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 phenotype of our constructed transgenic rice.

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

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. 2003) 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...
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 resources for identifying genes and functional analysis. In the case of rice, some genes for the enzymes involved in BR biosynthesis have been isolated and characterized (Olszewski et al. 2002). With the identification of the osble1 gene, mutations in which cause dwarfing, abnormal plant development and mortality (Hong et al. 2000), we were able to clone the OsBLE1 gene, which encodes the GA insensitive semi-dwarf d1 mutant Osble1 (Hong et al. 2000). The Osble1 (d1) mutant in rice is characterized by GA insensitive semi-dwarf phenotype, and cloners of the D1 locus revealed that it encodes the putative α-subunit of the heterotrimeric G protein (Ashikari et al. 1999). 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 gene transgenic rice for OsBLE1, OsBLE2, OsXTH8 or OsPDK1, the full-length OsBLE1, OsBLE2, OsXTH8 or OsPDK1 cDNA sequence was ampliﬁed by PCR using primer pairs 5′-GGGTTACCGAGCTTCCTCGTCTCTGATACCA-3′ (5′-end, underlining the XbaI site as a linker) and 5′-GGGTTACCGAGCTTCCTCGTCTCTGATACCA-3′ (3′-side, underlining the SalI site as a linker). The resulting PCR product was cut, puriﬁed and ligated between the CaMV 35S promoter and nos terminator in the binary vector pIG121-Hm (Ohta et al. 1990) in a position sandwiching the GUS coding region.

Novel brassinolide enhanced gene 1 (OsBLE1)

To understand the molecular mechanism of BR signal transduction, we developed cDNA microarrays containing 1,265 rice genes (Yazaki et al. 2000) that were 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 a gene up-regulated by BL (Yang and Komatsu 2000). We adopted this model system for analyzing BR effect on the changes of genes.

Transfer and selection of transgenic rice plant

After construction, the binary vector constructs were 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 (Yamamuro 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.
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 GA3, 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 AthPDF1 and WMS 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 GA3 restores ‘Tanginbozu’ leaf sheath growth whereas there is no significant effect of GA3 on gid1. The repressed leaf sheath growth of rice plants expressing antisense OsGAE1 was not completely reversed by application of GA3 (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 GA3 (Jan et al. 2006a). PDK is a negative regulator of the mPDH, and plays a pivotal role in controlling mPDH 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 GA3, 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 up-regulates OsPDK1. Further characterization of OsPDK1 showed that GA modulates the activity of mPDH.

Transgenic rice for GA- and BR-regulated genes. Setsuko Komatsu
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 kinase 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 caused by stunted growth of second, third, and fourth interodals produced plants with repressed growth (Fig. 3, right). These observations demonstrate that OsXTH8 is a unique gene that can be used to modify rice plant growth.

PROTEIN-PROTEIN INTERACTIONS OF GIBBERELLIN REGULATED PROTEINS

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.

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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 growth during the 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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