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Artificially Controlling Morphogenesis by Altering Plant Function Based on the Elucidation of Molecular Mechanism for Brassinosteroids and Gibberellins Signal Transduction

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ABSTRACT

Brassinosteroids (BRs) and gibberellins (GAs) are essential plant growth-promoting natural products that are required for normal plant elongation and during development. The underlying molecular mechanisms for signal transduction involving these phytohormones will be elucidated using the methods of molecular genetics and protein chemistry, and information from the rice genome. Altering plant function will help the next generation of rice plants with the ideal grass type having high-yield and improved grain quality, which will greatly contribute to and enhance agricultural productivity. In this review, we discuss the molecular mechanism of BR- and GA-regulated genes based on the phonotype of our constructed transgenic rice.

Keywords: rice, brassinosteroid, gibberellin, transgenic rice
Abbreviations: BR, brassinosteroid; **GA**, gibberellin

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INTRODUCTION

Rice is one of the world's most important agricultural resources, because it is indisputably the only plant species that feeds almost half of the world's population. Rice is also a model plant for biological research, because its genome is smaller than those of other cereals (Devos and Gale 2000) and it has an important syntenic relationship with the other cereal species (Gale and Devos 1998). The International Rice Genome Sequencing Project (2005) presented a map-based, finished-quality sequence that covers 95% of the 389 Mb genome of rice, including virtually all of the euchromatin and two complete centromeres. Once the rice genome is completely sequenced, the challenge ahead for the plant research community will be to identify the function of genes. To assign function to unknown genes, differential genomic methodologies, which are termed phenomics, transcriptomics, proteomics and metabolomics, are being developed and used (Holtorf *et al*. 2002).

Using cDNA microarray (Yang *et al*. 2004) and proteomic approaches (Komatsu *et al*. 2003), we have been systematically analyzing changes induced by phytohormones brassinosteroid (BR) and gibberellin (GA) both at transcriptional and translational levels in rice seedlings. Plant hormones play an important role in many aspects of signal transduction in cells, as well as in several growth and development pathways, such as seed dormancy, germination, stem elongation, leaf expansion and fruit development. BRs and GAs are two groups of plant growth regulators that are essential for normal growth and development (Mandava

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1988; Swan and Olszewski 1996). While rapid progress has been made in the study of the biosynthesis and metabolism of BRs (Schumacher and Chory 2000) and GAs (Hedden and Kamiya 1997) using biochemical techniques, as well as by the characterization of their biosynthetic mutants, not much is known about how they regulate a wide variety of physiological processes at the molecular level.

Mutants are very useful biological resource for identification of genes and functional analysis. In the case of rice, some genes for the enzymes involved in BR biosynthesis have been isolated and characterized. A rice gene that was mutated in a BR-deficient dwarf mutant, and which encodes a C-6 oxidase, was isolated and characterized, indicating that endogenous BRs were also important for normal growth and development in monocot plants (Hong *et al*. 2002). The genes for most of the enzymes involved in GA biosynthesis have been isolated and characterized (Olszewski *et al*. 2002). The *SD1* gene, mutations in which were responsible for the short stature of the semi-dwarf, high-yield rice variety IR8, has been cloned, and it was found to encode a GA_{20} oxidase, a key enzyme in the GA biosynthesis pathway, and its characterization provided useful information for regulating the heights of other crop plants by manipulating GA biosynthesis (Sasaki *et al*. 2002).

In the case of signal transduction, although the molecular mechanism by which plants respond to GA and BR is still largely unknown, several important components of the GA and BR signal transduction pathway have been identified. A gene encoding a putative protein kinase with a high similarity to BRI1 was isolated from BR-insensitive rice dwarf mutant d61 (Yamamuro *et al*. 2000). The dwarf1 (d1) mutant in rice is characterized by GA insensitive semi-dwarf phenotype, and cloning of the D1 locus revealed that it encodes the putative α-subunit of the heterotrimeric G protein (Ashikari *et al*. 1990). The major effects of BR and GA on plant growth and development are mediated via the modulation of gene expression, because inhibition of RNA and protein synthesis interfere with these processes. So, we constructed transgenic rice plants into which genes of sense or antisense direction and/or RNAi were introduced, to analyze the molecular mechanism of GA- and BR-regulated genes.

CONSTRUCTION OF TRANSGENIC RICE

Construction of antisense gene transgenic rice

For constructing antisense transgenic rice for *OsBLE1*, *OsBLE 2*, *OsBLE3* or *OsGAE1*, the full-length *OsBLE1*, *OsBLE*, *OsBLE3* or *OsGAE1* cDNA sequence in the pBluescript SK+ plasmid was amplified by PCR using primer pairs of 5′-GCTCTAGACTGGAACATCGTGGGGGTATT-3′ (5′-end, underlining the *Xba*I site as a linker) and 5′-GCGTGACCTATCTCACACATTGCGAGAGG-3′ (3′-side, underlining the *Sal*I site as a linker). The resulting PCR product was cut, purified and ligated between the *CaMV 35S* promoter and nopaline synthase (nos) terminator in the binary vector pIG121-Hm by replacing the GUS coding region (Ohta *et al*. 1990; Yang and Komatsu 2004; Yang *et al.* 2003; Jan *et al*. 2006b; Yang *et al.* 2006).

Construction of RNAi gene transgenic rice

The 3′UTR region of *OsXTH8* or *OsPDK1*was amplified in both sense and antisense orientations. The sense RNAi fragment was amplified using primer pairs 5′-GGGGTAC CAGGCTTCCCTGTCCTGATACCA -3′ (5′ region, *Kpn*I is underlined as a linker) and 5′-GCGTGACCTATCTCA CACATTGCGAGAGG-3′ (3′-side, underlining the *Sal*I site as a linker). The antisense RNAi fragment was amplified using primer pairs 5′-CGTCTAGAGATGAAGTTAG CTTACTACTGA-3′ (5′ region, *Xba*I is underlined as a linker) and 5′-CGGGATCCAGGCTTCCCTGTCCTGAT ACCA-3′ (3′ region, *Bam*HI is underlined as a linker). The resulting PCR fragments were ligated between the *CaMV 35S* promoter and nos terminator in the binary vector pIG121-Hm (Ohta *et al*. 1990) in a position sandwiching 700 bp of the partial GUS coding region. The pIG121-Hm/RNAi *OsXTH8* or *OsPDK1* constructs were confirmed by restriction mapping and sequencing (Jan *et al*. 2004, 2006a).

Transfer and selection of transgenic rice plant

After construction, the binary vector constructs were then transferred into *Agrobacterium tumefaciens* strain EHA101 or EHA105 (Hood *et al*. 1986) and transformed into rice (Toki 1997). Transgenic plants were selected on medium containing 50 μg/mL hygromycin. Hygromycin-resistant plants were transplanted to soil and grown to maturity at 30°C in 16 h light/8 h dark cycle in a closed greenhouse.

MOLECULAR MECHANISM OF BRASSINOSTEROID-REGULATED GENES

BRs are growth-promoting natural substances required for normal rice growth and development (Yamammuro *et al*. 2000; Hong *et al*. 2002a, 2003). To further understand the molecular mechanism by which BRs regulate the growth and development of rice plants, it is necessary to identify and analyze more genes that are controlled by BRs. The bending of the second leaf and its leaf sheath, which is a lamina joint, in rice is very sensitive to the concentration of BR. This unique characteristic of rice leaves has been used as a quantitative bioassay for BR (Wada *et al*. 1981; Yang and Komatsu 2000). We adopted this model system for analyzing BR effect on the changes of genes.

Novel brassinolide enhanced gene 1 (*OsBLE1*)

To understand the molecular mechanism of BR signal transduction, a cDNA microarray containing 1,265 rice genes (Yazaki *et al*. 2000) was analyzed for expression differences in the rice lamina joint after treatment with brassinolide (BL) (Yang *et al*. 2004). Using this cDNA microarray, OsBLE1 was originally identified, cloned and characterized as gene up-regulated by BL (Yang and Komatsu 2004). Northern blots analysis revealed that *OsBLE1* expression began to increase after BL treatment, and was most responsive to BL in lamina joint seedlings. In addition, OsBLE1 was expressed more, as revealed by *in situ* hybridization, in active tissues such as vascular bundles. To assess the effect of the loss of function of the *BLE1* gene on rice growth and development, the cDNA of OsBLE1 was introduced into rice plants in an antisense orientation. Transgenic rice expressing antisense OsBLE1 exhibits various degrees of repressed growth. They were 10-50% shorter compared to the vector control when they reached maturity. No other abnormal changes in morphology were observed (**Fig. 1**, left). These results suggest that *OsBLE1* might be involved in BL-regulated growth processes in rice seedlings (Yang and Komatsu 2004).

Novel brassinolide enhanced gene 2 (*OsBLE2*)

Furthermore, using the same cDNA microarray, a novel BL-enhanced gene designated *OsBLE2* was identified and cloned (Yang *et al*. 2003). There are no homologous proteins for this gene in the database. Southern blot results showed that *OsBLE2* may exist as a small family. *OsBLE2* expression was most responsive to BL in the lamina joint and leaf sheath in rice seedlings. In addition, auxin and GAs also increased its expression. *OsBLE2* was expressed more, as revealed by *in situ* hybridization, in vascular bundles and root primordia, where the cells are actively undergoing division, elongation and differentiation. Transgenic rice expressing antisense *OsBLE2* exhibits various degrees of repressed growth and plants were about 10-50% shorter compared to the vector control when they reach maturity, but no other abnormal morphological changes were observed (**Fig. 1**,

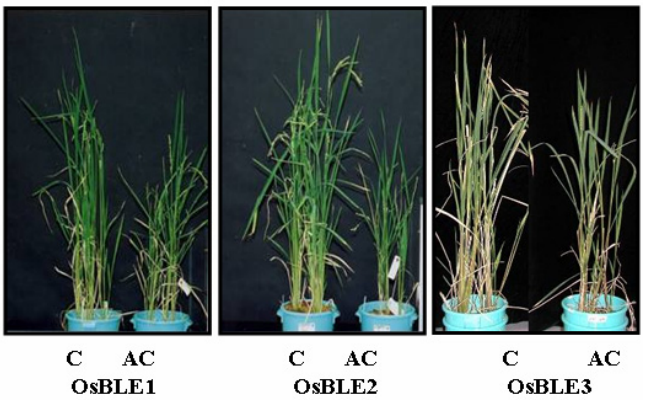

Fig. 1 Proposed model for the role of identified BL-regulated genes in rice plant growth. The BR regulation of rice plant growth and development by regulating important genes of different functions and coherent rice growth is achieved by coordinately regulating genes of different cascades. The expression of proteins in transgenic rice was tested using Northern blotting. C, vector control; AS, antisense transgenic rice. Photographs of OsBLE1 and OsBLE2 transgenic rice were adapted and modified from **Yang and Komatsu** (2004) *Plant Physiology and Biochemistry* **42**, 1-6 and **Yang *et al*.** (2003) *Plant Molecular Biology* **52**, 843-85, respectively.

middle). BL could not enhance its expression in transgenic rice expressing antisense *OsBRI1*, in which is a BR receptor (Yamamuro *et al*. 2000), indicating that BR signaling to the enhanced expression of *OsBLE2* is through *OsBRI1*. The BL effect in *d1* mutant rice, which is defective in the α-subunit of heterotrimeric G protein (Ashikari *et al*. 1990), was much weaker than that in wild type control. MAP kinase is involved in cell division and growth in plants, and its specific inhibitor, apiginin, could inhibit the BL enhanced expression of *OsBLE2*. Thus, the α-subunit of heterotrimeric G protein and MAP kinase may be components of BRs signaling. These results suggest that OsBLE2 is involved in BL-regulated growth and development processes in rice (Yang *et al*. 2003).

Novel brassinolide enhanced gene 3 (*OsBLE3*)

To investigate the mechanism of BR action in monocots, the original cDNA microarray containing 4,000 rice genes up- and down-regulated by BL was constructed and analyzed (Yang *et al*. 2004). Using this original cDNA microarray, a novel BL up-regulated gene designated *OsBLE3* was identified, cloned and characterized in rice (Yang *et al*. 2006). *OsBLE3* was mainly expressed in roots and leaf sheaths with levels of expression directly dependent on the dose of BL. *In situ* hybridization detected *OsBLE3* mRNA in the shoot apical meristem, organ primordia and vascular tissue. Furthermore, *OsBLE3* expression was enhanced by co-treatment with BL and low concentrations of auxin. Computer analyses using the PLACE signal scan program (Higo *et al*. 1999) revealed the existence of auxin response elements in the 5′-flanking region of the *OsBLE3* gene. Our results and computer analysis indicated that *OsBLE3* expression is under the control of both BR and auxin. The GUS reporter gene driven by a 2.3 kbp *OsBLE3* putative promoter was mainly expressed in vascular tissues, branch root primordia and was responsive to exogenous BL treatment. *OsBLE3* transcript levels were greatly reduced in *brd1* plants, a BL deficient mutant (Hong *et al*. 2002), compared to the wild type control. In transgenic rice expressing antisense *OsBRI1*, which is a BR receptor (Yamamuro *et al*. 2000), the exogenous BL treatment was significantly lower compared to that in control plants transformed with a vacant vector. Reduced *OsBLE3* expression and growth retardation was also observed in *OsBLE3* antisense transgenic rice plants. Internode cell length of the *OsBLE3* antisense transgenic lines was about 70% of that in vacant vector transformed control lines (**Fig. 1**, right). These results suggest that OsBLE3 is involved in cell elongation in rice through the dual regulation by BL and auxin (Yang *et al*. 2006).

MOLECULAR MECHANISM OF GIBBERELLIN REGULATED GENES

GAs control diverse growth and developmental processes, including seed germination, stem elongation, and flower development (Davies 1995). The GA biosynthetic pathway has been well characterized by using biochemical techniques as well as by studying mutants defective in biosynthesis (Hedden and Kamiya 1997). On the other hand, genetic and cell biological studies have revealed key components in the GA (Olszewski *et al*. 2002). However, additional GA signaling components and downstream cellular and biochemical events need to be investigated further to better understand the molecular nature of the GA response. We examined the progress of identifying new members of genes involved in GA-regulated rice leaf sheath growth.

Novel gibberellin enhanced gene 1 (*OsGAE1*)

Using an original cDNA microarray containing 4,000 rice genes up- and down-regulated by GA (Yang *et al*. 2004), a gene of unknown function was identified and analyzed (Jan *et al*. 2006b). It was up-regulated by GA_3, and was highly expressed in callus and at a moderate level in the leaf sheath. The gene from this clone was found to be a novel GA-enhanced gene and hence was designated as *OsGAE1*. Analysis of the OsGAE1 amino acid sequence revealed some similarity to the AtPDF1 and WM5 protein (Abe *et al*. 1999; Dong *et al*. 2005), however the *OsGAE1* gene was unique in the sense that it was hormonally regulated. *In situ* hybridization and promoter::GUS analysis revealed that *OsGAE1* was predominantly expressed in the stem, shoot apex meristem and young leaves. Computer analysis using the PLACE signal scan program (Higo *et al*. 1999) also revealed the presence of three potential GA response elements in the 1.5 kbp promoter region of *OsGAE1*. *OsGAE1* antisense transgenic plants were repressed in growth and the plants were almost 55 to 70% shorter than the vector control upon maturity. The typical phenotype of *OsGAE1* antisense transgenic plants resembled that of GA-deficient mutants. The complete GA signaling cascade is not yet fully understood and it is believed that *gid1* is a soluble GA receptor (Ueguchi-Tanaka *et al*. 2005) whereas the semi-dwarf stature of 'Tanginbozu' phenotype is caused by a defective early step of gibberellin biosynthesis, which is catalyzed by *ent*-kaurene oxidase (Ito *et al*. 2004). Exogenous application of GA_3 restores 'Tanginbozu' leaf sheath growth whereas there is no significant effect of GA_3 on *gid1*. The repressed leaf sheath growth of rice plants expressing antisense OsGAE1 was not completely reversed by application of GA_3 (**Fig. 2**, left). These observations indicate that *OsGAE1* is not involved in regulating a basic reaction shared by GA biosynthesis or signaling cascade rather that it is a downstream gene playing a vital function in the GA-mediated rice leaf sheath elongation.

Pyruvate dehydrogenase kinase 1 (*OsPDK1*)

Using an original microarray (Yang *et al*. 2004), *OsPDK1* was also identified as another gene that was up regulated by GA_3 (Jan *et al*. 2006a). PDK is a negative regulator of the mtPDH, and plays a pivotal role in controlling mtPDC activity, and hence, in the TCA cycle and cell respiration (Zou *et al*. 1999). Jan *et al*. (2006a) provided the first report of transcriptional up-regulation of plant PDK by GA_3, whereas transcriptional down regulation of *OsPDK1* gene expression by ABA using microarray was observed by Yazaki *et al*. (2003). Considering the antagonistic effects of GA and ABA (Koornneef *et al*. 1982), it is reasonable that GA_3 up-regulates *OsPDK1*. Further characterization of *OsPDK1* showed that GA modulates the activity of mtPDC

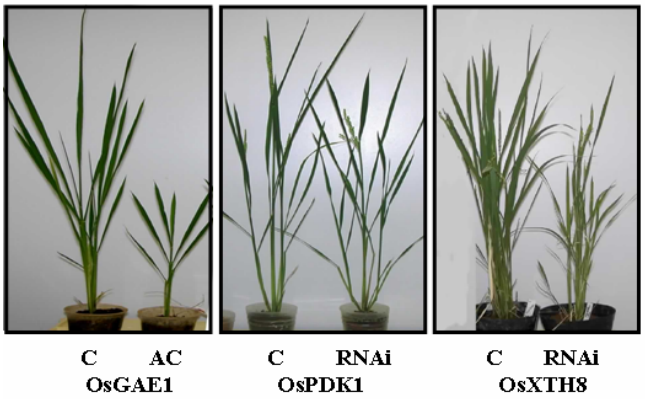

Fig. 2 Proposed model for the role of identified GA-regulated genes in rice plant growth. The GA regulation of rice plant growth and development by regulating important genes of different functions and coherent rice growth is achieved by coordinately regulating genes of different cascades. The expression of proteins in transgenic rice was tested using Northern blotting. C, vector control; AS, antisense transgenic rice; RNAi, RNAi transgenic control. Photographs of OsGAE1, OsPDK1 and OsXT8 were adapted and modified from **Jan *et al***. (2006b) *Plant Molecular Biology* **62**, 439-445, **Jan *et al***. (2006a) *Plant Cell Physiology* **47**, 244-225 and **Jan *et al***. (2004) *Plant Physiology* **136**, 3670-3368, respectively.

by regulating *OsPDK1* expression and subsequently controlling plant growth. Transgenic rice expressing RNAi *OsPDK1* had altered vegetative growth with reduced accumulation of vegetative tissues. RNAi *OsPDK1* transgenic plants developed normally, but were almost 10 to 30% shorter than controls. The possible explanation for reduced vegetative growth is that the reduction in *OsPDK1* expression causes increased mtPDH activity that allows enhanced conversion of pyruvate to acetyl-CoA and hence an increase in respiration. Tissue-specific repression of *AtPDK* increased the oil content in seeds (Marillia *et al*. 2003). In rice there was no significant effect of RNAi *OsPDK1* on reproductive growth traits like flowering time or the time to reach maturity (**Fig. 2**, middle). The effect of RNAi *OsPDK1* on the seed content in rice has yet to be examined, but may lead to insights on how the plant balances metabolic demands between developing seed grains and other tissues when primary metabolism is challenged at the entry point of the TCA cycle. These studies demonstrate that the *OsPDK1* gene can be exploited to challenged primary metabolism at the entry point of TCA cycle which will not only result in shaping the rice plant but also in the efficient use and conversion of different metabolites resources in different organs.

Xyloglucan endotransglucosylase/hydrolase 8 (*OsXTH8*)

Using an original microarray, Yang *et al*. (2004) isolated 4 clones representing a single *XTH* gene which were induced by GA_3 (Jan *et al*. 2004). XTHs catalyze the endo cleavage of xyloglucan polymers and the subsequent transfer of the newly generated reducing ends to other polymeric or oligomeric xyloglucan molecules (Fry *et al*. 1992; Nishitani and Tominaga 1992). The existence of a family of 29 *XTHs* genes in rice suggests that individual *XTHs* may exhibit distinct patterns of expression in terms of tissue specificity and responses to hormonal and environmental stimuli (Yokoyama *et al*. 2004). Out of 4 clones, *OsXTH8* was specifically up-regulated by GA_3 and not by any other hormones (Jan *et al*. 2004). Computer analysis using the PLACE signal scan program (Higo *et al*. 1999) also revealed the presence of three potential GA response elements in the 2.0 kbp sequence of *OsXTH8*. Northern blot analysis showed that the level of *OsXTH8* mRNA in 'Tanginbozu', a GA-deficient semi dwarf mutant (Ito *et al*. 2004), was lower than that in its wild-type. The expression of *OsXTH8* in the mutant was induced to exceed the wild-type level following treatment with GA_3 for 24 h while *OsXTH8* expression was quite high in *Slender rice 1*, a GA-insensitive mutant growing 2 to 3 times more than the wild-type (Ito *et al*. 2002). This finding confirms the correlation of *OsXTH8* with leaf sheath elongation. Transgenic rice expressing RNAi *OsXTH8* produced plants with repressed growth caused by stunted growth of second, third, and fourth internodes (Jan *et al*. 2004; **Fig. 2**, right). These observations demonstrate that *OsXTH8* is a unique gene that can be used to modify rice plant growth.

PROTEIN-PTOTEIN INTERACTIONS OF GIBBERELLIN REGULATED PROTEINS

Calcium is a ubiquitous signaling molecule and changes in cytosolic calcium concentration are involved in plant responses to various stimuli, including environmental stresses and plant hormones (Pooviah and Reddy 1993; Bush 1995). Increasing evidence shows that calcium-dependent protein kinases (CDPKs) (Asano *et al*. 2005) are also involved in environmental stress response and plant hormone signaling. To identify the crosstalk between environmental stress response and plant hormone signaling, calcium-signal transduction cascade in rice seedlings was analyzed using transgenic rice plant.

Calreticulin (OsCRT1)

To comprehend the molecular basis of interodal elongation in rice, a proteomics approach using differentially displayed proteins on two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) was carried out (Shen *et al*. 2003). Out of 352 proteins detected on 2D-PAGE, 32 proteins showed modulation in the expression levels in GA_3-treated leaf-sheaths for 48 h. One of them, calreticulin was identified as a GA-regulated protein. Calreticulin was also identified as a responsive protein catalyzed by phosphorylation in GA signaling (Khan *et al*. 2005). In addition, calreticulin was phosphorylated by cold stress (Li *et al*. 2003). Functional motifs found in calreticulin included a nuclear targeting signal, a praline-rich and N-glycosylation region, and an ER retention signal (Li and Komatsu 2000). To precisely determine the function of calreticulin in rice tissues, the full-length cDNA for calreticulin was introduced in the sense and antisense orientation. Twenty independent lines of transgenic plants were regenerated and were confirmed by immunoblotting. The over-expression of calreticulin inhibited callus regeneration and also the rate of seedling growth compared with the control and antisense rice (Shen *et al*. 2003; **Fig. 3**). These results suggest that the function of calreticulin might come from results of multiple locations and covalent modifications such as phosphorylation and/or calcium binding.

Calcium dependent protein kinase 13 (OsCDPK13)

Using an immuno-precipitation system, calreticulin was detected as interacting protein to OsCDPK13. Rice OsCDPK13 was cloned from rice seedlings and its transcript was shown to accumulate in response to cold stress and GA treatment (Yang *et al*. 2003). OsCDPK13 accumulated in 2-week-old leaf sheaths and callus, and became phosphorylated in response to cold and GA. OsCDPK13 gene expression and protein accumulation were up-regulated in response to GA treatment, but suppressed in response to abscisic acid and BL. Antisense OsCDPK13 transgenic lines were shorter than vector controls, and expression of OsCDPK13 was lower in dwarf mutants of rice than in their wild type. On the other hand, sense OsCDPK13 transgenic rice lines had higher recovery rates after cold stress than vector controls, and the expression of OsCDPK13 was stronger in cold-tolerant rice varieties than in cold-sensitive ones (Abbasi *et al*. 2004; **Fig. 3**). The results suggest that OsCDPK13 might be an important signaling component in the response of rice to GA and cold stress.

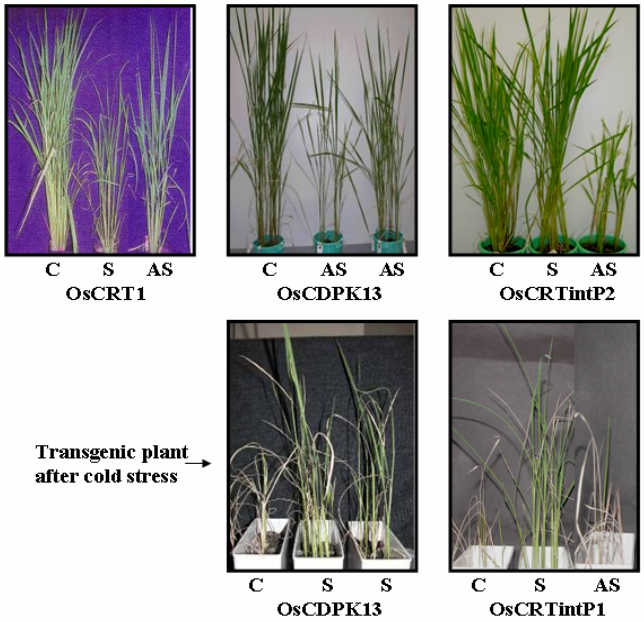

Fig. 3 Analysis of protein interactions during growth and/or stress conditions (e.g. calreticulin). Calreticulin was detected as GA- and cold-response proteins by proteomics technique. The immuno-precipitation system with calreticulin was used to identify calcium-dependent protein kinase. The yeast two-hybrid interaction- cloning system was used to identify novel calreticulin interacting. The expression of proteins in transgenic rice was tested using Western blotting. C, vector control; S, sense transgenic rice; AS, antisense transgenic rice. Photographs of OsCRT1 and OsCDPK13 were adapted and modified from **Shen *et al*.** (2003) *Biological Pharmaceutical Bulletin* **26**, 129-136 and **Abbasi *et al*.** (2004) *Plant Molecular Biology* **55**, 542-552, respectively.

Calreticulin-interacting proteins (OsCRTintPs)

Furthermore, using the screening of calreticulin-interacting proteins through yeast two-hybrid system, two novel proteins were identified in rice. cDNAs that showed with calreticulin from a rice suspension culture cell cDNA library and leaf sheath cDNA library were identified as calreticulin-interacting proteins and named OsCRTintP1 (Sharma *et al*. 2003) and OsCRTintP2 (unpublished data), respectively. OsCRTintP1 contains a nuclear localization signal site and studies on cellular localization using OsCRTintP1::GFP validated its nuclear localization. The expression of OsCRTintP1 increased in response to cold stress, indicating that it is a stress-responsive gene (Sharma *et al*. 2004). On the other hand, using an *in situ* hybridization system, OsCRTintP2 was expressed particularly in the shoot apical and nodal apical meristems, which are important in leaf sheath elongation. The average height of the various antisense OsCRTintP2 transgenic rice lines was 50% of that of the vector control (unpublished data; **Fig. 3**). These results suggest that the possible element involved in controlling stress-responsiveness and leaf sheath elongation, and cold tolerance and GA-dependent elongation may be regulated through distinct signaling pathways that crosstalk at the level of OsCRTintP1/CRTintP2.

CONCLUDING REMARKS

BRs and GAs are essential plant growth-promoting natural products that are required for normal plant elongation and during development. The underlying molecular mechanisms for signal transduction involving these phytohormones will be elucidated using the methods of molecular genetics and protein chemistry, and information from the rice genome. Altering plant function will help the next generation of rice plants with an ideal grass type having high-yield and improved grain quality, which will greatly contribute to and enhance agricultural productivity.

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