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 INSENSITIVE 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-

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perimentally (*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 tilling 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 (*33–35*), 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 GA_3-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 GA_3. The results indicated that 2.2% of the 4,000 randomly selected clones were affected by treatment with exogenous GA_3 (*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 GA_3. A total of 62 unique genes were identified as GA_3 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 GA_3 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 GA_3, which confirmed the microarray results. Using an original cDNA microarray, Yang *et al* (*21*) identified three new GA-regulated genes involved in rice seedlings, which displayed increased expression in response to GA_3 treatment. Using DNA microarray, Yamauchi *et al* (*20*) identified a subset of GA up-regulated GA biosynthesis genes, such as *AtGA3ox1* and *AtGA20ox1*, 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 endotransglucosylase/hydrolase 8 (*OsXTH8*) (*46*), pyruvate dehydrogenase kinase 1 (*OsPDK1*) (*47*), and a novel GA-enhanced gene 1 (*OsGAE1*) (*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 GA_3, 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 GA_3 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 GA_3 for 24 h, while *OsXTH8* expression was quite high in the Slender rice 1, which is a GA-insensitive mutant growing 2 to 3 times more than the 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 GA_3 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 (*53*). Jan *et al* (*47*) provided the first report of transcriptional up-regulation of plant PDK by GA_3, 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 GA_3 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 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 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 GA_3 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 AtPDF1 and WM5 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

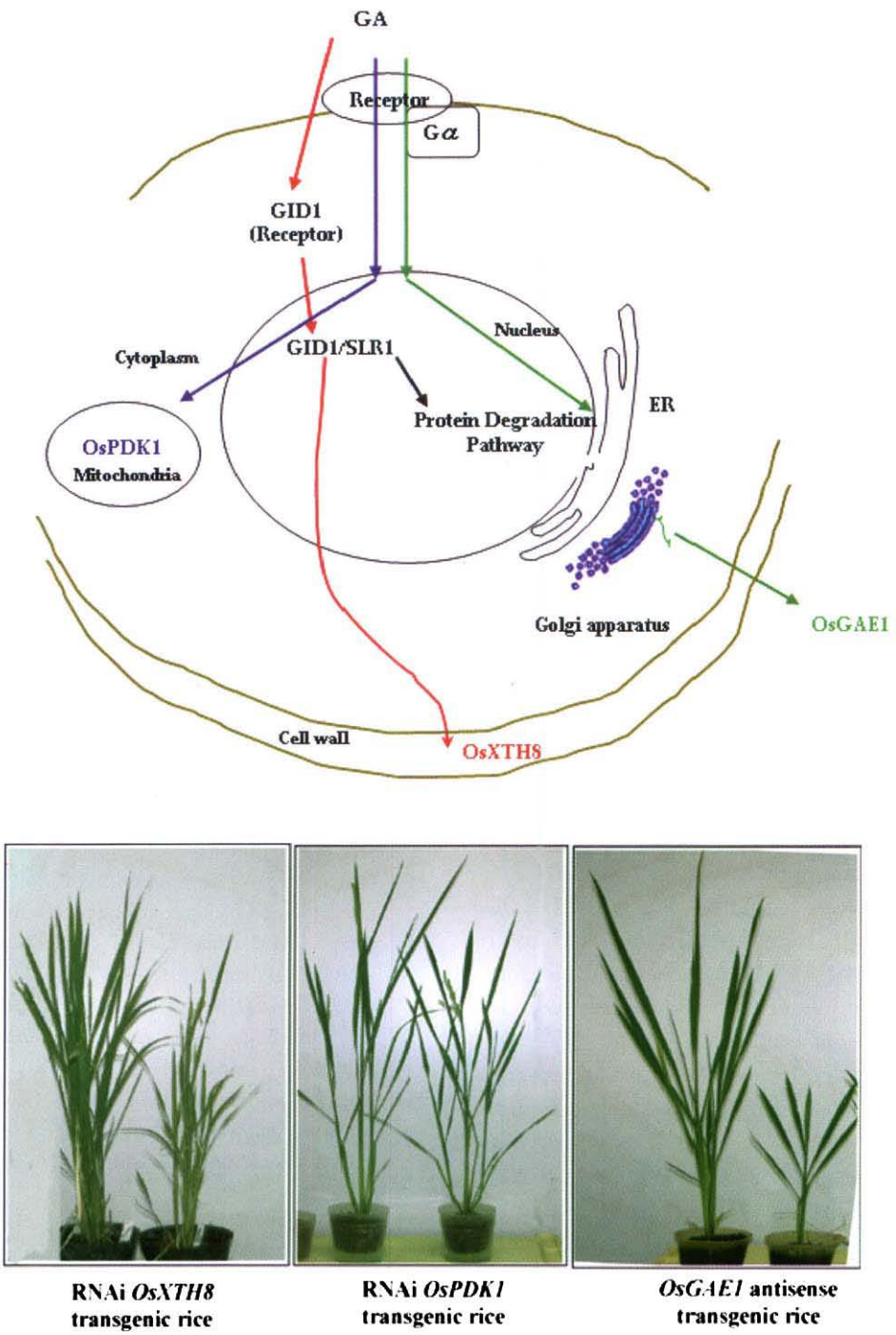

Fig. 1 Proposed model for the role of identified genes in rice plant growth. The GA regulation of rice plant growth and development by regulating important genes of different functions and coherent rice plant growth is achieved by coordinately regulating genes of different cascades [Modified from Jan *et al* (*46–48*)].

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 *OsGAE1* was not completely reversed by application of GA_3. These observations indicate that *OsGAE1* 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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