OsGRAS19 May Be a Novel Component Involved in the Brassinosteroid Signaling Pathway in Rice

Dear Editor,

The brassinosteroid (BR) signaling pathway has been well elucidated in *Arabidopsis*. The identification of BR-signaling components in rice suggested that the primary pathway is conserved in both dicot and monocot plants. However, some novel rice-specific components involved in BR signaling were reported in the past years, indicating a more complex BR-signaling pathway in monocot (Tong and Chu, 2012). The GRAS (GA INSENSITIVE (GAI), REPRESSOR OF *GAI* (RGA), and SCARECROW (SCR)) genes belong to a plant-specific gene family of putative transcription factors and play important roles in diverse processes of plant development and responses to environmental stresses (Bolle, 2004; Tian et al., 2004). Recent studies suggested that GRAS proteins are also involved in BR signaling (Tong et al., 2009, 2012).

With *SCR* as a query, 57 putative GRAS genes were previously identified in the rice genome (Tian et al., 2004). We selected 13 members of rice GRAS genes for further study by a reverse genetics approach. Among the obtained transformants, we found *OsGRAS19* RNAi and overexpression transgenic plants showed dramatic morphological changes. Therefore, we focused on this gene for an in-depth characterization.

OsGRAS19 encodes a polypeptide of 578 amino acid residues. Sequence alignment with SCR, SLENDER RICE 1 (SLR1), and MONOCULM (MOC1) revealed that OsGRAS19 contains all the five conserved motifs of VHIID, PFYRE, SAW, and two LHRs at its C-terminus and a highly variable sequence at its N-terminus (Supplemental Figure 1). In addition to these conserved motifs, OsGRAS19 contains another two signatures: ^{208}LQSLL^{212} and ^{414}LYHLL^{418}, which are identical to the consensus motif (LXXLL) that has been shown to mediate binding of transcriptional co-activators to nuclear receptors (Heery et al., 1997; Supplemental Figure 1).

By searching the *Arabidopsis* database, we identified two homologs of OsGRAS19: At5g66770 and At3g50650, which show 47.3% and 46.7% identities to OsGRAS19, respectively. Particularly, their foremost 25 amino acid residues at the N-termini are almost identical to OsGRAS19, which are not found in any other GRAS proteins (Supplemental Figure 1). This signature might be a novel functional motif that defines a new subgroup of GRAS family. Actually, Tian et al. (2004) categorized OsGRAS19 into LS subfamily, which consists of two subgroups; one comprises OsGRAS19, At5g66770, and At3g50650 and the other LAS, LS, MOC1, and OsGRAS7, implying that these two subgroups might play different roles in plant development.

To understand the roles of OsGRAS19, we first analyzed the expression pattern of *OsGRAS19* by RT–PCR. As shown in Supplemental Figure 2, *OsGRAS19* was detected in all examined organs, including roots, culms, shoot apexes, leaf blades, leaf sheathes, and panicles, with more abundance in young organs than in mature ones. To elucidate biological functions of *OsGRAS19*, we knocked down the *OsGRAS19* expression level in Nipponbare (hereafter refers to wild-type) via RNA interference (RNAi) (Supplemental Figure 3A and 3B). Compared with the wild-type, *OsGRAS19* RNAi plants exhibited erect leaves and panicles (Figure 1A and 1C, and Supplemental Figure 4A). In addition, *OsGRAS19* RNAi plants displayed a significant increase in the mechanical strength of panicle stems (Supplemental Figure 4B), which may result from increased vascular bundles, thicker sclerenchyma, and increased cell layers (Supplemental Figure 5). Furthermore, the first three internodes of these RNAi plants were all significantly shortened (Supplemental Table 1), resulting in reduced stature with an increase in diameters of culms and panicle stems (Supplemental Figure 6A and Supplemental Table 2).

In contrast, all the *OsGRAS19*-overexpressing plants showed essentially opposite phenotypes compared to that of *OsGRAS19* RNAi lines (Figure 1B and Supplemental Figure 3C and 3D). Particularly, *OsGRAS19*-overexpressing plants displayed narrow leaves (Supplemental Figure 6B), larger leaf angles (Figure 1C), thin culms and panicle stems (Supplemental Figure 6A and Supplemental Table 2).

The pleiotropic phenotypes of *OsGRAS19* transgenic plants suggested that *OsGRAS19* might be an important regulator in certain fundamental processes or signaling transduction pathways. In rice, erect leaf has long been considered as a representative phenotype of BR-deficient or insensitive mutants. In fact, the phenotypes of *OsGRAS19* RNAi lines were reminiscent of the *d61* mutant, which is a well characterized rice *BRI1* mutant (Yamamuro et al., 2000). Therefore, we presumed that *OsGRAS19* might be involved in the BR-signaling pathway.

To test our hypothesis, we carried out three experiments. First, we compared the lamina joint inclination between the wild-type and *OsGRAS19* RNAi plants after the treatment

doi:10.1093/mp/sst027, Advance Access publication 6 February 2013

Received 13 December 2012; accepted 2 February 2013

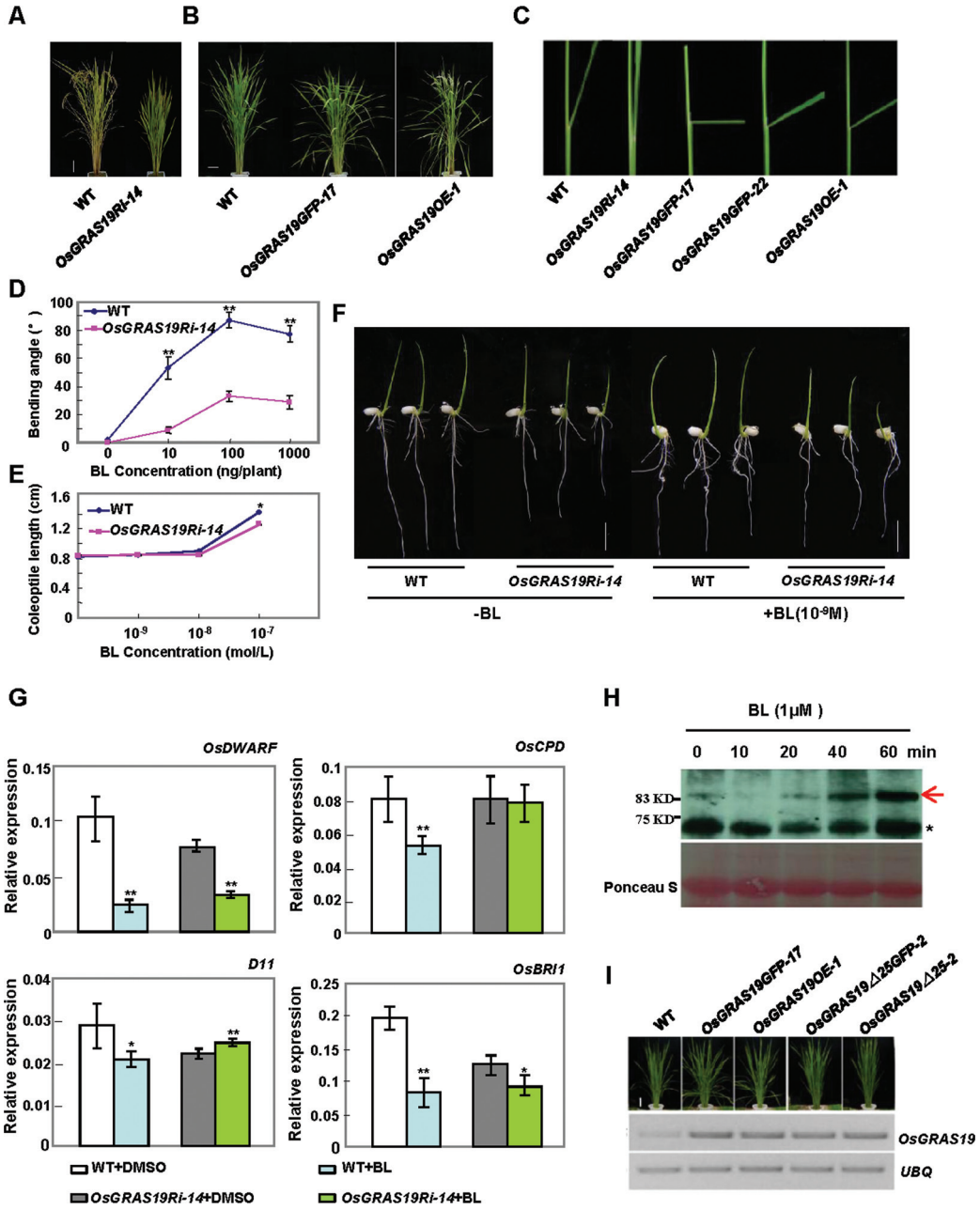

Figure 1. OsGRAS19 Acts as a Positive Regulator in the BR-Signaling Pathway.

(A) The *OsGRAS19* RNAi transgenic plant at mature stage. Bar = 10 cm.

(B) Phenotypes of *OsGRAS19* overexpressing plants at the mature stage. Bar = 10 cm.

(C) Leaf angle was altered in the *OsGRAS19* transgenic plant.

(D) *OsGRAS19* RNAi plants were less sensitive compared with the wild-type upon BL treatment in lamina joint inclination assay.

(E) Coleoptile elongation was less sensitive to BL treatment in OsGRAS19RNAi transgenic plants compared with the wild-type. Error bars represent SE of the mean in (D) and (E) ($n = 10$). The single and double asterisks represent significance difference determined by the Student's *t*-test at $P < 0.05$ and $P < 0.01$, respectively.

(F) Root inhibition was less sensitive in *OsGRAS19* RNAi plants compared with the wild-type upon BL treatment.

(G) Real-time PCR results showing the relative expression levels of genes involved in BR biosynthesis and signaling upon BL treatment in the wild-type and *OsGRAS19* RNAi plants. Error bars represent SE of the mean ($n = 3$). The single and double asterisks represent significance difference determined by the Student's *t*-test at $P < 0.05$ and $P < 0.01$, respectively.

(H) Time-course analysis of OsGRAS19 accumulation upon BL treatment. The arrow indicates OsGRAS19–GFP bands. The asterisk indicates the unspecific bands.

(I) The phenotypes of *OsGRAS19Δ25* and *OsGRAS199Δ25GFP*-overexpressing transgenic plants; the RT–PCR in the lower panel shows the expression levels of target genes in the transgenic plants. Bar = 10 cm.

of 24-epi-brassinolide (BL). In the absence of BL, leaves of wild-type and *OsGRAS19* RNAi plants were nearly erect. The leaf angle of the wild-type dramatically increased to ~90° with the exogenous supplement of BL, in contrast to ~35° in *OsGRAS19* RNAi seedlings (Figure 1D). Second, we compared the sensitivities of coleoptile elongation upon BL treatment between the wild-type and *OsGRAS19* RNAi seedlings. As shown in Figure 1E, the coleoptile elongation of *OsGRAS19* RNAi plants was significantly less than that of the wild-type at the presence of 10^{-7} M BL. Third, we compared the effect of BR on root growth between the wild-type and *OsGRAS19* RNAi seedlings. As shown in Figure 1F, the wild-type roots of the seedlings germinated on 1.0 nM BL-containing medium were shortened and twisted, indicating an inhibition effect of BL on the root growth. However, the inhibition effect of BL was not found in *OsGRAS19* RNAi roots (Figure 1F). Based on these results, we concluded that *OsGRAS19* RNAi plants were less sensitive to exogenous application of BL, suggesting that *OsGRAS19* is involved in the BR-signaling pathway.

In *Arabidopsis*, the exogenous treatment of BL leads to a decreased expression of *CONSTITUTIVE PHOTOMORPHOGENIC DWARF* (*CPD*), which encodes a critical enzyme in the BR synthetic pathway (He et al., 2005). Similarly, the expression levels of BR receptor gene *BR INSENSITIVE 1* (*OsBRI1*) and BR biosynthetic genes including *D2*, *D11*, and *OsDWARF* are also feedback-inhibited by BR in rice (Yamamuro et al., 2000; Hong et al., 2002, 2003; Tanabe et al., 2005). To further confirm the involvement of OsGRAS19 in BR signaling, we detected the expression levels of these feedback-regulated genes of the BR-signaling pathway in the wild-type and *OsGRAS19* RNAi plants in the absence or presence of 0.1 μM BL. The results showed that the expression of *OsBRI1*, *OsDWARF*, *OsCPD*, and *D11* was strongly inhibited by the exogenous supplement of BL in the wild-type. However, the feedback inhibition of these genes by BL was either completely released or much attenuated in *OsGRAS19* RNAi transgenic lines (Figure 1G), indicating that *OsGRAS19* is required for feedback regulation downstream of the BR-signaling pathway.

To explore whether OsGRAS19 mRNA or its translated protein is responsive to BL treatment, we first examined the *OsGRAS19* mRNA levels with or without BL treatment. The result showed that the expression level of *OsGRAS19* slightly decreased upon BL treatment (Supplemental Figure 7A). However, when the *OsGRAS19GFP-17* transgenic seedlings were treated with 1 μM BL, we found that the OsGRAS19–GFP protein level significantly increased after 40-min treatment and reached maximum after 1-h treatment (Figure 1H). This result implied that the stability of the OsGRAS19 protein may be dependent on BR signaling.

Sequence alignment revealed that OsGRAS19 contains a unique conserved domain consisting of 25 amino acid residues (25 aa) at the N-terminal, as its two *Arabidopsis* homologs (Supplemental Figure 1). To test whether the 25-aa residues are essential for its function, we overexpressed OsGRAS19Δ25GFP or OsGRAS19Δ25, which is a truncated OsGRAS19 lacking the N-terminal 25 aa (with or without a GFP tag) driven by the ubiquitin promoter. As shown in Figure 1I, neither OsGRAS19Δ25GFP nor OsGRAS19Δ25 could mimic the phenotypes of OsGRAS19GFP or OsGRAS19OE transgenic plants. Further, we analyzed the accumulation of OsGRAS19Δ25 in OsGRAS19Δ25GFP transgenic plants after the treatment with BL and found that the treatment of BL had no significant effect on the accumulation of OsGRAS19Δ25 (Supplemental Figure 7B). These results demonstrated that N-terminal 25 aa residues are essential for the function of OsGRAS19.

In conclusion, we have identified OsGRAS19, a new member of the GRAS family, as a positive regulator in the BR-signaling pathway. We found a novel motif that could mediate functions of OsGRAS19 and its homologs specific to the BR-signaling transduction pathway.

SUPPLEMENTARY DATA

Supplementary Data are available at *Molecular Plant Online*.

FUNDING

This work was supported by grants from the Ministry of Science and Technology of the People's Republic of China (2011CB100200) and the National Natural Science Foundation of China (31025004, 90817108). No conflict of interest declared.

Lisheng Chen[a,b,2], Guosheng Xiong[a,2], Xia Cui[a], Meixian Yan[c], Ting Xu[d], Qian Qian[c], Yongbiao Xue[d], Jiayang Li[a] and Yonghong Wang[a,1]

a State Key Laboratory of Plant Genomics and National Center for Plant Gene Research, Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing 100101, China
b Present address: Molecular, Cellular, and Developmental Biology, University of Michigan, Ann Arbor, MI 48105, USA
c State Key Laboratory of Rice Biology, China National Rice Research Institute, Chinese Academy of Agricultural Sciences, Hangzhou 310006, China
d State Key Laboratory of Molecular Developmental Biology, Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing 100101, China
[1] To whom correspondence should be addressed. E-mail yhwang@genetics.ac.cn, tel. +8610-64806008, fax +8610-64806595.
[2] These authors contributed equally to this work.

REFERENCES

Bolle, C. (2004). The role of GRAS proteins in plant signal transduction and development. Planta. **218**, 683–692.

He, J.X., Gendron, J.M., Sun, Y., Gampala, S.S., Gendron, N., Sun, C.Q., and Wang, Z.Y. (2005). BZR1 is a transcriptional repressor with dual roles in brassinosteroid homeostasis and growth responses. Science. **307**, 1634–1638.

Heery, D.M., Kalkhoven, E., Hoare, S., and Parker, M.G. (1997). A signature motif in transcriptional co-activators mediates binding to nuclear receptors. Nature. **387**, 733–736.

Hong, Z., Ueguchi-Tanaka, M., Shimizu-Sato, S., Inukai, Y., Fujioka, S., Shimada, Y., Takatsuto, S., Agetsuma, M., Yoshida, S., Watanabe, Y., 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. 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.

Tanabe, S., Ashikari, M., Fujioka, S., Takatsuto, S., Yoshida, S., Yano, M., Yoshimura, A., Kitano, H., Matsuoka, M., Fujisawa, Y., et al. (2005). A novel cytochrome P450 is implicated in brassinosteroid biosynthesis via the characterization of a rice dwarf mutant, *dwarf11*, with reduced seed length. Plant Cell. **17**, 776–790.

Tian, C., Wan, P., Sun, S., Li, J., and Chen, M. (2004). Genome-wide analysis of the GRAS gene family in rice and *Arabidopsis*. Plant Mol. Biol. **54**, 519–532.

Tong, H., and Chu, C. (2012). Brassinosteroid signaling and application in rice. J. Genet. Genomics. **39**, 3–9.

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

Tong, H., Liu, L., Jin, Y., Du, L., Yin, Y., Qian, Q., Zhu, L., and 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. Plant Cell. **24**, 2562–2577.

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**, 1591–1606.