGATGGAGTTGAGGAA

Table S6. Primers used in this study: For transgenic constructs

Construct	Forward primer (5′-3′)	Reverse primer (5′-3′)
BG1-OE	TCTAGAATGGAGAGGTGGGCGGCGCCCAAG	CTGCAGTCAGACAACTCTGGCGCTCCGT
BG1-Ri	CTCGAGCAGCCGTCTTTCTCATCCAC	GGATCCGCGAAGATGGAGTTGAGGAA
PRO_{BG1}:GUS	GGATCCGCTTGTCATTGCTGTACATG	GAATTCAGGAAACAAACGCCAAGAAG
BG1-eGFP	TCTAGAATGGAGAGGTGGGCGGCGCCCAAG	ACTAGTGACAACTCTGGCGCTCCGTCCAT
PRO_{BG1}:BG1-OE	GAATTCGCTTGTCATTGCTGTACATGCTGT	GGATCCCACACACATGTTCCCATTAGGTTT

The underlined nucleotides indicate the restriction sites for cloning.

Table S7. Primers used in this study: For RNA in situ hybridization

Gene	Forward primer (5′-3′)	Reverse primer (5′-3′)
BG1	GATGGAGAGCGACGAGGAC	CTCCGTCCATGGCCATACC