RESEARCH PAPER

SLG controls grain size and leaf angle by modulating brassinosteroid homeostasis in rice

Zhiming Feng1*, Chuanyin Wu2*, Chunming Wang1, Jeehee Roh3, Long Zhang1, Jun Chen2, Shengzhou Zhang1, Huan Zhang1, Chunyan Yang1, Jinlong Hu1, Xiaoman You1, Xi Liu1, Xiaoming Yang1, Xiuping Guo2, Xin Zhang2, Fuqing Wu2, William Terzaghi4, Seong-Ki Kim3, Ling Jiang1† and Jianmin Wan1,2†

1 State Key Laboratory for Crop Genetics and Germplasm Enhancement, Jiangsu Plant Gene Engineering Research Center, Nanjing Agricultural University, Nanjing 210095, China
2 National Key Facility for Crop Resources and Genetic Improvement, Institute of Crop Science, Chinese Academy of Agricultural Sciences, Beijing 100081, China
3 Department of Life Science, Chung-Ang University, Seoul 156-756, Korea
4 Department of Biology, Wilkes University, Wilkes-Barre, PA 18766, USA

* These authors contributed equally to this work.
† Corresponding authors: wanjm@njau.edu.cn, wanjianmin@caas.cn, or jiangling@njau.edu.cn

Received 21 February 2016; Accepted 3 May 2016

Editor: Dabing Zhang, Shanghai Jiao Tong University

Abstract

Grain size and leaf angle are two important traits determining grain yield in rice. However, the mechanisms regulating the two traits remain largely unknown. Here, we characterized a rice gain-of-function mutant, slender grain Dominant (slg-D), which exhibited longer and narrower grains and larger leaf angles, similar to plants with elevated brassinosteroid (BR) levels or strengthened BR signaling. The increased cell length is responsible for the mutant phenotypes in slg-D. We demonstrated that the phenotype of slg-D is caused by enhanced expression of SLG, a BAHD acyltransferase-like protein gene. SLG is preferentially expressed in young panicles and lamina joints, implying its role in controlling cell growth in those two tissues. slg-D was restored to wild type by treatment with brassinazole, an inhibitor of BR biosynthesis. Overexpression of SLG in d11-2 (deficient in BR synthesis) and d61-1 (deficient in BR signaling) did not change the existing phenotypes. The slg-D plants had elevated BR contents and, accordingly, expression of BR-related genes was changed in a manner similar to BR treatment. Moreover, SLG RNAi plants displayed mild BR-deficient phenotypes including shorter grains, smaller leaf angles, and compact semi-dwarf plant types. The in vitro biochemical assays and transgenic approaches collectively demonstrated that SLG functions as homomers. Taken together, we conclude that SLG is an important regulator in BR homeostasis and that manipulation of SLG expression to an optimal level may provide a way to develop an ideal plant type.

Key words: BR, grain size, homomer, leaf angle, rice, SLG.

Introduction

Rice (Oryza sativa) feeds more than half of the world’s population as one of the world’s most important cereal crops. Given the rapid increase in the world’s population and decrease in cultivated land area, improving rice production remains a great challenge for rice breeding programs. Grain size and leaf angle are two important traits determining rice...
Grain yield and have always been a consideration in breeding programs (Sinclair and Sheehy, 1999; Ikeda et al., 2013).

Grain size, determined by grain length, grain width, and grain thickness, not only contributes to grain yield, but also influences the appearance, processing, cooking, and eating quality of rice. For example, people in Japan, Korea, and Northern China favor medium length round grains, whereas people in the USA, Southeast Asian countries, and Southern China prefer long and slender grains (Unnewehr et al., 1992). The organ size is largely determined by the cell number and cell size during organogenesis (Potter and Xu, 2001; Sugimoto-Shirasu and Roberts, 2003). In recent years, several genes and quantitative trait loci (QTLs) that affect grain size by influencing cell number have been identified in rice, including GS3, GW2, GW5, GS5, GW8, qGL3, TGW6, GW6a, and BGL1 (Song et al., 2007, 2015; Weng et al., 2008; Mao et al., 2010; Li et al., 2011; Wang et al., 2012; Zhang et al., 2012; Ishimaru et al., 2013; Liu et al., 2015). Some other genes and QTLs that control grain size by influencing cell size have also been isolated in rice, including PGL1, GL7, and GS2/GL2 (Heang and Sassa, 2012; Che et al., 2015; Duan et al., 2015; Hu et al., 2015; Wang et al., 2015). It has been documented that at least some of those genes participate in seed size control by regulating biosynthesis and signaling of plant hormones, including brassinosteroids (BRs), cytokinins, gibberellins, and auxin (Ashikari et al., 1999, 2005; Hong et al., 2003; Tanabe et al., 2005; Ishimaru et al., 2013).

Leaf angle, the inclination between the leaf blade and vertical culm, is a key factor determining the plant architecture (Hoshikawa, 1989; Sinclair and Sheehy, 1999). A compact plant type with erect leaves is preferred since it increases photosynthetic efficiency and nitrogen storage for grain filling, and facilitates dense planting (Sinclair and Sheehy, 1999; Sakamoto et al., 2006). A number of genes or QTLs have been reported to have a role in controlling leaf angle, including Tal, OsDWARF4, D2, OsBRII, OsBU1, ILI, LC2, and ILA1 (Li et al., 1998, 1999; Sakamoto et al., 2006; Tanaka et al., 2009; Zhang et al., 2009; Zhao et al., 2010; Ning et al., 2011). The leaf lamina joint that connects the leaf blade and sheath is considered the most important tissue governing the leaf angle. The degree of the leaf angle largely depends on cell division and expansion as well as cell wall composition at the joint (Nakamura et al., 2009; Zhang et al., 2009; Zhao et al., 2010; Ning et al., 2011). Nevertheless, it is well known that BR treatment stimulates leaf inclination in rice (Wada et al., 1981).

BRs are a group of steroidal phytohormones that regulate diverse plant growth and developmental processes, including cell expansion and division, vasculature differentiation, root and leaf development, stem elongation, skotomorphogenesis, and grain filling (Clouse and Sasse, 1998; Fujioka and Yokota, 2003; Wu et al., 2008). In recent decades, researchers have clarified many genes and the main pathway of BR biosynthesis utilizing genetic studies, chemical feeding, and enzymatic analysis. Most of the enzymes known to catalyze BR biosynthesis belong to the cytochrome P450 protein family (Choe, 2006). The BR biosynthesis pathway mainly consists of the early and late C-22 oxidation pathway, and the early and late C-6 oxidation pathway (Choe, 2006). Similarly, research on BR signaling has also developed rapidly, and most of the main participants in BR signaling have been determined in Arabidopsis (Belkhadir and Chory, 2006). BRs are perceived by the receptor kinase BRI1 to transmit signaling (Li and Chory, 1997). In rice, it has been reported that BR plays important roles in the regulation of grain size, leaf angle, and yield potential. For example, most loss-of-function mutants in BR biosynthesis or signaling pathways, such as d2, d11, and d61, display short grains, erect leaves, and dwarf phenotypes (Yamamura et al., 2000; Hong et al., 2003; Tanabe et al., 2005), while some other mutants or transgenic plants with enhanced BR signaling or increased BR levels, such as GSK2 knockdown lines, the DLT overexpressor, and the D11 activation mutant m107, show longer grains and larger leaf angles (Tanabe et al., 2005; Wan et al., 2009; Tong et al., 2012). More importantly, modulating the expression level of BR-related genes such as OsDWARF4 and OsBRI1 has been proven to improve rice grain yield at higher planting densities (Morinaka et al., 2006; Sakamoto et al., 2006).

BR homeostasis is vital for normal growth and development of plants. BRs are synthesized in most plant tissues, and their level is the highest in young developing organs but low in mature organs (Shimada et al., 2003). Unlike the other plant hormones such as auxin that can be transported from the site of synthesis to a distant target site (Berleth and Sachs, 2001), BRs do not undergo long-distance transport and have the same site of synthesis and action (Symons and Reid, 2004). Therefore, there exist mechanisms that cells or tissues use to modulate levels of endogenous BRs precisely to keep cell expansion in balance and ensure normal plant growth and development (Symons and Reid, 2004). Negative feedback regulation is a common mechanism that also regulates BR homeostasis. It is reported that expression of many BR biosynthesis and signaling genes is inhibited by BR treatment, such as d2, D11, OsDWARF4, BRD1, OsBRII1, and DLT in rice (Yamamura et al., 2000; Hong et al., 2002, 2003; Tanabe et al., 2005; Sakamoto et al., 2006; Tong et al., 2009). However, BR homeostasis is still poorly understood.

In this study, we characterized a rice semi-dominant mutant, slender grain Dominant (slg-D), with slender grains and enlarged leaf angles, which are caused by enhanced expression of SLG, a BAHD acyltransferase-like protein gene. We provide genetic evidence that the BR contents are increased in slg-D mutants in BR biosynthesis or signaling pathways, such as d2, d11, and d61, display short grains, erect leaves, and dwarf phenotypes (Yamamura et al., 2000; Hong et al., 2003; Tanabe et al., 2005), while some other mutants or transgenic plants with enhanced BR signaling or increased BR levels, such as GSK2 knockdown lines, the DLT overexpressor, and the D11 activation mutant m107, show longer grains and larger leaf angles (Tanabe et al., 2005; Wan et al., 2009; Tong et al., 2012). More importantly, modulating the expression level of BR-related genes such as OsDWARF4 and OsBRI1 has been proven to improve rice grain yield at higher planting densities (Morinaka et al., 2006; Sakamoto et al., 2006).

BR homeostasis is vital for normal growth and development of plants. BRs are synthesized in most plant tissues, and their level is the highest in young developing organs but low in mature organs (Shimada et al., 2003). Unlike the other plant hormones such as auxin that can be transported from the site of synthesis to a distant target site (Berleth and Sachs, 2001), BRs do not undergo long-distance transport and have the same site of synthesis and action (Symons and Reid, 2004). Therefore, there exist mechanisms that cells or tissues use to modulate levels of endogenous BRs precisely to keep cell expansion in balance and ensure normal plant growth and development (Symons and Reid, 2004). Negative feedback regulation is a common mechanism that also regulates BR homeostasis. It is reported that expression of many BR biosynthesis and signaling genes is inhibited by BR treatment, such as d2, D11, OsDWARF4, BRD1, OsBRII1, and DLT in rice (Yamamura et al., 2000; Hong et al., 2002, 2003; Tanabe et al., 2005; Sakamoto et al., 2006; Tong et al., 2009). However, BR homeostasis is still poorly understood.

In this study, we characterized a rice semi-dominant mutant, slender grain Dominant (slg-D), with slender grains and enlarged leaf angles, which are caused by enhanced expression of SLG, a BAHD acyltransferase-like protein gene. We provide genetic evidence that the BR contents are increased in slg-D mutants in BR biosynthesis or signaling pathways, such as d2, d11, and d61, display short grains, erect leaves, and dwarf phenotypes (Yamamura et al., 2000; Hong et al., 2003; Tanabe et al., 2005), while some other mutants or transgenic plants with enhanced BR signaling or increased BR levels, such as GSK2 knockdown lines, the DLT overexpressor, and the D11 activation mutant m107, show longer grains and larger leaf angles (Tanabe et al., 2005; Wan et al., 2009; Tong et al., 2012). More importantly, modulating the expression level of BR-related genes such as OsDWARF4 and OsBRI1 has been proven to improve rice grain yield at higher planting densities (Morinaka et al., 2006; Sakamoto et al., 2006).

**Materials and methods**

**Plant materials**

The slg-D mutant (3A-10513) was isolated from a collection of activation-tagging T-DNA insertion rice lines (Jeon et al., 2000; Jeong et al., 2006), and kindly provided by Professor Gynheung An. The wild type (WT) of slg-D was Dongjin, a japonica cultivar. d61-1, d11-2, and m107 were kindly provided by Professor Chenciui Chu (Tong et al., 2012). The WT of d61-1 and d11-2 was a japonica cultivar, Zhonghua11, and the WT of m107 was a japonica cultivar, Nipponbare. Rice plants were cultivated in an experimental field under natural long-day conditions in Nanjing, China.
Scanning electron microscopy (SEM) and light microscopy

For SEM, lemmae were harvested from florets after flowering and fixed in 2.5% formaldehyde glutaraldehyde. Fixed samples were soaked in 2% OsO4 for 2h, dehydrated in a graded ethanol series, infiltrated and embedded in buty1 methyl methacrylate, treated with critical point drying, and then sputter coated with platinum. The outer and inner epidermal cells of lemmae were observed using a HITACHI S-3400N scanning electron microscope. For light microscopy, laminae joints of the second leaves were harvested 10 d after flowering and fixed with FAA solution, followed by a graded series of dehydration and infiltration steps. Fixed tissues were embedded in paraffin. After sectioning, 10 μm thick sections were dewaxed with xylene, rehydrated, stained with 1% toluidine blue, and observed with a Leica DM5000B microscope. Cell lengths and widths of each organ were measured with IMAGEJ software.

Isolation, cloning, and RNAi suppression of the SLG gene

To identify the T-DNA insertion locus in slg-D, we searched the flanking sequence database (Jeong et al., 2006; http://orygenesdb.cirad.fr/). The T-DNA loci were confirmed by PCR genotyping, using the primers P1, P2, and P3 (see Supplementary Table S1 at JXB online). To recapitulate the phenotype of slg-D, full-length cDNAs of $Loc_{Os08g44830}$ and $Loc_{Os08g44840}$ were amplified by PCR and cloned into the binary vector pCUbi1390 under the control of the maize $Ub$ promoter to create pl390-Ubi-slg-D, and p1390-Ubi-slg-D constructs, respectively. These constructs were then transformed into the rice variety Dongjin according to a published Agrobacterium-mediated method (Hiei et al., 1994).

To obtain SLG RNAi plants, the construct pCUbi1390-AFAD2 (an FAD2 intron and ubiquitin promoter inserted into pCUbi1390) was used as an RNAi vector ( Wu et al., 2007). Both sense and anti-sense versions of a specific 305 bp fragment from the cDNA of SLG were amplified with primer pairs SLG-RNAiL and SLG-RNAiR (Supplementary Table S1), and cloned into pCUbi1390-AFAD2 to create the pUbi-dsRNAiSLG construct, which was then transformed into the rice variety Dongjin by the Agrobacterium-mediated method described above.

RNA extraction and quantitative RT-PCR

Total RNA from roots, leaves, leaf sheaths, lamina joints, shoot apices, culms, and different stages of panicles were isolated using the RNAprep Pure Plant Kit (TIANGEN, Beijing, China). First-strand cDNA was reverse transcribed from 1 μg of total RNA using the PrimeScript 1st Strand cDNA Synthesis Kit (TaKaRa). Quantitative RT-PCR was performed using a SYBR Premix Ex Taq™ kit (TaKaRa) on an ABI prism 7500 Real-Time PCR System according to the manufacturer’s instructions, and the $ACTIN1$ gene was used as an internal control. The primers for quantitative RT-PCR analysis are listed in Supplementary Table S1.

GUS staining

To analyze the expression pattern of SLG, an ~2.5 kb promoter fragment was cloned into the pCAMBIA1381Z vector to create the $PRO_{SLG}^{GUS}$ (β-glucuronidase) reporter gene construct, which was then transformed into the rice variety Dongjin by the Agrobacterium-mediated method. GUS staining was performed on $PRO_{SLG}^{GUS}$ T1 generation transgenic plants according to a method described previously (Jefferson et al., 1987). Images were taken using a Nikon CDS5ri1P camera. Primers used to clone the promoter fragment are listed in Supplementary Table S1.

In situ hybridization

RNA in situ hybridization was performed as described previously (Bradley et al., 1993). A 305 bp gene-specific region of SLG amplified with primers SLG-PF and SLG-PR (see Supplementary Table S1) was cloned into the pGEM-T Easy vector (Promega). The linearized templates were amplified from the pGEM-T plasmid containing the gene-specific region of SLG using primers Y17 and Ysp6. Digoxigenin-labeled RNA probes were transcribed in vitro using T7 and SP6 RNA polymerases, respectively, using a DIG Northern Starter Kit (Cat. No. 2039672, Roche) following the manufacturer’s instructions. Images were taken using a Leica DMS500B microscope.

Subcellular localization of SLG

To determine the subcellular localization of SLG, green fluorescent protein (GFP) was fused to the C-terminus of SLG under the control of the 35S promoter in the PAN580 vector. In addition, the nuclear marker D53–mCherry was constructed. The SLG–GFP fusion construct was transiently co-transferred into rice protoplasts with the D53–mCherry constructs according to the method described previously (Chen et al., 2006). Next, GFP was fused to the C-terminus of SLG under the control of the Cauliflower mosaics virus (CaMV) 35S promoter in the pCAMBIA1305.1 vector. The pCAMBIA1305-SLG-GFP construct was transformed into the rice variety Dongjin by the Agrobacterium-mediated method. GFP fluorescence was examined in the young roots of 2-week-old T1 transgenic plants.

Fluorescence images were observed using a Zeiss LSM510 confocal laser microscope. Primers used to make these constructs are listed in Supplementary Table S1.

BR and BRZ treatment

The lamina joint bending assay using excised leaf segments was performed as described by Wada et al. (1981). Seeds were germinated for 2 d and then grown in the dark for 8 d at 30 °C. Segments of 2 cm comprising the second leaf blade, lamina joint, and leaf sheath were floated on distilled water for 24 h and then incubated in 2.5 mM maleic acid potassium solution containing various concentrations of brassinolide (BL), Sigma, http://www.sigmaaldrich.com/) for 48 h in the dark. Lamina joint angles were measured using IMAGEJ software. The coleoptile and root elongation tests were performed using a previously described method (Yamamuro et al., 2000). Seeds were germinated on agar plates containing various concentrations of BL, and then the coleoptile and root lengths were measured 1 d after germination.

To measure the effect of brassinazole (BRZ; TCI) treatment on lamina joint bending, the leaf tips of 8-day-old seedlings of slg-D and the WT were spotted with 1 μl of DMSO containing 0 or 10 μM BRZ daily for 3 d, followed by 7 d growth in a controlled growth chamber under long-day conditions (16 h light at 28 °C/8 °C dark at 24 °C). The angles of the third lamina joints were measured using IMAGEJ software.

Measuring endogenous BRs

BR contents were analyzed using gas chromatography–mass spectrometry (GC-MS) as described previously (Kim et al., 2005). Four-week-old shoots of the WT (88.50 g FW) and slg-D (65.43 g FW) were harvested, lyophilized, and extracted three times with 500mL of 80% methanol. Deuterium-labeled 6-deoxocastasterone (6-deoxoCS), tephysterol (TV), castasterone (CS), and BL were added as internal standards for quantitative analysis of the extracts (200 ng each).

 Yeast two-hybrid assay

The full-length cDNA of SLG was cloned into pGBKTK7 (Clontech, http://www.clontech.com). Full-length SLG as well as its N- and C-terminal truncated deletions were then subcloned into pGADT7 (Clontech) and all vectors were transformed into yeast strain AH109. A yeast two-hybrid library was constructed from the mRNA of young rice panicles 0.1–5 cm long. Yeast transformation and screening procedures were performed according to the manufacturer’s instructions.
instructions (Clontech). Primers used to make these constructs are listed in Supplementary Table S1.

Bimolecular fluorescence complementation (BiFC) assay
The full-length SLG cDNA was cloned into the vector pSPYCE(M), and the SLG cDNA and its truncated deletions were then subcloned into the vector pSPYNE173. The plasmids were transiently expressed in Nicotiana benthamiana leaves as described previously (Waadt and Kudla, 2008). Yellow fluorescent protein (YFP) fluorescent signals were observed under a Zeiss LSM510 confocal laser microscope between 48 h and 72 h post-transfection. Primers used to make these constructs are listed in Supplementary Table S1.

Pull-down assay
The SLG cDNA was cloned into the vectors pMAL-c2x and pGEX4T-2 to generate fusions with maltose-binding protein (MBP) and glutathione S-transferase (GST), respectively. Expression of MBP-SLG, GST-SLG, and GST in BL21 Rosetta cells was induced with 0.5 mM isopropyl-β-d-thiogalactoside at 16 °C for 20 h. The total protein concentration was quantified using the Bio-Rad protein assay reagent. The pull-down assay was performed as reported previously (Miernyk and Thelen, 2008). The proteins were separated on a 10% SDS–PAGE gel and immunoblotted with anti-GST or anti-MBP antibodies (Abmart, http://www.ab-mart.com). The primers used to make these constructs are listed in Supplementary Table S1.

Results

Phenotypic characterization of the semi-dominant mutant slg-D
To identify new components involved in regulating rice grain size, we screened a collection of activation-tagging T-DNA insertion rice lines (Jeon et al., 2000; Jeong et al., 2006). As a result, we isolated a mutant (3A-10513) with a slender-grain phenotype, and named it slender grain Dominant (slg-D). slg-D showed less compact plant architecture than the WT at both the vegetative and mature stages (Fig. 1A, B). In slg-D, the grain length was significantly increased while the grain width decreased, and the 1000-grain weight was slightly decreased (Fig. 1C–F). The lamina joint bending angles of slg-D were larger than those of the WT, especially for the flag leaves (Fig. 1G, H). Together, these results indicate that slg-D displays slender grain and an enlarged leaf angle.

The F1 plants from the cross slg-D×WT exhibited an intermediate phenotype in grain shape and leaf angle, indicating a semi-dominant nature of the mutation (Supplementary Fig. S1A–F). Genetic analyses of an F2 population derived from the same cross showed a segregation ratio of 1:2:1 (64

Fig. 1. Phenotype of the slg-D mutant. (A, B) Gross morphologies of the WT and slg-D at the vegetative (A) and mature (B) stages. (C) Slender grains of slg-D compared with the WT. (D–F) Measurements of traits showing longer but narrower grains (D and E), and reduced seed weight (F) in slg-D. (G) Comparison of the lamina joint angle of the flag (1), second (2), third (3), and fourth (4) leaves between slg-D and the WT (counted from the flag leaf downwards). (H) Quantification of the flag leaf lamina joint angles. Values are given as means ± SD (n=10 in D–F, H). **P<0.01 compared with the WT by Student’s t-test. Scale bars=10 cm (A, B), 2 mm (C), or 2 cm (G).
SLG controls grain size and leaf angle. SLG controls grain size and leaf angle

controls grain size and leaf angle suggesting that slg-D is a single locus mutation (Supplementary Fig. S1G). This observation provides a hint that the semi-dominant nature of slg-D might be associated with an insertion of the activation-tagging T-DNA.

Cell length change in slg-D determines the mutant phenotypes

To investigate the mutant phenotypes in slg-D at a cell level, we performed SEM and light microscopy on slg-D plants along with the WT. The observations on the outer and inner epidermal cells of lemmas, which determine the shape and size of grains, showed that those cells were stretched longitudinally in slg-D, such that the slender-grain phenotype was developed (Fig. 2A–G). The histological analysis on the second leaf lamina joints indicated that there was no significant alteration in cell size in the abaxial sides between slg-D and the WT (Fig. 2H–K). In contrast, the cell length of the adaxial sides was increased in slg-D (Fig. 2H, L–N). Therefore, it is the asymmetric cell expansion at the opposite sides of the lamina joint that causes a larger leaf bending in slg-D. Overall, these results indicate that the changes in cell length are responsible for the phenotype in slg-D.

Enhanced expression of Loc_Os08g44840 in slg-D leads to the mutant phenotypes

To isolate the gene for slg-D, we searched the T-DNA insertion database and obtained a genomic flanking sequence (Jeong et al., 2006; http://orygenesdb.cirad.fr/). Based on this information, we designed three PCR primers (P1, P2, and P3) and confirmed the site of the T-DNA insertion in slg-D (Fig. 3A, B). To understand whether the mutation in slg-D is related to an insertion of the activation-tagging T-DNA, we PCR-genotyped the mutant F₂ plants derived from the cross slg-D × WT and found that the mutant phenotypes were always associated with the presence of the T-DNA (Supplementary Fig. S2). A BLAST search (http://www.ncbi.nlm.nih.gov/) showed that the T-DNA was inserted in an intergenic region, with Loc_Os08g44830 2000 bp upstream and Loc_Os08g44840 4500 bp downstream (Fig. 3A).

Fig. 2. The changes in cell length are responsible for the phenotype in slg-D. (A) Spikelets just before heading. The red asterisk indicates the site used for detailed observation in (B–E). pa, palea; le, lemma. (B, C) SEM images of outer epidermal cells of lemmas. (D, E) SEM images of inner epidermal cells of lemmas. (F, G) Average length (F) and average width (G) of inner epidermal cells of lemmas. (H) Comparison of second leaf lamina joints (counted from the flag leaf downwards) between the WT and slg-D. Red lines indicate cut sites. ad, adaxial; ab, abaxial. (I, J) Longitudinal sections of the adaxial sides of the second leaf lamina joint shown in (H). (K) Average lengths of cells shown in (I) and (J). (L, M) Longitudinal sections of the adaxial sides of the second leaf lamina joints shown in (H). (N) Average lengths of cells shown in (L) and (M). Values are given as means ± SD (n=3 in F, G, K, N). **P<0.01 compared with the WT by Student’s t-test. Scale bars=2 mm (A), 50 μm (B–E), 2 cm (H), or 100 μm (I, J, L, M).
Another nearby gene is \textit{Loc\_Os08g44820}, upstream of \textit{Loc\_Os08g44830} (Fig. 3A). Next we examined if the four enhancer repeats in the T-DNA had an influence on the expression level of the three nearby genes, and found that two of them (\textit{Loc\_Os08g44830} and \textit{Loc\_Os08g44840}) had elevated expression (Fig. 3C). This result suggests that the changed expression level of the two genes might be responsible for the mutant phenotypes in \textit{slg-D}.

We assumed that overexpression of \textit{Loc\_Os08g44830}, \textit{Loc\_Os08g44840}, or both in the WT might recapitulate the phenotypes observed in \textit{slg-D}. To test this assumption, \textit{Loc\_Os08g44840} and \textit{Loc\_Os08g44830}, under control of the maize \textit{Ubi} promoter, were individually overexpressed in WT plants. Interestingly, only plants overexpressing \textit{Loc\_Os08g44840}, not \textit{Loc\_Os08g44830}, showed varying degrees of enlarged leaf angle and slender grain, thus phenocopying \textit{slg-D} (Fig. 3D–I; Supplementary Fig. S5). The phenotypic variation in the transgenic plants was well correlated with the expression level of \textit{Loc\_Os08g44840} (Fig. 3D–I). We concluded that it is the enhanced expression of \textit{Loc\_Os08g44840} that causes the phenotypic changes in \textit{slg-D}. We designated \textit{Loc\_Os08g44840} as a \textit{SLENDER GRAIN} (SLG) gene.

\textbf{Expression pattern and subcellular localization of SLG}

Our quantitative RT-PCR analysis showed that expression of \textit{SLG} is strong in young panicles, relatively high in lamina joints, low in shoot apices, culms and leaves, and very little in roots and leaf sheaths (Fig. 4A). Further, \textit{SLG} has the highest expression level during early panicle development, but drops dramatically as the spikelets reach their final size (Fig. 4B). To investigate further the expression pattern of \textit{SLG}, a genomic sequence \textasciitilde 2.5 kb upstream of the translation start site was cloned and introduced into the pCAMBIA1381Z vector, resulting in the \textit{PRO\_SLG\_GUS} reporter construct. Analysis of GUS activity in transgenic lines showed that...
strong GUS staining was observed in young panicles, lamina joints, and young stem nodes, with faint staining in leaf veins, but not visible in roots and leaf sheaths (Fig. 4C, parts 1–8). Cross-sectioning of the GUS-stained leaf lamina joint and the bottom portion of the stained internode further showed that the GUS signals were mainly restricted to vasculature regions (Fig. 4C, parts 9–12). We also performed RNA in situ hybridization to localize SLG expression during early panicle development more precisely. Strong SLG expression was detected in spikelet meristem primordia, floral meristem primordia, lemma and palea primordia, and vasculature regions (Fig. 4D). The predominant expression of SLG in young panicles and lamina joints implies its role in controlling grain shape and leaf angle.

To determine the subcellular localization of SLG, we fused the green fluorescent protein (GFP) to the C-terminus of SLG. Transient expression of this fusion protein in rice protoplasts revealed that the GFP signals were found in both the cytoplasm and the nucleus (Fig. 4E). Transgenic rice plants harboring the same fusion construct also showed the cytoplasmic and nuclear localization pattern of SLG (Fig. 4F).

**SLG positively regulates endogenous BR levels**

The slg-D phenotype resembles that of an activation mutant or transgenic plants with elevated BR accumulation (Wu et al., 2008; Wan et al., 2009), and that of...
transgenic plants with enhanced BR signaling (Tanaka et al., 2009; Tong et al., 2012), leading us to hypothesize that SLG may be involved in regulating the BR pathway. To determine whether slg-D responds differently to BR treatment, we first performed lamina joint bending assays using excised leaf segments (Wada et al., 1981). We measured the effects of a range of 24-epibrassinolide (BL; a type of active BR) concentrations on the angle of the lamina joints, and found that lamina joint bending was increased in a dose-dependent manner and the sensitivity to BL treatment was similar in slg-D and the WT (Fig. 5A, B). Next we performed another BR response assay involving coleoptile and root elongation using a previously described method (Yamamuro et al., 2000). Comparison of coleoptile and root lengths also showed that slg-D had a similar response to BL as the WT (see Supplementary Fig. S6). These results indicate that BR signaling is not altered in slg-D.

To investigate whether SLG functions in regulating endogenous BR levels, we first tested the effect of brassinazole (BRZ; a specific BR biosynthesis inhibitor; Asami et al., 2000) on slg-D in a lamina joint bending experiment. The leaf tips of 8-day-old seedlings of slg-D and the WT were spotted with 10 μM BRZ daily for 3 d, followed by 7 d growth in a chamber. We observed that the leaf angle of slg-D was restored to the WT level by BRZ treatment, whereas the WT seedlings had a milder response to the same treatment (Fig. 5C, D), indicating that slg-D is more sensitive to BRZ. A similar result was also seen when the D11 activation line m107, a BR overproduction mutant, was treated with BRZ (see Supplementary Fig. S7). Next we introduced the SLG-overexpressing construct into d61-1 (a loss-of-function mutant of the BR receptor gene OsBRI1; Yamamuro et al., 2000) and d11-2 (a mutant deficient in BR biosynthesis; Tanabe et al., 2005), and found that the d61-1 SLG:OE and d11-2 SLG:OE plants still retained the dwarfism, smaller and round grains, and erect leaves (Fig. 5E–G). Those results suggest a role for SLG in regulating BR levels. Consistent with this, our chemical analysis indeed showed a higher content of 6-deoxocastasterone (6-deoxoCS), typhasterol (TY), and castasterone (CS) in slg-D than in the WT (Supplementary Fig. S8).

It is known that excessive BRs down-regulate the BR-related genes D2, D11, OsDWARF4, BRD1, OsBRI1, and DLT, but up-regulate OsBZR1 as a feedback mechanism (Yamamuro et al., 2000; Hong et al., 2002, 2003; Wang et al., 2002; He et al., 2005; Tanabe et al., 2005; Sakamoto et al., 2006; Tong et al., 2009). We analyzed the expression level of those genes in slg-D and found that all except BRD1 had the expected transcription level change as a response to the elevated BR levels (Fig. 5H). As an alternative control, we also measured expression of those BR genes in the mutant m107, where D11 was dramatically enhanced and BR levels increased, and found similar expression changes (see Supplementary Fig. S9). Those results further confirm higher BR contents in slg-D. However, SLG itself did not respond to the exogenous BL treatment (Supplementary Fig. S10).

Fig. 5. SLG is involved in regulating endogenous BR levels but not BR signaling. (A) Response of the second leaf lamina joint to 10 μM BL treatment by the excised leaf segment method. (B) Dose response of the bending angle to various concentrations of BL. (C) Response of the third leaf lamina joint to 10 μM BRZ. (D) Measurement of the lamina joint angles after the 10 μM BRZ treatment shown in (C). (E, F) Gross morphologies (E) and grains (F) of WT, WT SLG:OE, d61-1, d61-1 SLG:OE, d11-2, and d11-2 SLG:OE. (G) Expression levels of SLG in the different lines shown in (E). (H) Quantitative RT-PCR analysis of BR-related genes in young panicles of slg-D and the WT. Values are given as means ±SD (n=10 in B, D; n=3 in G, H). Different letters (D) indicate P<0.01 (LSD multiple range tests). *P<0.05; **P<0.01 (H) compared with the WT by Student’s t-test. Scale bars=2 cm (A, C), 10 cm (E), or 2 mm (F).
Taken together, these results suggested that SLG positively regulates endogenous BR levels and is a new regulator of BR homeostasis in rice.

**Suppression of SLG leads to BR-deficient phenotypes**

To explore further the function of SLG, a SLG RNAi vector was constructed and introduced into WT plants. The SLG RNAi plants displayed a more compact architecture, reduced plant height, smaller leaf angle, and shorter and rounder grain (Fig. 6A–G). These phenotypes are similar to those of BR-deficient mutants, such as d61 and d11 (Hong et al., 2003; Tanabe et al., 2005). In addition, we investigated expression changes of the genes involved in BR synthesis or signaling in R7, a typical SLG RNAi line with greatly reduced leaf angle and SLG expression (Fig. 6F, G). Six of the genes detected, D2, D11, OsDWARF4, BRD1, OsBR11, and DLT were up-regulated, but OsBZR1 was down-regulated in R7 compared with the WT (Fig. 6H). The feedback regulation of those BR-related genes caused by knock-down of SLG further supports involvement of SLG in regulating the BR level. These results further highlight a role for SLG in regulating BR homeostasis. This observation also suggests that an optimized expression level of SLG may help create a compact and semi-dwarf ideal plant type.

**SLG functions as homomers**

It has been reported that enzyme proteins often function as homomers or heteromers (Ali and Imperiali, 2005). To investigate the functional forms of SLG, the full-length SLG protein was used as a bait to screen a yeast two-hybrid library prepared from young rice panicles. We identified four positive clones that contain different SLG cDNA fragments from ~1 million yeast transformants. To confirm the self-interaction of SLG, different truncated SLG proteins were used for interaction analysis. As shown in Fig. 7A, a 30 amino acid region in the N-terminus of SLG (SLGΔC4), rather than the two conserved motifs, was required for the self-interaction of SLG. An *in vitro* GST pull-down assay also confirmed the self-interaction (Fig. 7B). In addition, BiFC analysis also showed that SLG physically interacted with itself and this interaction required the N-terminal 30 amino acid region (Fig. 7C).

To study the importance of SLG self-interaction, two truncated SLG CDS, SLGΔC1 with only the 190 N-terminal amino acids, and SLGΔN3 without the 30 N-terminal amino acids, were individually overexpressed in slg-D and the WT. We found that overexpression of SLGΔC1 but not SLGΔN3 in both the WT and slg-D resulted in shorter and rounder grains, smaller leaf angles, and dwarf phenotypes, similar to those of SLG RNAi plants (Fig. 7; Supplementary Fig. S11). The truncated SLGΔC1 protein might interfere with

---

**Fig. 6.** Phenotypes of SLG RNAi transgenic plants. (A–C) Gross morphologies (A), grains (B), and leaf angles (C) of SLG RNAi transgenic plants in the WT background. R3 and R7 are two independent lines. (D–F) Quantitative comparisons of grain lengths (D), grain widths (E), and leaf angles (F) of the lines shown in (A). Leaf angles were measured at the positions indicated by the arrowheads in (C). (G) SLG expression in the lines shown in (A). (H) Quantitative RT-PCR analysis of BR-related genes in R7 and the WT. Values are given as means ±SD (n=10 in D–F; n=3 in G, H). *P<0.05; **P<0.01 compared with the WT by Student’s *t*-test. Scale bars=10 cm (A) or 2 mm (B).
formation of functional homomers between the intact SLG proteins, thus leading to a dominant negative mutant phenotype. When the interaction region was removed in SLGΔN3, however, the truncated protein did not exert any effect on SLG, thus providing genetic evidence that SLG indeed functions as homomers in vivo.

Discussion

In this study, we have provided evidence that SLG is involved in BR homeostasis by positively regulating endogenous BR levels to control grain size and leaf angle in rice. First, the activation-tagging mutant slg-D and transgenic plants overexpressing SLG displayed longer and narrower grains and larger leaf angles that are similar to the mutants or transgenic plants with enhanced BR signaling or increased BR levels. Secondly, slg-D and the WT had similar sensitivity to BL treatment in lamina joint bending, coleoptile elongation, and root elongation assays. Thirdly, the BRZ treatment restored slg-D to the WT. Fourthly, overexpression of SLG in the BR-related mutants, d61-1 and d11-2, did not lead to slender grains and enlarged leaf angles. Fifthly, the major BRs were increased in slg-D. Sixthly, feedback regulation on expression of the known BR genes was seen in slg-D. Lastly, knockdown of SLG resembled mild BR-deficient mutants.

The size of an organ is determined by cell proliferation and cell expansion (Potter and Xu, 2001; Sugimoto-Shirasu and Roberts, 2003). Our results showed that SLG is required for cell expansion in grains. To investigate the possible regulatory relationship between SLG and other previously identified genes that control grain size by influencing cell expansion, such as PGL1, GL7, and GS2/GL2 (Heang and Sassa, 2012; Che et al., 2015; Duan et al., 2015; Hu et al., 2015; Wang et al., 2015), we examined the transcript level of these genes and found no obvious difference between slg-D and the WT (see Supplementary Fig. S12). This result suggests that SLG may regulate grain size in a pathway independent of PGL1, GL7, and GS2/GL2.

SLG is predicted to encode a BAHD acyltransferase-like protein. Previous studies of BAHD acyltransferase family
members have shown that this family is capable of using CoA thioesters and catalyzing the formation of a wide variety of plant metabolites by generating ester or amide bonds (D’Auria, 2006). In Arabidopsis, two BAHD acyltransferases, BIA1 and BAT1, are involved in BR homeostasis, probably by conversion of active BR intermediates into inactive acylated BR conjugates (Roh et al., 2012; Choi et al., 2013). Overexpression of BIA1 or BAT1 results in decreased levels of active BRs and typical BR-deficient phenotypes (Roh et al., 2012; Choi et al., 2013). In our study, SLG, as a BAHD acyltransferase, probably converts an as yet unidentified substrate to the corresponding acyl conjugate to affect endogenous BR levels in an opposite way. Overexpression of SLG induced increased levels of active BRs and BR-overproduction phenotypes. The difference in Arabidopsis and rice implies that the function of BAHD acyltransferases in BR homeostasis has been differentiated. On the other hand, most of the enzymes known to catalyze BR biosynthesis belong to the cytochrome P450 protein family (Choe, 2006), implying that SLG, as a BAHD acyltransferase, may not work directly on the known BR intermediates, or that it may represent a different class of enzymes mediating BR synthesis. Further studies are needed to clarify how SLG participates in BR homeostasis.

In many cases, homomer formation is an essential biochemical process as it forms the complex quaternary structure of proteins to regulate selectivity against different substrates, enzyme activity, or stability (Dayhoff et al., 2010). Here, we showed that SLG interacted with itself, and its N-terminal 30 amino acid region was required for the interaction. It is likely that the self-interaction of SLG forms a functional enzyme complex with a special quaternary structure that binds the target substrates effectively. A truncated protein SLGΔC1 lacking a 255 amino acid C-terminus is still able to interact with the intact version but may form a complex unable to function properly due to a change in the quaternary structure, thus leading to a dominant negative phenotype. This finding also suggests that the substrate recognition and/or catalyzing domain may be located in the C-terminus. The version without the N-terminal interaction region failed to create a dominant negative phenotype. This finding also suggests that the homomer formation of SLG indeed exists in vivo. It will be interesting to investigate further the number of SLG proteins required to form a functional enzyme complex and its structural organization.

The plant architecture determines planting density, and thus yield. In rice, BR-deficient or -insensitive mutants show the erect leaf phenotype, such as d2, d11, and d61 (Yamamura et al., 2000; Hong et al., 2003; Tanabe et al., 2005). More erect leaves that increase light capture and thus enhance photosynthetic efficiency and nitrogen storage for grain filling can be combined with high planting densities to improve grain yield and biomass in rice (Sinclair and Sheehy, 1999; Morinaka et al., 2006; Sakamoto et al., 2006). For example, modulating the expression levels of OsDWARF4 and OsBR11 led to the erect leaf phenotype and efficiently improves rice grain yield and biomass in dense planting conditions (Morinaka et al., 2006; Sakamoto et al., 2006). SLG, when knocked-down by RNAi or interfered with by a truncated version, can create a compact semi-dwarf plant type with smaller leaf angles. Therefore, SLG can be used as an alternative to manipulate plant height for lodging resistance and leaf angle for planting density by optimizing its expression level, offering the potential for improving rice production.

Supplementary data
Supplementary data are available at JXB online.

Figure S1. The slg-D mutation behaves in a semi-dominant manner.

Figure S2. Co-segregation analysis of phenotypes and genotypes in F2 progeny.

Figure S3. Overexpression of Loc_Os08g44830 does not phenocopy the phenotypes of slg-D.

Figure S4. Phylogenetic tree of SLG homologs.

Figure S5. Alignment of the monocot group of SLG homologs.

Figure S6. Sensitivities of roots and coleoptiles to BL are not altered in slg-D.

Figure S7. Responses of WT and m107 leaf lamina joint angles to BRZ.

Figure S8. Measurements of endogenous BR intermediates.

Figure S9. Quantitative RT-PCR analysis of BR-related genes in young m107 and WT panicles.

Figure S10. Quantitative RT-PCR analysis of SLG expression in WT seedlings treated with BL.

Figure S11. Overexpression of SLGΔN3 does not change the phenotypes of the WT and slg-D.

Figure S12. Quantitative RT-PCR analysis of several genes that control grain size by influencing cell expansion in slg-D and the WT.

Table S1. Primers used in this study.

Acknowledgements
We owe special thanks to Professor G. An (Department of Plant Systems Biotech, Kyung Hee University, Korea) for providing slg-D lines, and to Professor Chencui Chu (Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, China) for providing d61-1, d11-2, and m107 lines. We also thank the supports by Key Laboratory of Biology, Genetics and Breeding of Japonica Rice in Mid-lower Yangtze River, Ministry of Agriculture, P.R.China, and Jiangsu Collaborative Innovation Center for Modern Crop Production, This research was supported by grants from the National Natural Science Foundation (91535302 and 31571601), 973 National Basic Research Program (2014CB943403), National Transformation Science and Technology Program of China (2014ZX08001006), 863 National High-tech R&D Program of China (2014AA10A603-15), Jiangsu Science and Technology Development Program (BE2014394), and the National Research Foundation of Korea (NRF-2014R1A1A2056102 to S-KK).

References
All MH, Imperialis B. 2005. Protein oligomerization: how and why. Bioorganic and Medicinal Chemistry 13, 5013–5020.
Asami T, Min YK, Nagata N, Yamagishi K, Takatsuto S, Fujioka S, Murofushi N, Yamaguchi I, Yoshida S. 2000. Characterization of brassinazole, a triazole-type brassinosteroid biosynthesis inhibitor. Plant Physiology 123, 93–100.
Ashikari M, Sakakibara H, Lin S, Yamamoto T, Takashii T, Nishimura A, Angeles ER, Qian Q, Kitano H, Matsuoka M. 2005. Cytokinin oxidase regulates rice grain production. Science 309, 741–745.
Ashikari M, Wu J, Yano M, Sasaki T, Yoshimura A. 1999. Rice gibberellin-insensitive dwarf mutant gene Dwarf 1 encodes the
alpha-subunit of GTP-binding protein. Proceedings of the National Academy of Sciences, USA 96, 10284–10289.

Belkhadir Y, Chory J. 2006. Brassinosteroid signaling: a paradigm for steroid hormone signaling from the cell surface. Science 314, 1410–1411.

Berleth T, Sachs T. 2001. Plant morphogenesis: long-distance coordination and local patterning. Current Opinion in Plant Biology 4, 57–62.

Bradley D, Carpenter R, Sommer H, Hartley N, Coen E. 1993. Complementary floral homeotic phenotypes result from opposite orientations of a transposon at the plena locus of Antirrhinum. Cell 72, 85–95.

Che R, Tong H, Shi B, et al. 2015. Control of grain size and rice yield by GL2-mediated brassinosteroid responses. Nature Plants 2, 15195.

Chen S, Tao L, Zeng L, Vega-sanchez ME, Umemura K, Wang G. 2006. A highly efficient transient protoplast system for analyzing defence gene expression and protein–protein interactions in rice. Molecular Plant Pathology 7, 417–427.

Choe S. 2006. brassinosteroid biosynthesis and inactivation. Physiologia Plantarum 126, 539–548.

Choi S, Cho YH, Kim K, Matsu M, Son SH, Kim SK, Fujioka S, Hwang I. 2013. BAT1, a putative acyltransferase, modulates brassinosteroid levels in Arabidopsis. The Plant Journal 73, 380–391.

Clouse SD, Sasse JM. 1998. Brassinosteroids: essential regulators of plant growth and development. Annual Review of Plant Physiology and Plant Molecular Biology 49, 427–451.

D’Auria JC. 2006. Aciytransferases in plants: a good time to be BAHD. The Plant Journal 45, 314–340.

Dayhoff JE, Shoemaker BA, Bryant SH, Panchenko AR. 2010. Evolution of protein binding modes in homooligomers. Journal of Molecular Biology 395, 860–870.

Duan P, Ni S, Wang J, Zhang B, Xu R, Wang Y, Chen H, Zhu X, Li Y. 2015. Regulation of OsGFR4 by OsmR396 controls grain size and yield in rice. Nature Plants 2, 15023.

Fujioka S, Yokota T. 2003. Biosynthesis and metabolism of brassinosteroids. Annual Review of Plant Biology 54, 137–164.

He JX, Gendron JM, Sun Y, Gampala SS, Gendron N, Sun CQ, Wang ZY. 2005. BZR1 is a transcriptional repressor with dual roles in brassinosteroid homeostasis and growth responses. Science 307, 1634–1638.

Heang D, Sassa H. 2012. Antagonistic actions of HLH/bHLH proteins are involved in grain length and weight in rice. PLoS One 7, e31325.

Hiei Y, Ohta S, Komari T, Kumashiro T. 1994. Efficient transformation of rice (Oryza sativa L.) mediated by Agrobacterium and sequence analysis of the boundaries of the T-DNA. The Plant Journal 6, 271–282.

Hong Z, Ueguchi-Tanaka M, Shimizu-Sato S, et al. 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. The Plant Journal 32, 495–508.

Hong Z, Ueguchi-Tanaka M, Umemura K, Ouzo S, Fujioka S, Takatsuto S, Yoshida S, Ashikari M, Kitano H, 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. The Plant Cell 15, 2900–2910.

Hoshikawa K. 1989. Leaf. In: The growing rice plant. Tokyo: Nobunkyo.

Hu J, Wang Y, Fang Y, et al. 2015. A rare allele of GS2 enhances grain size and grain yield in rice. Molecular Plant 8, 1445–1465.

Ikeda M, Miura K, Aya K, Kitano H, Matsuoka M. 2013. Genes offering the potential for designing yield-related traits in rice. Current Opinion in Plant Biology 16, 213–220.

Ishimaru K, Hirotsu N, Madoka Y, et al. 2013. Loss of function of the IAA-glucose hydrolase gene TGW6 enhances rice grain weight and increases yield. Nature Genetics 45, 707–711.

Jefferson RA, Kavanagh TA, Bevan MW. 1987. GUS fusions: β-glucoronidase as a sensitive and versatile gene fusion marker in higher plants. EMBO Journal 6, 3901–3907.

Jeon JS, Lee S, Jung KH, et al. 2000. T-DNA insertional mutagenesis for functional genomics in rice. The Plant Journal 22, 561–570.

Jeong DH, An S, Park S, et al. 2006. Generation of a flanking sequence-tag database for activation-tagging lines in japonica rice. The Plant Journal 45, 123–132.

Kim T, Hwang JY, Joo S, Chang S, Lee J, Takatsuto S, Kim S. 2005. Arabidopsis CYP85A2, a cytochrome P450, mediates the Baeyer–Villiger oxidation of castasterone to brassinolide in brassinosteroid biosynthesis. The Plant Cell 17, 2397–2412.

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

Li Y, Fan C, Xing Y, et al. 2011. Natural variation in GS5 plays an important role in regulating grain size and yield in rice. Nature Genetics 43, 1266–1269.

Li Z, Paterson AH, Pinson SRM, Stansel JW. 1999. RFLP facilitated analysis of tiller and leaf angles in rice (Oryza sativa L.). Euphytica 109, 79–84.

Li ZK, Paterson AH, Pinson SRM, Khush G. 1998. A major gene, Ta1 and QTLs affecting tiller and leaf angles in rice. Rice Genetics Newsletter 15, 79–84.

Liu L, Tong H, Xiao Y, Che R, Xu F, Hu B, Liang C, Chu J, Liu C, Chu G. 2015. Activation of Big Grain1 significantly improves grain size by regulating auxin transport in rice. Proceedings of the National Academy of Sciences, USA 112, 11102–11107.

Mao H, Sun S, Yao J, Wang C, Yu S, Xu C, Li X, Zhang Q, et al. 2010. Linking differential domain functions of the GS3 protein to natural variation of grain size in rice. Proceedings of the National Academy of Sciences, USA 107, 19579–19584.

Miernyk JA, Thelen JJ. 2008. Biochemical approaches for discovering protein–protein interactions. The Plant Journal 53, 597–609.

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

Nakamura A, Fujioka S, Takatsuto S, Tsujimoto M, Kitano H, Yoshida S, Asami T, Nakano T. 2009. Involvement of C-22-hydroxylated brassinosteroids in auxin-induced lamina joint bending in rice. Plant and Cell Physiology 50, 1627–1635.

Ning J, Zhang B, Wang N, Zhou Y, Xiong L. 2011. Increased leaf angle1, a Raf-like MAPKKK that interacts with a nuclear protein family, regulates mechanical tissue formation in the lamina joint of rice. The Plant Cell 23, 4334–4347.

Potter CJ, Xu T. 2001. Mechanisms of size control. Current Opinion in Genetics and Development 11, 279–286.

Roh H, Jeong CW, Fujioka S, Kim YK, Lee S, Ahn JH, Choi YD, Lee JS. 2012. Genetic evidence for the reduction of brassinosteroid levels by a BAHD acyltransferase-like protein in Arabidopsis. Plant Physiology 159, 696–709.

Sakamoto T, Morinaka Y, Ohnishi T, et al. 2006. Erect leaves caused by brassinosteroid deficiency increase biomass production and grain yield in rice. Nature Biotechnology 24, 105–109.

Shimada Y, Goda H, Nakamura A, Takatsuto S, Fujioka S, Yoshida S. 2003. Organ-specific expression of brassinosteroid biosynthetic genes and distribution of endogenous brassinosteroids in Arabidopsis. Plant Physiology 131, 287–297.

Sinclair TR, Sheehy JE. 1999. Erect leaves and photosynthesis in rice. Science 283, 1455.

Song XJ, Huang W, Shi M, Zhu MZ, Lin HX. 2007. A QTL for rice grain width and weight encodes a previously unknown RING-type E3 ubiquitin ligase. Nature Genetics 39, 623–630.

Song XJ, Kouroha T, Aymo M, et al. 2015. Rare allele of a previously unidentified histone H4 acetyltransferase enhances grain weight, yield, and plant biomass in rice. Proceedings of the National Academy of Sciences, USA 112, 76–81.

Sugimoto-Shirasu K, Roberts K. 2003. ‘Big it up’: endoreduplication and cell-size control in plants. Current Opinion in Plant Biology 6, 544–553.

Symons GM, Reid JB. 2004. Brassinosteroids do not undergo long-distance transport in pea. Implications for the regulation of endogenous brassinosteroid levels. Plant Physiology 135, 2196–2206.

Tanabe S, Ashikari M, Fujioka S, et al. 2005. A novel cytochrome P450 is implicated in brassinosteroid biosynthesis via the characterization of a rice dwarf mutant, dwart1, with reduced seed length. The Plant Cell 17, 776–790.

Tanaka A, Nakagawa H, Tomita C, et al. 2009. BRASSINOSTEROID UPREGULATED1, encoding a helix–loop–helix protein, is a novel gene
involved in brassinosteroid signaling and controls bending of the lamina joint in rice. Plant Physiology 151, 669–680.

Tong H, Jin Y, Liu W, Li F, Fang J, Yin Y, Qian Q, Zhu L, Chu C. 2009. DWARF AND LOW-TILLERING, a new member of the GRAS family, plays positive roles in brassinosteroid signaling in rice. The Plant Journal 58, 803–816.

Tong H, Liu L, Jin Y, Du L, Yin Y, Qian Q, Zhu L, Chu C. 2012. DWARF AND LOW-TILLERING acts as a direct downstream target of a GSK3/SHAGGY-like kinase to mediate brassinosteroid responses in rice. The Plant Cell 24, 2562–2577.

Unnevehr LJ, Duff B, Juliano VO. 1992. Consumer demand for rice grain quality: terminal report of IDRC projects National Grain Quality (Asia) and International Grain Quality Economics (Asia). International Rice Research Institute.

Waadt R, Kudla J. 2008. In planta visualization of protein interactions using bimolecular fluorescence complementation (BiFC). CSH Protocols 2008, t4995.

Wada K, Marumo S, Ikekawa N, Morisaki M, Mori K. 1981. Brassinolide and homobrassinolide promotion of lamina inclination of rice seedlings. Plant and Cell Physiology 22, 323–325.

Wan S, Wu J, Zhang Z, et al. 2009. Activation tagging, an efficient tool for functional analysis of the rice genome. Plant Molecular Biology 69, 69–80.

Wang S, Wu K, Yuan Q, et al. 2012. Control of grain size, shape and quality by OsSPL16 in rice. Nature Genetics 44, 950–954.

Wang Y, Xiong G, Hu J, et al. 2015. Copy number variation at the GL7 locus contributes to grain size diversity in rice. Nature Genetics 47, 944–948.

Wang ZY, Nakano T, Gendron J, et al. 2002. Nuclear-localized BZR1 mediates brassinosteroid-induced growth and feedback suppression of brassinosteroid biosynthesis. Developmental Cell 2, 505–513.

Weng J, Gu S, Wan X, et al. 2008. Isolation and initial characterization of GW5, a major QTL associated with rice grain width and weight. Cell Research 18, 1199–1209.

Wu CY, Trieu A, Radhakrishnan P, et al. 2008. Brassinosteroids regulate grain filling in rice. The Plant Cell 20, 2130–2145.

Wu Z, Zhang X, He B, et al. 2007. A chlorophyll-deficient rice mutant with impaired chlorophyllide esterification in chlorophyll biosynthesis. Plant Physiology 145, 29–40.

Yamamuro C, Ihara Y, Wu X, Noguchi T, Fujioka S, Takatsu S, Ashikari M, Kitano H, Matsuoka M. 2000. Loss of function of a rice brassinosteroid insensitive1 homolog prevents internode elongation and bending of the lamina joint. The Plant Cell 12, 1591–1606.

Zhang LY, Bai MY, Wu J, et al. 2009. Antagonistic HLH/bHLH transcription factors mediate brassinosteroid regulation of cell elongation and plant development in rice and Arabidopsis. The Plant Cell 21, 3767–3780.

Zhao SQ, Hu J, Guo LB, Qian Q, Xue HW. 2010. Rice leaf inclination 2, a VIN3-like protein, regulates leaf angle through modulating cell division of the collar. Cell Research 20, 935–947.