Functional Characterization of Gibberellin-Regulated Genes in Rice Using Microarray System

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Gibberellin (GA) is collectively referred to a group of diterpenoid acids, some of which act as plant hormones and are essential for normal plant growth and development. DNA microarray technology has become the standard tool for the parallel quantification of large numbers of messenger RNA transcripts. The power of this approach has been demonstrated in dissecting plant physiology and development, and in unraveling the underlying cellular signaling pathways. To understand the molecular mechanism by which GA regulates the growth and development of plants, with reference to the monocot model plant—rice, it is essential to identify and analyze more genes and their products at the transcription and translation levels that are regulated by GA. With the availability of draft sequences of two major rice types, indica and japonica rice, it has become possible to analyze global expression profiles of genes on a genome scale. In this review, the progress made in finding new genes in rice leaf sheath using microarray system and their characterization is discussed. It is believed that the findings made in this regard have important implications for understanding the mechanism by which GA regulates the growth and development of rice.

Key words: gibberellin, gene expression, microarray, rice

Introduction

Gibberellin (GA) is considered to control diverse growth and developmental processes, including seed germination, stem elongation, and flower development (1). Despite its complexity, the GA biosynthetic pathway has been well characterized by using biochemical techniques as well as by studying mutants defective in biosynthesis (2). On the other hand, genetic and cell biological studies have revealed key components in the GA response pathway (3). However, additional GA signaling components and downstream cellular and biochemical events need to be investigated further to better understand the molecular nature of GA response. The genes for most of the enzymes involved in GA biosynthesis have been isolated and characterized (3). Several important components of the GA signal transduction pathway have been identified. The dwarf1 (d1) mutant in rice is characterized by a GA-insensitive semi-dwarf phenotype, and cloning of the D1 locus has revealed that it encodes the putative α-subunit of the heterotrimeric G protein (4). The DELLA proteins function as negative regulators of GA signaling, and their degradation through the ubiquitin/proteasome pathway is considered as a key event in the regulation of GA-stimulated processes (5). The GID2 gene of GA-insensitive dwarf phenotype, gid2, encodes a putative F-box protein, and is expected to form a Skp1-cullin-F-box complex and to function as E3 ubiquitin ligase (5). Recently, GIBBERELLIN INSSENSITIVE DWARF1 (gid1) has been characterized to show similarity to hormone sensitive lipase and it serves as a soluble receptor for GA (6).

Complete genome sequences of Arabidopsis and rice have yielded a wealth of information about plants (7, 8). These accomplishments promise to provide detailed insights into the understanding of plant physiology and the molecular mechanisms of different signal transduction pathways. However, knowing the exact sequence and location of all genes of a given organism is only the first step towards understanding how all parts of a biological system work together. Although 25,426 genes have been identified in Arabidopsis thaliana, less than 10% have been documented ex-
permentally (9). Significant progress has been made in annotating the genomes of A. thaliana and rice during the past few years and by now, most of the predicted genes are supported by full-length cDNAs (10,11).

To assign function to unknown genes, different functional genomic methodologies are currently being developed and used. DNA microarray technology uses hundreds and thousands of DNA probes arrayed on a solid surface to examine the abundance and/or binding ability of DNA or RNA target molecules. Depending on the DNA probes used, DNA microarrays are categorized into cDNA microarrays and DNA oligonucleotide probe microarrays (12). Because of a high-throughput manner analysis of thousands of genes, DNA microarrays have proved to be a powerful tool for the analysis of global gene expression patterns. Moreover, gene functions can be inferred by comparing and making association of expression patterns of different samples for a particular trait (13), which can be exploited for plant improvement (14). Promoter microarrays with chromatin immunoprecipitation have been used to identify target genes and their regulatory domains on a genome scale (15,16). Similarly, tiling microarrays using tiling probes of the entire genome have been used to discover new transcript types (17). Therefore, it can be strongly argued that DNA microarrays hold tremendous promise for dissecting the regulatory mechanisms and networks of genes and consequently their products that govern plant phenotype.

Effects of GA on plant growth and development are mediated through gene expression modulation as RNA and protein synthesis inhibitors interfere with these processes. To further understand the molecular mechanism by which GA regulates the growth and development of plants, it is necessary to identify and analyze more genes that are controlled by GA. Microarrays provide high-throughput, simultaneous analysis of mRNA for hundreds and thousands of genes (18); however, there are only few reports on the microarray analysis of GA-regulated gene expression in Arabidopsis and rice (19–22). A throughput analysis of transcript profiles in GA-regulated gene expression using different plant tissues and organs remains pertinent, and a further characterization of the individual genes will help in understanding how GA regulates the growth and development of plants. In this review, we discuss the progress of identifying new members of genes involved in GA-regulated rice leaf sheath growth using microarray system.

GA-Regulated Gene Expression

Although fine progress has been made in the study of the biosynthesis and metabolism of GA (23) using biochemical techniques with the characterization of its biosynthetic mutants, not much is known about how it regulates a wide variety of physiological processes at the molecular level. Progress has been made towards an understanding of the mechanism of GA action in the cereal aleurone, where GA induces the synthesis and secretion of a number of hydrolytic enzymes (24). Although some other GA-regulated genes have been identified in shoot (25), leaf (26), flower (27), and stem (28) in various plants, how GA regulates the growth and development of these organs is still not clear.

GA plays an important role in regulating many physiological processes in the growth and development of plants, including seed germination, shoot and stem elongation, and flower development (29). It is known that GA regulates shoot elongation by affecting cell division and elongation, though its precise mode of action in shoot growth is not yet clear. Cell elongation is controlled by the turgor pressure and cell wall extensibility in a particular direction, which is in turn regulated by the orientation of both cellulose microfibrils and the cell wall matrix containing polysaccharides and proteins (30,31). Similarly, the process of cell elongation in plants requires loosening of the cell wall structure and the deposition of new materials to maintain cell wall integrity. Auxin, GA, and brassinosteroid promote stem elongation, whereas cytokinin, ethylene, and abscisic acid have a growth-inhibiting effect (32). Although researchers have provided information on the signal mediators transmitting signals from plant hormones for cell elongation, the mechanism for regulating cell elongation is still poorly understood at the molecular level.

While rapid progress has been made in the study of the biosynthesis and metabolism of GA (23), in contrast, much remains to be learned about the GA signal transduction pathways that lead to stem elongation and other GA-regulated processes. The d1 mutant in rice is characterized by a GA-insensitive semi-dwarf phenotype, and cloning of the D1 locus revealed that it encodes the putative α-subunit of the heterotrimeric G protein (4). Genetic analysis of GA-response mutants of rice and Arabidopsis and cloning of the respective genes revealed that DELLA proteins function as negative regulators of the GA signaling
pathway (38–35), and gid1 has been identified as a soluble GA-receptor in rice (6).

Efforts have been made to determine precisely where the bioactive GA is synthesized in plants, and which cells/tissues are the targets to initiate GA-mediated biological actions. Combined gas chromatography mass spectrometry analysis and bioassays with dwarf plants have revealed that GA is mainly present in actively growing and elongating tissues, such as shoot apices, young leaves, and flowers (36–38). Contradictorily, there is evidence for the presence of GA in xylem and phloem exudates (39,40), indicating a long-distance transport of GA. However, the expression analysis of some genes involved in GA biosynthesis and GA signaling has confirmed that GA is synthesized at the site of their action (41).

Rice leaf sheath is an important part where considerable critical metabolic and regulatory activities take place, which eventually control rice height and robustness. Rice leaf sheath elongates rapidly with the treatment of GA (42). To understand the mechanism by which GA regulates rice leaf sheath growth, it is necessary to identify more genes involved in it.

**Microarray Analysis of GA-Regulated Gene Expression in Rice**

The use of cDNA microarrays for monitoring gene expression provides an efficient high-throughput approach to assessing the possible functions of large numbers of genes. There are only few reports on the microarray analysis of GA-regulated gene expression in A. thaliana (19,20) and rice (22,43), but the results obtained by different groups were quite different. This might be due to the differences in the experimental conditions and materials they used. These microarrays were either Affymetrix GeneChips or were made of ESTs or oligonucleotides representing the gene expression profiles during normal growth conditions (44). In the study of Yang et al (21), a rice cDNA library was prepared from GA3-treated rice seedlings with the aim to enrich it for novel GA-regulated genes. The original cDNA microarray containing 4,000 clones was constructed from this enriched GA-regulated cDNA library and was analyzed for expression differences in rice seedlings that had been treated with GA3. The results indicated that 2.2% of the 4,000 randomly selected clones were affected by treatment with exogenous GA3 (21). A total of 29 unique cDNA clones were identified as being up-regulated, while 33 unique cDNA clones were identified as down-regulated by GA3. A total of 62 unique genes were identified as GA3 responsive, of which 37 genes had potential functions in signal transduction, transcription, metabolism, cellular organization, and defense or anti-stress responses based on BLAST homology searches. These results indicate that GA3 is involved in regulating a wide range of growth and development processes.

Ten clones with high induction ratio were further analyzed by Northern blot analysis and they were found to be up-regulated by GA3, which confirmed the microarray results. Using an original cDNA microarray, Yang et al (21) identified three new GA-regulated genes involved in GA biosynthesis and GA signaling, with increased expression in response to GA3 treatment. Using DNA microarray, Yamauchi et al (20) identified a subset of GA up-regulated GA biosynthesis genes, such as AtGA3ox1 and AtGA8ox1, and analyzed GA deficient mutants and cold stress response in A. thaliana. Genes involved in GA biosynthesis (45), which might be subject to feedback regulation, were not identified in the original cDNA microarray analysis by Yang et al (21), which perhaps because these genes were not included in the original cDNA microarray. In order to identify GA-regulated genes in rice, the use of microarrays containing more genes, with detailed analysis on GA deficient and insensitive mutants, and on the timing and tissue specificity of expression are required (21). Despite the number of unique genes in the original cDNA microarray in Yang et al (21) is less than 4,000, many genes that were not identified previously (22,43) were identified using the original cDNA microarray (21). Among them, three genes, namely xyloglucan endotransglycosylase/hydrolase 8 (OsXTH8) (46), pyruvate dehydrogenase kinase 1 (OsPDK1) (47), and a novel GA-enhanced gene 1 (OsGAEl) (48), showed clearly GA-differential expression when analyzed by Northern blot analysis. The three genes were selected for further characterization in order to elucidate their functions in rice growth and development.

**OsXTH8**

The results of OsXTH8 were reported by Jan et al (46). Four clones representing a single XTH gene were induced by GA3, implying a role in regulat-
ing cell elongation and cell wall organization. XTH catalyzes the endo cleavage of xyloglucan polymers and the subsequent transfer of the newly generated reducing ends to other polymeric or oligomeric xyloglucan molecules (49, 50). The existence of a family of 29 XTH genes in rice suggests that individual XTH may exhibit distinct patterns of expression in terms of tissue specificity and responses to hormonal and environmental stimuli (51). The OsXTH8 gene identified in the original microarray was specifically up-regulated by GA3 and not by any other hormones (46). Computer analysis using the PLACE signal scan program (52) also revealed the presence of three potential GA response elements in the 2-kb sequence of OsXTH8. Northern blot analysis showed that the level of OsXTH8 mRNA in Tanginbozu, a GA-deficient semi-dwarf mutant, was lower than that in its wild type. The expression of OsXTH8 in the mutant was induced to exceed wild-type level following treatment with GA3 for 24 h, while OsXTH8 expression was quite high in the Slender rice 1, which is a GA-insensitive mutant growing in its wild type. The expression of OsXTH8 in the mutant was induced to exceed wild-type level following treatment with GA3 for 24 h, while OsXTH8 expression was quite high in the Slender rice 1, which is a GA-insensitive mutant growing in its wild type (33). This finding confirms the correlation of OsXTH8 with leaf sheath elongation. RNAi OsXTH8 expressed under the control of CaMV 35S promoter produced plants with repressed growth caused by stunted growth of the second, third, and fourth internode (50). These observations demonstrate that OsXTH8 is a unique gene that can be used to modify rice plant growth (Figure 1).

**OsPDK1**

OsPDK1 was identified as a gene up-regulated by GA3 using the cDNA microarray (47). PDK is a negative regulator of mitochondrial pyruvate dehydrogenase (mtPDH), and plays a pivotal role in controlling mitochondrial pyruvate dehydrogenase complex (mtPDC) activity, and hence, in the tricarboxylic acid (TCA) cycle and cell respiration (59). Jan et al (47) provided the first report of transcriptional up-regulation of plant PDK by GA3, whereas transcriptional down-regulation of OsPDK1 gene expression by abscisic acid (ABA) using microarray has been observed by Yazaki et al (43). Considering the antagonistic effects of GA and ABA (54), it is reasonable that GA3 up-regulates OsPDK1 identified in the original microarray (47). Further characterization of OsPDK1 showed that GA modulates the activity of mtPDC by regulating OsPDK1 expression and subsequently controlling plant growth. Transgenic rice expressing RNAi PDK1 altered vegetative growth with reduced accumulation of vegetative tissues. RNAi transgenics developed normally, but were almost 10% to 30% shorter in height compared to control. The possible explanation for the 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 the respiration. Tissue-specific repression of AtPDK increased the oil content in seeds (55). In rice, there is no significant effect of RNAi OsPDK1 on reproductive growth traits like flowering time or the time to reach maturity. The effect of RNAi OsPDK1 on reproductive growth traits 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 TCA cycle. This study demonstrated 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 metabolite resources in different organs (Figure 1).

**OsGAE1**

In the study by Jan et al (48), a novel gene of unknown function that was up-regulated by GA3 was identified and analyzed, which expressed highly in callus and at a moderate level in leaf sheath. The gene from this clone was found to be a novel GA-enhanced gene and hence was designated as OsGAE1 (48). Analysis of the OsGAE1 amino acid sequence revealed some similarity to the A1PD1 and WMS5 protein (56, 57), 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 stem, shoot apex meristem, and young leaves. Computer analysis using the PLACE signal scan program (52) also revealed the presence of three potential GA response elements in the 1.5-kb promoter region of OsGAE1. OsGAE1 antisense transgenic plants were repressed in growth and the plants were almost 55% to 70% shorter than the control upon maturity. The typical phenotype of OsGAE1 antisense transgenics 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 (6) whereas the semi-dwarf stature of Tanginbozu phenotype is caused
by a defective early step of GA biosynthesis, which is catalyzed by *ent*-kaurene oxidase (58). 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 *OsGAEl* was not completely reversed by application of GA\(_3\). These observations indicate that *OsGAEl* is not involved in regulating a basic reaction shared by GA biosynthesis or signaling cascade rather than it is a downstream gene playing a vital function in the GA-mediated rice leaf sheath elongation (Figure 1).
Conclusion

Current researches have indicated that suitable rice morphogenesis can be achieved by cleverly tailoring GA-regulated genes. Sakamoto et al (59) modified the level of GA by overproduction of a GA catabolic enzyme, GA 2-oxidase. When the gene encoding GA 2-oxidase, OsGA2ox1, was constitutively expressed by the actin promoter, transgenic rice showed severe dwarfism and the plants failed in seed setting because GA is involved in both shoot elongation and reproductive development. In contrast, OsGA2ox1 ectopic expression at the site of bioactive GA synthesis in shoots under the control of the promoter of a GA biosynthesis gene, OsGA3ox2 (D18), resulted in a semi-dwarf phenotype that was normal in flowering and grain development (59). In molecular studies of rice and wheat varieties, the phytohormone GA has been identified as a key player in controlling crop plant architecture (60). However, along controlling plant architecture, grain numbers and grain quality are also parameters of prime importance. Recently it has been demonstrated that cytokinin metabolism also contributes to crop productivity. As cytokinin controls cell division and lateral meristem activity, its accumulation in the inflorescence meristem can cause higher grain numbers (61). Jan et al (46–48) showed that GA regulates plant growth and development by regulating important genes of different functions. Identification of agronomically important genes and pyramiding of such genes presents a useful strategy for efficient crop development. Wise tailoring of such genes of different check points will greatly facilitate artificially controlling the morphogenesis of rice plant, which will result in the development of next generation of rice plant with ideal grass type having high yield and improved grain quality.

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