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DNA Replication Factor C1 Mediates Genomic Stability and Transcriptional Gene Silencing in *Arabidopsis* [W][OA]

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Genetic screening identified a suppressor of *ros1-1*, a mutant of *REPRESSOR OF SILENCING1* (*ROS1*; encoding a DNA demethylation protein). The suppressor is a mutation in the gene encoding the largest subunit of replication factor C (RFC1). This mutation of RFC1 reactivates the unlinked *35S-NPTII* transgene, which is silenced in *ros1* and also increases expression of the pericentromeric *Athila* retrotransposons named *transcriptional silent information* in a DNA methylation-independent manner. *rfc1* is more sensitive than the wild type to the DNA-damaging agent methylmethane sulphonate and to the DNA inter- and intra- cross-linking agent cisplatin. The *rfc1* mutant constitutively expresses the G2/M-specific cyclin *CycB1;1* and other DNA repair-related genes. Treatment with DNA-damaging agents mimics the *rfc1* mutation in releasing the silenced *35S-NPTII*, suggesting that spontaneously induced genomic instability caused by the *rfc1* mutation might partially contribute to the released transcriptional gene silencing (TGS). The frequency of somatic homologous recombination is significantly increased in the *rfc1* mutant. Interestingly, *ros1* mutants show increased telomere length, but *rfc1* mutants show decreased telomere length and reduced expression of telomerase. Our results suggest that RFC1 helps mediate genomic stability and TGS in *Arabidopsis thaliana*.

INTRODUCTION

During DNA replication, DNA polymerase α interacts with DNA primase to form a complex for initiating synthesis of a 15-20 mer DNA primer using an RNA primer. Replication factor C (RFC), a clamp-loader complex consisting of five different subunits, binds DNA at the template–primer junctions and displaces polymerase α to terminate DNA primer synthesis. The binding of RFC to DNA creates a loading site for recruiting the DNA sliding clamp proliferating cell nuclear antigen (PCNA), a ring-shaped homotrimer. RFC is an AAA+-type ATPase that requires ATP hydrolysis for opening and closing PCNA around DNA during DNA replication, repair, and recombination (Majka and Burgers, 2004).

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The formation of the DNA replication fork can be stalled or arrested during DNA replication if the DNA structure is chemically or physically altered by double-strand breaks (DSBs). DSBs can be repaired by homologous recombination (HR), which will reestablish a formal replication fork. DNA damage can activate the S-phase replication checkpoint pathway that helps stabilize replication forks and prevents the breakdown of replication forks (Branzei and Foiani, 2009). Chromosome ends called telomeres are natural DNA breaks. However, telomeres have specific DNA structures that are protected by various proteins with negative or positive effects on telomere length (Smolikov et al., 2004). When the DNA replication machinery meets telomeres, it competes with telomerase (Shore and Bianchi, 2009). Telomeres can be elongated by some mutations, such as in Rfc1 and DNA polymerase α, suggesting that the replication machinery regulates telomere length (Adams and Holm, 1996). Abnormal regulation of telomere maintenance may evoke a DNA damage response leading to the repair of telomeres by HR.

DNA replication is a highly regulated process that accurately replicates both the primary DNA sequence and chromatin structure from the parent strands. The transmission of heterochromatin structure and DNA methylation is an essential process after the DNA has been replicated and is tightly regulated by various proteins (Shultz et al., 2007; Kloc and Martienssen, 2008;

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Cucumber Mosaic Virus Movement Protein Severs Actin Filaments to Increase the Plasmodesmal Size Exclusion Limit in Tobacco [W][OA]

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Plant viral movement proteins (MPs) enable viruses to pass through cell walls by increasing the size exclusion limit (SEL) of plasmodesmata (PD). Here, we report that the ability of *Cucumber mosaic virus* (CMV) MP to increase the SEL of the PD could be inhibited by treatment with the actin filament (F-actin)–stabilizing agent phalloidin but not by treatment with the F-actin–destabilizing agent latrunculin A. In vitro studies showed that CMV MP bound globular and F-actin, inhibited actin polymerization, severed F-actin, and participated in plus end capping of F-actin. Analyses of two CMV MP mutants, one with and one without F-actin severing activities, demonstrated that the F-actin severing ability was required to increase the PD SEL. Furthermore, the *Tobacco mosaic virus* MP also exhibited F-actin severing activity, and its ability to increase the PD SEL was inhibited by treatment with phalloidin. Our data provide evidence to support the hypothesis that F-actin severing is required for MP-induced increase in the SEL of PD. This may have broad implications in the study of the mechanisms of actin dynamics that regulate cell-to-cell transport of viral and endogenous proteins.

INTRODUCTION

Plasmodesmata (PD) are channels within plant cell walls that enable communication between adjacent cells. It is well established that PD allow trafficking of macromolecules. Such cell-to-cell movement of macromolecules can be specific or nonspecific and is regulated during plant development and differentiation (for reviews, see Haywood et al., 2002; Roberts and Oparka, 2003; Cilia and Jackson, 2004; Ruiz-Medrano et al., 2004; Lough and Lucas, 2006). Therefore, the regulation of PD is an important mechanism of developmental regulation in plants (Zambryski and Crawford, 2000; Kim et al., 2002, 2005; Zambryski, 2004; Kobayashi et al., 2007).

Viral movement proteins (MPs) are crucial for the spread of viruses from cell to cell. MPs enable viral pathogens to pass through cell walls by increasing the size exclusion limit (SEL) of PD (for review, see Lucas, 2006). Previous studies have demonstrated that the introduction of viral MPs by microinjection or expression of green fluorescent protein (GFP)–tagged MPs, including the *Cucumber mosaic virus* (CMV) MP, results in an increase in the SEL of the PD in various types of plant cells (Fujiwara et al., 1993; Vaquero et al., 1994; Ding et al., 1995; Itaya et al., 1997). Therefore, MPs are useful tools for studying the mechanisms of plasmodesmal regulation (Lazarowitz and Beachy, 1999). Models have been proposed to describe how viral MPs increase the PD SEL to facilitate viral RNA (vRNA) trafficking (Heinlein et al., 1995, 1998; Carrington et al., 1996; Lazarowitz and Beachy, 1999; Boyko et al., 2000a, 2000b; Tzfira et al., 2000; Zambryski and Crawford, 2000; Aaziz et al., 2001; Boevink and Oparka, 2005). These models suggest that cytoskeletal components are involved in the transport of MPs to PD sites, as well as in the transfer of the viral genome from cell to cell through the PD.

It has been shown that microtubules can interact with the MP of *Tobacco mosaic virus* (TMV) and, together with the endoplasmic reticulum (ER), play a role in viral movement (Heinlein et al., 1995, 1998; Más and Beachy, 1999, 2000; Boyko et al., 2000a, 2000b, 2002; Sambade et al., 2008). Tobamovirus MPs possess a conserved sequence motif, which shares similarity with a region in tubulins that mediates lateral contact between microtubule protofilaments (Boyko et al., 2000b). Therefore, MPs may mimic tubulin assembly surfaces to propel vRNA transport through a dynamic process that is driven by microtubule polymerization (Boyko et al., 2000b). In addition, it has been reported that some host proteins, such as movement protein binding 2C and microtubule end binding protein 1a, bind both microtubules and TMV MP (Kragler et al., 2003; Curin et al., 2007; Brandner et al., 2008) and play significant roles in virus infectivity (Brandner et al., 2008; Ruggenthaler et al., 2009).

Studies of TMV have indicated that microtubules may be involved in MP degradation but not in cell-to-cell trafficking (Reichel and Beachy, 1998; Más and Beachy, 1999; Gillespie et al., 2002). However, there is also evidence that opposes the

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ORIGINAL ARTICLE

Potassium channel α-subunit AtKC1 negatively regulates AKT1-mediated K^+ uptake in *Arabidopsis* roots under low-K^+ stress

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Potassium transporters play crucial roles in K^+ uptake and translocation in plants. However, so far little is known about the regulatory mechanism of potassium transporters. Here, we show that a *Shaker*-like potassium channel AtKC1, encoded by the *AtLKT1* gene cloned from the *Arabidopsis thaliana* low-K^+ (LK)-tolerant mutant *Atlkt1*, significantly regulates AKT1-mediated K^+ uptake under LK conditions. Under LK conditions, the *Atkc1* mutants maintained their root growth, whereas wild-type plants stopped their root growth. Lesion of *AtKC1* significantly enhanced the tolerance of the *Atkc1* mutants to LK stress and markedly increased K^+ uptake and K^+ accumulation in the *Atkc1*-mutant roots under LK conditions. Electrophysiological results showed that AtKC1 inhibited the AKT1-mediated inward K^+ currents and negatively shifted the voltage dependence of AKT1 channels. These results demonstrate that the 'silent' K^+ channel α-subunit AtKC1 negatively regulates the AKT1-mediated K^+ uptake in *Arabidopsis* roots and consequently alters the ratio of root-to-shoot under LK stress conditions.

Keywords: *Arabidopsis*; potassium channel; low-K^+ stress; AKT1; AtKC1
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Introduction

Potassium is an essential mineral element for plant growth and development, and it plays essential roles in many important physiological and biochemical processes in living plant cells, such as regulation of enzyme activation, electrical neutralization, osmoregulation, control of membrane potential, co-transport of sugars, and so on [1, 2]. Plant growth and development need millimolar K^+ in the soil or growth medium, but typical K^+ concentration at the interface of roots and soils is within micromolar range [3]. Thus, plants often encounter low-K^+ (LK) stress under natural conditions. Although different plants or different genotypes of a plant species show varied K^+ utilization efficiency [4], most plants show K^+-deficient symptom under LK stress, typically leaf chlorosis and subsequent inhibition of plant growth and development [5].

Absorption of K^+ by plant cells and K^+ translocation between different tissues and organs in plants are mediated by plant K^+ transporters and channels [2, 6, 7]. Over the past decade, a large number of genes encoding plant K^+ transporters and channels, particularly for *Arabidopsis*, have been characterized [6-8]. These K^+ transporters vary in K^+ affinity, kinetics, transcriptional modulation, regulatory mechanism, etc [2, 6-8], and they compose a complex system for plant K^+ uptake and translocation. Among these K^+ transporters and channels, members of the *Shaker* K^+ channel family are well characterized for their potential functions and are probably the most important for K^+ uptake and transport in *Arabidopsis* [8]. Most members in this family have been functionally characterized except *AKT5* and *AtKC1* [2, 6, 8]. How-

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PMRD: plant microRNA database

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ABSTRACT

MicroRNAs (miRNA) are ~21 nucleotide-long non-coding small RNAs, which function as post-transcriptional regulators in eukaryotes. miRNAs play essential roles in regulating plant growth and development. In recent years, research into the mechanism and consequences of miRNA action has made great progress. With whole genome sequence available in such plants as *Arabidopsis thaliana*, *Oryza sativa*, *Populus trichocarpa*, *Glycine max*, etc., it is desirable to develop a plant miRNA database through the integration of large amounts of information about publicly deposited miRNA data. The plant miRNA database (PMRD) integrates available plant miRNA data deposited in public databases, gleaned from the recent literature, and data generated in-house. This database contains sequence information, secondary structure, target genes, expression profiles and a genome browser. In total, there are 8433 miRNAs collected from 121 plant species in PMRD, including model plants and major crops such as *Arabidopsis*, rice, wheat, soybean, maize, sorghum, barley, etc. For *Arabidopsis*, rice, poplar, soybean, cotton, medicago and maize, we included the possible target genes for each miRNA with a predicted interaction site in the database. Furthermore, we provided miRNA expression profiles in the PMRD, including our local rice oxidative stress related microarray data (LC Sciences miRPlants_10.1) and the recently published microarray data for poplar, *Arabidopsis*, tomato, maize and rice. The PMRD database was constructed by open source technology utilizing a user-friendly web interface, and multiple search tools. The PMRD is freely available at http://bioinformatics.cau.edu.cn/PMRD. We expect PMRD to be a useful tool for scientists in the miRNA field in order to study the function of miRNAs and their target genes, especially in model plants and major crops.

INTRODUCTION

MicroRNAs (microRNA) are ~21 nucleotide-long endogenetic non-coding small RNAs and function as post-transcriptional regulators in eukaryotes. The processing of miRNA is well studied and is comprised of a discrete series of steps (1). miRNA genes are transcribed and excised into miRNAs, then miRNA is recruited by RISC (RNA-induced silencing complex, including Argonaute and other proteins) which combines with mRNA to inhibit or degrade the target mRNA (1) and thus translation is interrupted.

MiRNAs play essential roles in regulating plant growth and development (2). Research into miRNA function on target genes has progressed at a very rapid rate in plants. As examples, Archak *et al.* (3) discovered that miRNAs may participate in diverse functions including transcription, catalysis, binding and transporter activity. Some miRNAs related to abiotic stress responses such as cold stress and nutrient deprivation were identified in *Arabidopsis thaliana* using transcriptome analysis (4,5); Lu *et al.* (6) analyzed miRNA regulatory roles in the response *of Populus trichocarpa* to the stressful environment incurred over their long-term growth; Morin *et al.* conducted comparative analyses on the conservation of miRNAs between *Pinus contorta* and *Oryza sativa* and discovered that important RNA silencing processes were highly developed in the earliest spermatophytes (7).

Recently, more and more miRNAs have been identified in plant genomes. Jones-Rhoades *et al.* (8) developed comparative genomic approaches to systematically identify both miRNAs and target genes, which enlarged the miRNAs family and the number of target genes in *A. thaliana*. Using high-throughput sequencing, Rajagopalan *et al.* (9) identified 38 new miRNAs in

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agriGO: a GO analysis toolkit for the agricultural community

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ABSTRACT

Gene Ontology (GO), the *de facto* standard in gene functionality description, is used widely in functional annotation and enrichment analysis. Here, we introduce agriGO, an integrated web-based GO analysis toolkit for the agricultural community, using the advantages of our previous GO enrichment tool (EasyGO), to meet analysis demands from new technologies and research objectives. EasyGO is valuable for its proficiency, and has proved useful in uncovering biological knowledge in massive data sets from high-throughput experiments. For agriGO, the system architecture and website interface were redesigned to improve performance and accessibility. The supported organisms and gene identifiers were substantially expanded (including 38 agricultural species composed of 274 data types). The requirement on user input is more flexible, in that user-defined reference and annotation are accepted. Moreover, a new analysis approach using Gene Set Enrichment Analysis strategy and customizable features is provided. Four tools, SEA (Singular enrichment analysis), PAGE (Parametric Analysis of Gene set Enrichment), BLAST4ID (Transfer IDs by BLAST) and SEACOMPARE (Cross comparison of SEA), are integrated as a toolkit to meet different demands. We also provide a cross-comparison service so that different data sets can be compared and explored in a visualized way. Lastly, agriGO functions as a GO data repository with search and download functions; agriGO is publicly accessible at http://bioinfo.cau.edu.cn/agriGO/.

INTRODUCTION

The availability of high-throughput techniques allows biologists to monitor changes and regulation at a genome-wide level under certain conditions. Such experiments normally generate huge data sets of genes' expression values under different treatments. There are challenges in the analysis and interpretation of these data sets with one promising strategy to solve these problems being gene-annotation enrichment analysis. The bioinformatics community has developed multiple enrichment tools which were compared and summarized by Huang *et al*. (1). The majority of these tools (2–12) employ Gene Ontology (GO) (3) as their annotation resource, since GO is a controlled vocabulary system with rich content for gene function description at a molecular level and is supported by many consortia focusing on different organisms. Unfortunately, most GO enrichment tools have limited support for agricultural species. Recently, four applications enabling analysis of agricultural species data were evaluated by Berg *et al*. (13). Among four tools, only EasyGO (12) is designed to especially serve the agricultural community. Since its release, this tool has processed >20 000 analysis requests from all around the world and is referenced by 20 publications. After 3 years of continued maintenance, we developed the successor of EasyGO, a web-based toolkit named agriGO with enhanced and novel functionalities.

Retaining the advanced features of EasyGO, agriGO also continues to focus on agricultural species. The enrichment analysis approach used in EasyGO is categorized as SEA (Singular enrichment analysis) in Huang's survey (1). We kept this method because although SEA is the most traditional strategy, it is still very efficient and such continuity will not reduce its accessibility to past users. However, new features were added to meet current complex demands. First, new tools including PAGE (Parametric Analysis of Gene set Enrichment), BLAST4ID (Transfer IDs by BLAST) and SEACOMPARE (Cross comparison of SEA) were developed. The arrival of these tools provides users with possibilities for data mining and systematic result exploration and will allow better data analysis and interpretation. Second, the exploratory capability and result

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Mutation in the catalytic subunit of DNA polymerase α influences transcriptional gene silencing and homologous recombination in Arabidopsis

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SUMMARY

***REPRESSOR OF SILENCING 1* (*ROS1*) encodes a DNA demethylase that actively removes DNA methylation. Mutation in *ROS1* leads to transcriptional gene silencing of a T-DNA locus that contains two genes, *RD29A-LUC* and *35S-NPTII*, originally expressed in the C24 wild type. These units have different silencing regulation mechanisms: the former mechanism is dependent on small interfering RNA (siRNA)-directed DNA methylation, but the latter is not. We studied the latter gene silencing mechanism by screening the suppressors of the *ros1* mutant using the silenced *35S-NPTII* as a selection marker gene. The *polα/incurvata2* (*icu2*) gene was isolated as one *ros1* suppressor because its mutation leads to the reactivation of the silenced *35S-NPTII* gene. *POLα/ICU2* encodes a catalytic subunit of DNA polymerase α. Mutation of *POLα/ICU2* did not affect DNA methylation, but reduced histone H3 Lys9 dimethylation (H3K9me2) modification in the *35S* promoter. The *polα* mutation also influences the development of the shoot apical meristem, and delays the G2/M phase with high expression of a G2/*M* marker gene *CycB1;1:GUS*. Furthermore, the frequency of homologous recombination is greater in the *polα/icu2* mutant than in the C24 wild type. Our results suggest that DNA polymerase α is involved in mediating epigenetic states and in DNA homologous recombination in Arabidopsis.**

Keywords: DNA polymerase alpha, epigenetic, homologous recombination, DNA methylation, Arabidopsis.

INTRODUCTION

During DNA replication, not only nucleic acids but also DNA methylation, histone modifications, and chromatin structure are accurately replicated. Understanding the stable transmission of epigenetic information through multiple cell cycles has been the main focus of recent studies concerning epigenetic regulation. Various proteins and DNA polymerases participate in DNA replication, repair and recombination; these include chromatin assembly factors, DNA polymerase α, DNA polymerase δ, DNA polymerase ε, DNA primase, replication factor C, PCNA (a ring-shaped homotrimeric protein) and other proteins (Waga and Stillman, 1998; Garg and Burgers, 2005; Moldovan *et al.*, 2007). DNA polymerase α is responsible for initiating replication at both origins and the lagging strand (Kunkel and Burgers, 2008), and works together with DNA primase to form a four-subunit complex for initiating DNA replication (Muzi-Falconi *et al.*, 2003). In *Schizosaccharomyces pombe*, DNA polymerase α interacts with Swi6, a heterochomatin protein homolog. Mutation in DNA polymerase α influences the location of Swi6, suggesting that DNA polymerase α may play a role in maintaining epigenetic states (Ahmed *et al.*, 2001; Nakayama *et al.*, 2001). Arabidopsis contains only one copy of the catalytic subunit of DNA polymerase α (*INCURVATA2* or *ICU2*), and the interaction between ICU2 and LHP1 (LIKE HETEROCHROMATIN PROTEIN 1, an ortholog of SWI6) is detected *in vitro* (Barrero *et al.*, 2007). *ICU2* genetically interacts with *TERMINAL FLOWER 2* (*LHP1*) and with the Polycomb group (PcG) gene *CURLY LEAF* (Barrero *et al.*, 2007).

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OsSPX1 suppresses the function of OsPHR2 in the regulation of expression of *OsPT2* and phosphate homeostasis in shoots of rice

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SUMMARY

Phosphate (Pi) homeostasis in plants is required for plant growth and development, and is achieved by the coordination of Pi acquisition, translocation from roots to shoots, and remobilization within plants. Previous reports have demonstrated that over-expression of *OsPHR2* (the homolog of *AtPHR1*) and knockdown of *OsSPX1* result in accumulation of excessive shoot Pi in rice. Here we report that OsPHR2 positively regulates the low-affinity Pi transporter gene *OsPT2* by physical interaction and upstream regulation of *OsPHO2* in roots. OsPT2 is responsible for most of the OsPHR2-mediated accumulation of excess shoot Pi. OsSPX1 suppresses the regulation on expression of *OsPT2* by OsPHR2 and the accumulation of excess shoot Pi, but it does not suppress induction of *OsPT2* or the accumulation of excessive shoot Pi in the *Ospho2* mutant. Our data also show that OsSPX1 is a negative regulator of OsPHR2 and is involved in the feedback of Pi-signaling network in roots that is defined by OsPHR2 and OsPHO2. This finding provides new insight into the regulatory mechanism of Pi uptake, translocation, allocation and homeostasis in plants.

Keywords: *Oryza sativa* L., OsPT2, OsSPX1, Pi homeostasis, Pi signaling.

INTRODUCTION

Maintenance of phosphate (Pi) homeostasis in plants is essential for plant growth and development, and is achieved by coordination of acquisition of Pi from soils, translocation of Pi from roots to shoots, and remobilization of internal Pi (Poirier and Bucher, 2002). In yeast (*Saccharomyces cerevisiae*), the regulatory mechanism of Pi homeostasis driven by the phosphate signal transduction (PHO) pathway is thoroughly understood (Pinson *et al.*, 2004). However, full knowledge regarding the regulatory mechanism of Pi homeostasis in plants is still lacking.

AtPHR1, a transcription factor with a MYB domain, is a key regulator in the Pi-signaling pathway in Arabidopsis (Rubio *et al.*, 2001). Over-expression of *AtPHR1* leads to an increased concentration of Pi in the shoot tissues, together with induction of a range of Pi-starvation induced genes that encode Pi transporters, phosphatases and RNase (Nilsson *et al.*, 2007). Schachtman and Shin (2007) proposed a possible regulatory system downstream of AtPHR1, whereby *miR399* (a PHR1 target) reciprocally regulates the gene *PHO2* at the post-transcriptional level (Fujii *et al.*, 2005; Bari *et al.*, 2006; Chiou *et al.*, 2006). *miR399*-mediated *PHO2* cleavage is controlled by *IPS1*, a non-coding RNA, through target mimicry (Franco-Zorrilla *et al.*, 2007). PHO2 functions as a ubiquitin-conjugating E2 enzyme (UBC24), and loss of function of PHO2 leads to accumulation of excess shoot Pi (Aung *et al.*, 2006; Bari *et al.*, 2006). This regulatory system is

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The Arabidopsis eukaryotic translation initiation factor 3, subunit F (*At*eIF3f), is required for pollen germination and embryogenesis

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SUMMARY

Previous studies have shown that subunits E (eIF3e), F (eIF3f) and H (eIF3h) of eukaryotic translation initiation factor 3 play important roles in cell development in humans and yeast. eIF3e and eIF3h have also been reported to be important for normal cell growth in Arabidopsis. However, the functions of subunit eIF3f remain largely unknown in plant species. Here we report characterization of mutants for the Arabidopsis *eIF3f* (*AteIF3f*) gene. *AteIF3f* encodes a protein that is highly expressed in pollen grains, developing embryos and root tips, and interacts with Arabidopsis eIF3e and eIF3h proteins. A *Ds* insertional mutation in *AteIF3f* disrupted pollen germination and embryo development. Expression of some of the genes that are essential for pollen tube growth and embryogenesis is down-regulated in *ateif3f-1* homozygous seedlings obtained by pollen rescue. These results suggested that *At*eIF3f might play important roles in Arabidopsis cell growth and differentiation in combination with eIF3e and eIF3h.

Keywords: *AteIF3f*, translation initiation, MPN domain, male gametophyte, embryogenesis, Arabidopsis.

INTRODUCTION

In higher plants, pollen tube elongation in the pistil is a crucial step for sexual reproduction. Many important biological processes are involved. For example, a tip-focused calcium gradient plays a central role in orienting tip growth (Dumas and Gaude, 2006). Cell wall-modifying enzymes are required for normal pollen tube elongation (Krichevsky *et al.*, 2007). Exocytosis is also one of the major processes responsible for polarized cell growth (Malho *et al.*, 2006). To carry out these biological processes, numerous proteins need to be synthesized precisely during pollen tube growth and elongation.

In protein synthesis processes, mRNA translation is regulated at both global and message-specific levels, especially at the step of translation initiation. The normal initiation of eukaryotic protein synthesis is facilitated by at least 12 eukaryotic translation initiation factors (eIFs), several of which are multiprotein complexes. The largest, eukaryotic translation initiation factor 3 (eIF3) (approximately 650 kDa), participates in most translation initiation processes (Asano *et al.*, 1997a; Sonenberg *et al.*, 2000; Kawaguchi and Bailey-Serres, 2002). eIF3 helps to maintain the 40S and 60S ribosomal subunits in a dissociated state, and stabilizes binding of the eIF2–GTP–Met–tRNAi^{Met} ternary complex to the 40S subunit (Chaudhuri *et al.*, 1999; Sonenberg *et al.*, 2000). It promotes binding of the 43S pre-initiation complex to the 5′ end of mRNA (Kolupaeva *et al.*, 2005; Hinnebusch, 2006). Moreover, it also plays roles in scanning for and recognizing AUG start codons (Lukaszewicz *et al.*, 2000; Dever, 2002; Nielsen *et al.*, 2004; Valasek *et al.*, 2004).

The components of eIF3 have been identified in many species. In humans, the functional core of eIF3 is made up of six (eIF3a, eIF3b, eIF3c, eIF3e, eIF3f, eIF3h) of 11 subunits. These are conserved in mammals, *Triticum aestivum* (wheat), *Arabidopsis thaliana*, *Saccharomyces pombe* and *Drosophila melanogaster* (Asano *et al.*, 1997b; Burks *et al.*, 2001; Masutani *et al.*, 2007; Zhou *et al.*, 2008). However, only eIF3a, eIF3b and eIF3c are found in the eIF3 core of *Saccharomyces cerevisiae*, which has five subunits (eIF3a, eIF3b, eIF3c, eIF3g and eIF3i), indicating that eIF3e, eIF3f and eIF3h may not be necessary for global translation initiation (Asano *et al.*, 1998; Burks *et al.*, 2001; Zhou *et al.*, 2005).

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A mutant CHS3 protein with TIR-NB-LRR-LIM domains modulates growth, cell death and freezing tolerance in a temperature-dependent manner in *Arabidopsis*

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SUMMARY

Low temperature is one of environmental factors that restrict plant growth homeostasis and plant–pathogen interactions. Recent studies suggest a link between temperature responses and defense responses; however, the underlying molecular mechanisms remain unclear. In this study, the *chilling sensitive 3* (*chs3-1*) mutant in *Arabidopsis* was characterized. *chs3-1* plants showed arrested growth and chlorosis when grown at 16°C or when shifted from 22 to 4°C. *chs3-1* plants also exhibited constitutively activated defense responses at 16°C, which were alleviated at a higher temperature (22°C). Map-based cloning of *CHS3* revealed that it encodes an unconventional disease resistance (R) protein belonging to the TIR-NB-LRR class with a zinc-binding LIM domain (Lin-11, Isl-1 and Mec-3 domains) at the carboxyl terminus. The *chs3-1* mutation in the conserved LIM-containing domain led to the constitutive activation of the TIR-NB-LRR domain. Consistently, the growth and defense phenotypes of *chs3-1* plants were completely suppressed by *eds1*, *sgt1b* and *rar1*, partially by *pad4* and *nahG*, but not by *npr1* and *ndr1*. Intriguingly, *chs3-1* plants grown at 16°C showed enhanced tolerance to freezing temperatures. This tolerance was correlated with growth defect and cell death phenotypes caused by activated defense responses. Other mutants with activated defense responses, including *cpr1*, *cpr5* and *slh1* also displayed enhanced freezing tolerance. These findings revealed a role of an unconventional mutant *R* gene in plant growth, defense response and cold stress, suggesting a mutual interaction between cold signaling and defense responses.

Keywords: CHS3, disease resistance (R) protein, low temperature, cell death, Arabidopsis.

INTRODUCTION

As sessile organisms, plants have evolved a variety of tolerance mechanisms by triggering a cascade of regulatory events via changes in gene expression, and subsequent biochemical and physiological modifications, to withstand environmental stresses, including low temperature and pathogen attack.

Advances have been made in understanding plant responses to low temperatures (Thomashow, 1999; Chinnusamy *et al*., 2007; Hua, 2009). Cold stress induces a transient increase in cytosolic Ca^{2+} levels and activates the expression of the C-repeat binding transcription factors *CBF/DREB1* (Thomashow, 1999). *CBF/DREB1* in turn triggers the expression of a subset of cold responsive (*COR*) genes (Gilmour *et al*., 1992). *CBF3* is transcriptionally regulated by the transcription factors ICE1 (inducer of CBF expression 1) and MYB15 (Chinnusamy *et al*., 2003; Agarwal *et al*., 2006). Besides the ICE1-CBF-COR cascade, which is one of the primary cold signaling pathways involved in plant responses to cold stress (Chinnusamy *et al*., 2007), other important components have been identified for cold responses in CBF-independent pathways (Xin and Browse, 1998; Zhu *et al*., 2005, 2008; Wang *et al*., 2009).

Plants use multiple mechanisms to fight against pathogen infection. Disease resistance (*R*) gene-mediated defense is one of the major mechanisms. The majority of R proteins fall into five classes. The largest class includes those proteins containing NB-LRR (nucleotide binding domain leucine-rich repeats) domains with either a coiled-coil (CC) domain or a

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ABO3, a WRKY transcription factor, mediates plant responses to abscisic acid and drought tolerance in Arabidopsis

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SUMMARY

The biological functions of WRKY transcription factors in plants have been widely studied, but their roles in abiotic stress are still not well understood. We isolated an ABA overly sensitive mutant, *abo3*, which is disrupted by a T-DNA insertion in *At1g66600* encoding a WRKY transcription factor AtWRKY63. The mutant was hypersensitive to ABA in both seedling establishment and seedling growth. However, stomatal closure was less sensitive to ABA, and the *abo3* mutant was less drought tolerant than the wild type. Northern blot analysis indicated that the expression of the ABA-responsive transcription factor *ABF2/AREB1* was markedly lower in the *abo3* mutant than in the wild type. The *abo3* mutation also reduced the expression of stress-inducible genes *RD29A* and *COR47*, especially early during ABA treatment. ABO3 is able to bind the W-box in the promoter of *ABF2 in vitro*. These results uncover an important role for a WRKY transcription factor in plant responses to ABA and drought stress.

Keywords: WRKY transcription factor, abscisic acid, Arabidopsis, drought stress.

INTRODUCTION

Drought stress is one of the most severe environmental culprits that greatly restrict plant distribution and crop production (Zhu, 2002). Drought stress induces the accumulation of the plant hormone abscisic acid (ABA), which leads to stomatal closure for maintaining water status in plant cells under water-deficit conditions. The increased ABA interacts with the ABA receptors PYR/PYLs of START proteins, which interact with PP2C proteins and release the inhibition of PP2Cs on SnRK2 protein kinases. The activated SnRK2s phosphorylate downstream transcriptional factors such as ABSCISIC ACID RESPONSIVE ELEMENTS-BINDING FACTOR 2 (ABF2) and ABI5 (ABA insensitive 5, which is a bZIP protein) to regulate the expression of ABA response genes (Fujii and Zhu, 2009; Ma *et al.*, 2009; Nakashima *et al.*, 2009; Park *et al.*, 2009). Various genes are up- or downregulated at the transcriptional level by both drought stress and ABA treatment. Analyzing the promoters of ABA-inducible genes has identified some conserved *cis*-elements, one of which is ABRE (ABA-responsive element, PyACGTGGC) (Guiltinan *et al.*, 1990; Yamaguchi-Shinozaki *et al.*, 1990; Iwasaki *et al.*, 1995; Shen *et al.*, 1996). Transcription factors such as AREB (ABRE binding protein)/ABFs, and ABI5 could bind ABRE and regulate the expression of ABA-responsive genes (Uno *et al.*, 2000; Carles *et al.*, 2002; Casaretto and Ho, 2003). The transcripts of ABFs, including *ABF1*, *ABF2/AREB1*, *ABF3* and *ABF4/AREB2*, are also highly induced by the application of exogenous ABA (Choi *et al.*, 2000; Uno *et al.*, 2000). Plants overexpressing *ABF3* and *ABF4* showed increased ABA sensitivity in seed germination and seedling growth, reduced transpiration and more drought tolerance than wild-type plants (Kang *et al.*, 2002). However, constitutive overexpression of *ABF2/AREB1* did not increase the expression of downstream ABA-responsive genes, because the activation of ABF2/AREB1 needs ABA-triggered protein phosphorylation (Fujita *et al.*, 2005). An ABA-activated 42-kDa kinase can phosphorylate and activate ABF2/AREB1 (Fujita *et al.*, 2005;

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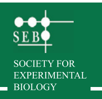

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ABA overly-sensitive 5 (*ABO5*), encoding a pentatricopeptide repeat protein required for *cis*-splicing of mitochondrial *nad2* intron 3, is involved in the abscisic acid response in Arabidopsis

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SUMMARY

To study the molecular mechanism of abscisic acid (ABA) regulation of root development, we screened the root growth of Arabidopsis mutants for sensitivity to ABA. *ABA overly-sensitive 5* (*ABO5*/*At1g51965*) was identified, and was determined to encode a pentatricopeptide repeat protein required for *cis*-splicing of mitochondrial *nad2* intron 3 (*nad2* is one subunit in complex I). Under constant light conditions (24-h light/0-h dark photoperiod), *abo5* mutants exhibited various phenotypes and expressed lower transcripts of stress-inducible genes, such as *RD29A*, *COR47* and *ABF2*, and photosynthesis-related genes *proton gradient regulation 5* (*PGR5*) and *PGR5-like photosynthetic phenotype* (*PGRL1*), but higher levels of nuclear-encoded genes *alternative oxidase 1a* (*AOX1a*) and *oxidative signal-inducible 1* (*OXI1*). Prolonged ABA treatment increased the expression of the *cox2* gene in complex IV and *nad* genes in complex I to a higher level than no ABA treatment in the wild type, but only to a moderate level in *abo5*, probably because *abo5* already expressed high levels of mitochondrial-encoded *cox2* and *nad* genes under no ABA treatment. More H_2O_2 accumulated in the root tips of *abo5* than in the wild type, and H_2O_2 accumulation was further enhanced by ABA treatment. However, these growth phenotypes and gene-expression defects were attenuated by growing *abo5* plants under short-day conditions (12-h light/12-h dark photoperiod). Our results indicate that ABO5 is important in the plant response to ABA.

Keywords: mitochondria, PPR protein, ABA signaling, oxidative stress.

INTRODUCTION

Plants have evolved a series of mechanisms to limit stress and damage caused by unfavorable environmental conditions. Abscisic acid (ABA), a hormone produced when plants are stressed by drought, salt and cold, is an important signal molecule. ABA helps plants cope with these unfavorable stresses, and also plays essential roles in seed development and seedling growth. Genetic screening using seed germination sensitivity to ABA has identified several key mediators in the ABA signaling pathway, including (ABA insensitive 1) ABI1, ABI2, ABI3, ABI4 and ABI5 (Finkelstein and Lynch, 2000; Finkelstein *et al.*, 1998). ABI1 and ABI2, which are phosphatase type-2C proteins with negative regulation roles in ABA signaling, physically interact with and inhibit downstream target proteins of ABA signals, such as serine/threonine protein kinase OPEN STOMATA1 (OST1), when ABA content is limited. The increased levels of ABA under abiotic stress cause the ABA receptors PRY1/PYLs to interact with these PP2C proteins and relieve the inhibition on their downstream targeted protein kinases (Fujii *et al.*, 2009; Ma *et al.*, 2009; Nishimura *et al.*, 2009; Park *et al.*, 2009; Santiago *et al.*, 2009). ABI5 is a basic leucine zipper transcription factor that can be phosphorylated and activated

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Overexpression of AtDOF4.7, an Arabidopsis DOF Family Transcription Factor, Induces Floral Organ Abscission Deficiency in Arabidopsis[1[C][W]]

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After flower pollination, a programmed process called abscission occurs in which unwanted floral organs are actively shed from the main plant body. We found that a member of the DOF (for DNA binding with one finger) transcription factor family, Arabidopsis (*Arabidopsis thaliana*) DOF4.7, was expressed robustly in the abscission zone. The Arabidopsis *35S::AtDOF4.7* lines with constitutive expression of *AtDOF4.7* exhibited an ethylene-independent floral organ abscission deficiency. In these lines, anatomical analyses showed that the formation of the abscission zone was normal. However, dissolution of the middle lamella failed to separate between the cell walls. AtDOF4.7 was identified as a nucleus-localized transcription factor. This protein had both in vitro and in vivo binding activity to typical DOF cis-elements in the promoter of an abscission-related polygalacturonase (PG) gene, *PGAZAT*. Overexpression of *AtDOF4.7* resulted in down-regulation of *PGAZAT*. AtDOF4.7 interacted with another abscission-related transcription factor, Arabidopsis ZINC FINGER PROTEIN2. Taken together, our results suggest that AtDOF4.7 participates in the control of abscission as part of the transcription complex that directly regulates the expression of cell wall hydrolysis enzymes.

Organ shedding, or abscission, is a critical cell separation process that may occur throughout the life cycle of plants (Roberts et al., 2002; Lewis et al., 2006). Abscission can be an adaptation to environment stresses, such as water or nutrient deficiency, oxygen damage, and pathogen attack (Taylor and Whitelaw, 2001), or a developmentally controlled program that occurs after leaf senescence, flower pollination, and fruit maturation (van Doorn and Stead, 1997; Roberts et al., 2002). At the predetermined abscission position, an abscission zone (AZ) develops. The AZ is composed of a few layers of dense cytoplasmic cells (Sexton and Roberts, 1982; Patterson, 2001). The AZ cells recognize abscission signals that activate cell wall-loosening protein factors. Several wall-loosening proteins have been well documented for their AZ expression, including endoglucanases (del Campillo et al., 1990; Tucker et al., 1991; Tucker and Milligan, 1991; Taylor et al., 1994; del Campillo, 1999; Mishra et al., 2008), polygalacturonases (PGs; del Campillo et al., 1990; Taylor et al., 1991, 1993; Kalaitzis et al., 1995; del Campillo, 1999; Gonzalez-Carranza et al., 2002, 2007a; Jiang et al., 2008), and expansins (Cho and Cosgrove, 2000; Belfield et al., 2005). The middle lamellae of AZ cell walls are presumably loosened and dissolved by at least some of the listed wall proteins, resulting in organ detachment from the main body of the plant.

Floral organ abscission in Arabidopsis (*Arabidopsis thaliana*) has been used as a model system for studying the genetics underlying the abscission process (Bleecker and Patterson, 1997; Patterson, 2001). Certain genes involved in upstream signaling of abscission have been identified in this system. Ethylene is considered to be a fundamental regulator of the abscission rate (Bleecker and Patterson, 1997; Patterson, 2001; Taylor and Whitelaw, 2001; Patterson and Bleecker, 2004). Defects in the components of the ethylene perception and signaling pathways will delay abscission to various degrees. In ethylene-insensitive mutants, such as *etr1* and *ein2*, both floral organ abscission and senescence are delayed (Patterson and Bleecker, 2004; Lewis et al., 2006).

Ethylene is not the only regulator of abscission. A ligand gene family, including *INFLORESCENCE*

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Breakthrough Technologies

MISSA Is a Highly Efficient in Vivo DNA Assembly Method for Plant Multiple-Gene Transformation[1[C][W]]

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We describe a highly efficient in vivo DNA assembly method, multiple-round in vivo site-specific assembly (MISSA), which facilitates plant multiple-gene transformation. MISSA is based on conjugational transfer, which is driven by donor strains, and two in vivo site-specific recombination events, which are mediated by inducible Cre recombinase and phage λ site-specific recombination proteins in recipient strains, to enable in vivo transfer and in vivo assembly of multiple transgenic DNA. The assembly reactions can be performed circularly and iteratively through alternate use of the two specially designed donor vectors. As proof-of-principle experiments, we constructed a few plant multigene binary vectors. One of these vectors was generated by 15 rounds of MISSA reactions and was confirmed in transgenic Arabidopsis (*Arabidopsis thaliana*). As MISSA simplifies the tedious and time-consuming in vitro manipulations to a simple mixing of bacterial strains, it will greatly save time, effort, and expense associated with the assembly of multiple transgenic or synthetic DNA. The principle that underlies MISSA is applicable to engineering polygenic traits, biosynthetic pathways, or protein complexes in all organisms, such as *Escherichia coli*, yeast, plants, and animals. MISSA also has potential applications in synthetic biology, whether for basic theory or for applied biotechnology, aiming at the assembly of genetic pathways for the production of biofuels, pharmaceuticals, and industrial compounds from natural or synthetic DNA.

The vast majority of agronomic traits, including crop production traits, metabolic pathways such as carotenoid biosynthesis pathways, signal pathways such as abscisic acid (ABA) signal transduction, and multimeric proteins such as vacuolar H^+-ATPase, are controlled by polygenes. Genetic manipulation of polygenic traits, pathways, or protein complexes is having a profound impact on basic plant research and biotechnology and is presenting a clear challenge for plant genetic engineers, along with the prospect of continued developments in functional genomics (Daniell and Dhingra, 2002; Halpin, 2005; Dafny-Yelin and Tzfira, 2007). The transgenic golden rice (*Oryza sativa*; Ye et al., 2000), purple tomato (*Solanum lycopersicum*; Butelli et al., 2008), red corn (*Zea mays*; Zhu et al., 2008), among others, demonstrated the promising future of plant multigene transformation. Several approaches, such as cotransformation (Chen et al., 1998; Zhu et al., 2008), retransformation (Li et al., 2003), multigene linking and sexual crosses (Zhao et al., 2003), can be used for the delivery of multiple genes into plant cells. The stacking of multiple expression cassettes onto a single binary plasmid sometimes has a profound advantage over the use of the other approaches mentioned above (Dafny-Yelin and Tzfira, 2007). The homing endonuclease-based pRCS/pAUX and pSAT vector systems (Goderis et al., 2002; Tzfira et al., 2005; Dafny-Yelin and Tzfira, 2007), Cre/*loxP* recombination (Lin et al., 2003), MultiSite Gateway (Karimi et al., 2007), and MultiRound Gateway (Chen et al., 2006a) have been specially developed in order to assemble multiple genes. In spite of the success of the vector systems in simplifying the assembly of multigene cassettes and enabling greater numbers of transgenes to be directly linked, they still require large amounts of time, effort, and expense. The MAGIC technology based on in vivo transfer and in vivo homologous recombination is an efficient, inexpensive, and time-saving method (Li and Elledge, 2005), but it is not suitable for the assembly of multiple transgenes. Therefore, a method having the advantages of MAGIC for the assembly of multiple genes will be greatly beneficial for multiple-gene transformation in plants or other organisms.

In this report, we describe a highly efficient, inexpensive, and labor-saving in vivo DNA assembly system based on in vivo transfer and in vivo site-specific recombination methods. This system, named MISSA (for multiple-round in vivo site-specific assembly), can be used not only for the assembly of plant multiple transgenes but also for the assembly of genetic pathways for the production of biofuels, pharmaceuticals, and industrial compounds from natural

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A Gain-of-Function Mutation in the Arabidopsis Disease Resistance Gene *RPP4* Confers Sensitivity to Low Temperature[1[W][OA]]

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How plants adapt to low temperature is not well understood. To identify components involved in low-temperature signaling, we characterized the previously isolated *chilling-sensitive2* mutant (*chs2*) of Arabidopsis (*Arabidopsis thaliana*). This mutant grew normally at 22°C but showed phenotypes similar to activation of defense responses when shifted to temperatures below 16°C. These phenotypes include yellowish and collapsed leaves, increased electrolyte leakage, up-regulation of *PATHOGENESIS RELATED* genes, and accumulation of excess hydrogen peroxide and salicylic acid (SA). Moreover, the *chs2* mutant was seedling lethal when germinated at or shifted for more than 3 d to low temperatures of 4°C to 12°C. Map-based cloning revealed that a single amino acid substitution occurred in the TIR-NB-LRR (for Toll/Interleukin-1 receptor- nucleotide-binding Leucine-rich repeat)-type resistance (R) protein RPP4 (for Recognition of *Peronospora parasitica4*), which causes a deregulation of the R protein in a temperature-dependent manner. The *chs2* mutation led to an increase in the mutated *RPP4* mRNA transcript, activation of defense responses, and an induction of cell death at low temperatures. In addition, a *chs2* intragenic suppressor, in which the mutation occurs in the conserved NB domain, abolished defense responses at lower temperatures. Genetic analyses of *chs2* in combination with known SA pathway and immune signaling mutants indicate that the *chs2*-conferred temperature sensitivity requires *ENHANCED DISEASE SUSCEPTIBILITY1*, *REQUIRED FOR Mla12 RESISTANCE*, and *SUPPRESSOR OF G2 ALLELE OF skp1* but does not require *PHYTOALEXIN DEFICIENT4*, *NONEXPRESSOR OF PR GENES1*, or SA. This study reveals that an activated TIR-NB-LRR protein has a large impact on temperature sensitivity in plant growth and survival.

For optimal growth and survival, plants have evolved unique and sophisticated defense mechanisms against multiple stresses, both abiotic and biotic. Cold stress has a significant limiting effect on the geographic location of plants and on crop productivity (Guy, 1990). It can disrupt cellular homeostasis by altering the fatty acid composition of membrane lipids, which can deactivate membrane proteins and uncouple major physiological processes (Los and Murata, 2004). Plants respond and adapt to cold stress in many biochemical and physiological processes. A number of genes are involved in the DREB/CBF (for DRE-binding protein/C-repeat-binding factor)-dependent pathway to control cold acclimation (Gilmour et al., 1992, 2004), and DREB/CBF-independent pathways have been identified as important for cold responses as well (Xin and Browse, 1998; Dong et al., 2006; Lee et al., 2006; Xin et al., 2007; Zhu et al., 2008).

Plants have evolved at least two layers of defense mechanisms against pathogens. One of them is mediated by resistance (R) proteins. Interaction of an R protein with a specific pathogen avirulence protein triggers the hypersensitive response (HR), which is a form of programmed cell death that limits pathogen growth and spread (Scheel, 1998). Most of the characterized R proteins encode proteins with nucleotide-binding Leu-rich repeat (NB-LRR) domains. A well-conserved ARC (for Apaf-1, R protein, and CED4) domain is found just after the NB domain, and these two domains are often referred to as the NB-ARC domain. The NB-LRR proteins can be grouped into two main classes based on their N-terminal structure, which has either a Toll/Interleukin-1 receptor (TIR) domain or a coiled-coil domain (Meyers et al., 2003).

The Arabidopsis (*Arabidopsis thaliana*) *RPP5* (for *Recognition of Peronospora parasitica5*) locus in Columbia-0 (Col) is composed of seven TIR-NB-LRR class *R* genes, including *RPP4* and *SNC1* (for *Suppressor of npr1-1, constitutive 1*) genes (Noel et al., 1999). *RPP4* plays an important role in resistance to *Hyaloperonospora parasitica* through multiple signaling components, in-

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Nitric Oxide Acts Downstream of Auxin to Trigger Root Ferric-Chelate Reductase Activity in Response to Iron Deficiency in Arabidopsis[1][C][W][OA]

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In response to iron (Fe) deficiency, dicots employ a reduction-based mechanism by inducing ferric-chelate reductase (FCR) at the root plasma membrane to enhance Fe uptake. However, the signal pathway leading to FCR induction is still unclear. Here, we found that the Fe-deficiency-induced increase of auxin and nitric oxide (NO) levels in wild-type Arabidopsis (*Arabidopsis thaliana*) was accompanied by up-regulation of root FCR activity and the expression of the basic helix-loop-helix transcription factor (*FIT*) and the ferric reduction oxidase 2 (*FRO2*) genes. This was further stimulated by application of exogenous auxin (α-naphthaleneacetic acid) or NO donor (*S*-nitrosoglutathione [GSNO]), but suppressed by either polar auxin transport inhibition with 1-naphthylphthalamic acid or NO scavenging with 2-(4-carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide, tungstate, or N$^{\omega}$-nitro-L-arginine methyl ester hydrochloride. On the other hand, the root FCR activity, NO level, and gene expression of *FIT* and *FRO2* were higher in auxin-overproducing mutant *yucca* under Fe deficiency, which were sharply restrained by 2-(4-carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide treatment. The opposite response was observed in a basipetal auxin transport impaired mutant *aux1-7*, which was slightly rescued by exogenous GSNO application. Furthermore, Fe deficiency or α-naphthaleneacetic acid application failed to induce Fe-deficiency responses in *noa1* and *nial nia2*, two mutants with reduced NO synthesis, but root FCR activities in both mutants could be significantly elevated by GSNO. The inability to induce NO burst and FCR activity was further verified in a double mutant *yucca noa1* with elevated auxin production and reduced NO accumulation. Therefore, we presented a novel signaling pathway where NO acts downstream of auxin to activate root FCR activity under Fe deficiency in Arabidopsis.

Iron (Fe) deficiency is one of the major limiting factors affecting crop production in calcareous soils worldwide (Imsande, 1998). Fortunately, many plants have developed various strategies to cope with Fe deficiency in those soils. These strategies are classified as strategy I in nongraminaceous monocots and dicots, and strategy II in graminaceous monocots (Römheld and Marschner, 1981). Strategy I plants employ a range of responses to Fe-deficiency stress to acquire Fe from the soil, including: (1) induction of both a plasmalemma ferric-chelate reductase (FCR; Robinson et al., 1999) and plasmalemma Fe(II) transporter in root cells (Eide et al., 1996; Vert et al., 2002), (2) enhanced release of protons and reductants such as phenolic compounds into the rhizosphere (Curie and Briat, 2003; Jin et al., 2006, 2007), and (3) changes in root architecture, including enhanced root branching (Jin et al., 2008) and subapical root hair development (Römheld and Marschner, 1981; Schmidt, 1999; Santi and Schmidt, 2008). Among these responses, the activation of FCR has been suggested to be a key component (Curie and Briat, 2003) as the strategy I plants must enzymatically reduce Fe(III) before their root cells can take it up in the form of Fe(II) (Chaney et al., 1972). Although FCR induction in Fe-deficient roots of different plant species and its function have been well documented (Robinson et al., 1999; Connolly et al., 2003), the signals involved in the regulatory cascade leading to the activation of FCR are still not well understood.

It has been demonstrated that the regulation of Fe-deficiency responses does not depend solely on root Fe content, but is far more complex and may also involve signals originating from the shoot (Romera et al., 1992; Grusak and Pezeshgi, 1996; Forde, 2002; Vert et al., 2003; Zheng et al., 2003; Enomoto and Goto, 2008). Shoot-derived auxin is a promising candidate as a signal molecule transmitting Fe-deficiency information, since its synthesis is enhanced under Fe starvation (Römheld

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Arabidopsis Calcium-Dependent Protein Kinase CPK10 Functions in Abscisic Acid- and Ca^{2+}-Mediated Stomatal Regulation in Response to Drought Stress[1][W][OA]

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Plant calcium-dependent protein kinases (CDPKs) may function as calcium sensors and play important roles in the regulation of plant growth and development and in plant responses to biotic and abiotic stresses. The Arabidopsis (*Arabidopsis thaliana*) genome encodes 34 CDPKs, and most of them have not been functionally characterized. Here, we report the functional characterization of CPK10 in Arabidopsis response to drought stress. The *cpk10* mutant, a T-DNA insertion mutant for the Arabidopsis *CPK10* gene, showed a much more sensitive phenotype to drought stress compared with wild-type plants, while the *CPK10* overexpression lines displayed enhanced tolerance to drought stress. Induction of stomatal closure and inhibition of stomatal opening by abscisic acid (ABA) and Ca^{2+} were impaired in the *cpk10* mutants. Using yeast two-hybrid methods, a heat shock protein, HSP1, was identified as a CPK10-interacting protein. The interaction between CPK10 and HSP1 was further confirmed by pull-down and bimolecular fluorescence complementation assays. The *HSP1* knockout mutant (*hsp1*) plants showed a similar sensitive phenotype under drought stress as the *cpk10* mutant plants and were similarly less sensitive to ABA and Ca^{2+} in regulation of stomatal movements. Electrophysiological experiments showed that ABA and Ca^{2+} inhibition of the inward K^+ currents in stomatal guard cells were impaired in the *cpk10* and *hsp1* mutants. All presented data demonstrate that CPK10, possibly by interacting with HSP1, plays important roles in ABA- and Ca^{2+}-mediated regulation of stomatal movements.

Plants are subjected to various environmental stresses during their growth and development and have developed various mechanisms to adapt to these stresses. As an important cytoplasmic second messenger, Ca^{2+} plays critical roles in plant responses to environmental stresses (Rudd and Franklin-Tong, 2001; Sanders et al., 2002; Kudla et al., 2010). Specific calcium signatures may be recognized by different sensor proteins. Three major families of Ca^{2+} sensors have been identified in higher plants: calmodulins (CaMs) and CaM-like proteins (McCormack et al., 2005); calcineurin B-like (CBL) proteins (Kolukisaoglu et al., 2004; Luan, 2009; Weinl and Kudla, 2009); and calcium-dependent protein kinases (CDPKs; Harmon et al., 2000; Cheng et al., 2002; Harper et al., 2004; Harper and Harmon, 2005). CaMs and CBLs are small proteins and transmit the Ca^{2+} signal through interacting target proteins and regulating their activities. The CBLs not only regulate the activities of CBL-interacting protein kinases, but at least some of them are also involved in recruiting the kinases to different membranes (Luan, 2009). CDPKs are activated upon binding Ca^{2+} to their CaM-like domain and then relay the signaling to their downstream targets (Harmon et al., 2000; Cheng et al., 2002; Harper et al., 2004; Harper and Harmon, 2005).

CDPKs are found in a wide range of vascular and nonvascular plants as well as in green algae and certain protozoa (Harmon et al., 2001), suggesting their potential importance in Ca^{2+} signaling in plant cells. The CDPKs are encoded by multigene families and have been identified in various plant species, such as Arabidopsis (*Arabidopsis thaliana*; Harmon et al., 2001; Cheng et al., 2002), rice (*Oryza sativa*; Asano et al., 2005; Wan et al., 2007), cotton (*Gossypium hirsutum*; Huang et al., 2008), and wheat (*Triticum aestivum*; Li et al., 2008). Some CDPKs are expressed ubiquitously, whereas others are present in specific tissues or their expression is regulated by different stimuli (Hrabak et al., 2003). It is also known that different CDPKs have different subcellular locations, including cytosol, nucleus, the plasma membrane, endoplasmic reticulum, peroxisomes, mitochondrial outer membrane, and oil bodies (Harper et al., 2004), indicating their possible diverse functions. A number of studies have demonstrated that CDPKs play important roles in plant responses to various abiotic stresses, including cold,

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The *Arabidopsis LSD1* gene plays an important role in the regulation of low temperature-dependent cell death

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Key words: *Arabidopsis*, cell death, low temperature, LSD1, reactive oxygen species (ROS).

Summary

- In higher plants, the crosstalk between cold stress responses and reactive oxygen species (ROS) signaling is not well understood.
- Two chilling-sensitive mutants, *chs4-1* and *chs4-3*, were characterized genetically and molecularly.
- The *CHS4* gene, identified by map-based cloning, was found to be identical to *LESION SIMULATING DISEASE RESISTANCE 1* (*LSD1*). We therefore renamed these two alleles *lsd1-3* and *lsd1-4*, respectively. These two mutants exhibited an extensive cell death phenotype under cold stress conditions. Consistently, *lsd1-3* plants exposed to cold showed up-regulation of the *PR1* and *PR2* genes, and increased accumulation of salicylic acid. These results indicate that low temperature is another trigger of cell death in *lsd1* mutants. Furthermore, *lsd1-3* plants accumulated higher concentrations of H_2O_2 and total glutathione under cold conditions than wild-type plants. Genetic analysis revealed that *PAD4* and *EDS1*, two key signaling regulators mediating resistance responses, are required for the chilling-sensitive phenotype of *lsd1-3*.
- These findings reveal a role of LSD1 in regulating cell death trigged by cold stress and a link between cold stress responses and ROS-associated signaling.

Introduction

Temperature is one of the major environmental factors that influence plant growth and development as well as distribution. In order to survive, plants respond and adapt to stress through various biochemical and physiological processes. When exposed to low temperatures, plant cytosolic Ca^{2+} concentrations increase transiently, followed by altered expression of diverse cold-regulated (*COR*) genes (Gilmour *et al.*, 1992; Nordin *et al.*, 1993; Yamaguchi-Shinozaki & Shinozaki, 1994). Some of these genes are regulated by C-repeat/dehydration responsive element-binding factor (CBF) transcription factors, which are considered to be the central components responsible for cold tolerance (Shinwari *et al.*, 1998).

Several lines of evidence suggest that plant responses to cold stress are directly linked to reactive oxygen species (ROS) signaling (Lee *et al.*, 2002; Davletova *et al.*, 2005a; Vogel *et al.*, 2005; Einset *et al.*, 2007a,b, 2008). ROS are key signal transduction molecules during responses to environmental stresses and developmental stimuli (Mittler *et al.*, 2004). During environmental stresses, ROS activate stress-response pathways and induce defense mechanisms. On the other hand, excess ROS produced under stress and the failure to maintain ROS balance cause oxidative damage to cells, leading to growth defects or initiation of cell death (Torres & Dangl, 2005). Cold stress increases transcript abundance and protein concentrations of ROS-scavenging enzymes, as well as ROS accumulation (O'Kane *et al.*, 1996). Several genes have been implicated in both cold stress and ROS signaling. For example, overexpression of *ZAT12*, a C2H2 zinc finger-type transcription factor gene, induces cold-inducible genes and confers increased cold tolerance in plants when overexpressed. Furthermore, *ZAT12* plays a central role and regulates a number of genes involved in plant responses to oxidative stress (Davletova *et al.*, 2005b). Glycine betaine (GB) is a chemical that improves tolerance to stress caused by chilling (Park *et al.*, 2004). A membrane trafficking protein RabA4c and a ferric reductase FRO2 play roles in GB's effect on ROS accumulation

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Research

Identification of a novel mitochondrial protein, short postembryonic roots 1 (SPR1), involved in root development and iron homeostasis in *Oryza sativa*

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Key words: chlorosis, iron homeostasis, iron, map-based cloning, rice (*Oryza sativa*), root elongation.

Summary

- A rice mutant, *Oryza sativa short postembryonic roots 1* (*Osspr1*), has been characterized. It has short postembryonic roots, including adventitious and lateral roots, and a lower iron content in its leaves.
- *OsSPR1* was identified by map-based cloning. It encodes a novel mitochondrial protein with the Armadillo-like repeat domain.
- *Osspr1* mutants exhibited decreased root cell elongation. The iron content of the mutant shoots was significantly altered compared with that of wild-type shoots. A similar pattern of alteration of manganese and zinc concentrations in shoots was also observed. Complementation of the mutant confirmed that *OsSPR1* is involved in post-embryonic root elongation and iron homeostasis in rice. *OsSPR1* was found to be ubiquitously expressed in various tissues throughout the plant. The transcript abundance of various genes involved in iron uptake and signaling via both strategies I and II was similar in roots of wild-type and mutant plants, but was higher in the leaves of mutant plants.
- Thus, a novel mitochondrial protein that is involved in root elongation and plays a role in metal ion homeostasis has been identified.

Introduction

Iron (Fe) is an essential co-factor for several enzymes involved in crucial cellular processes ranging from oxygen and electron transport to hormone production and DNA synthesis (Briat & Lobreaux, 1997). In addition, plants require Fe for several processes that occur in plastids and an adequate Fe supply is essential to maintain photosynthetic function at optimal rates (Briat *et al.*, 2007). However, excess Fe generates hydroxyl radicals via the Fenton reaction, which can damage biological molecules (Grotz & Guerinot, 2006).

Although abundant in soils, Fe often forms insoluble ferric hydroxide precipitates that limit its uptake by plants. Therefore, higher plants have evolved two distinct strategies to solubilize and acquire Fe from the rhizoshpere (Jeong & Guerinot, 2009; Morrissey & Guerinot, 2009). Nongraminaceous plants use the strategy I system, which involves the induction of membrane-bound Fe(III)-chelate reductases, which reduce Fe(III) to the more soluble form of Fe(II), followed by uptake of Fe(II) via the Fe(II) transporter iron-regulated transporter 1 (IRT1) (Eide *et al.*, 1996). By contrast, graminaceous plants use strategy II, which is mediated by the synthesis and secretion of natural Fe chelators, the mugineic acid (MA) family of phytosiderophores. The secreted MAs solubilize Fe(III) in the rhizosphere, and the resulting Fe(III)–MA complexes are absorbed into the root cells by the yellow-stripe-like (YSL) transporters (Curie *et al.*, 2001). Rice (*Oryza sativa*) can use both strategies I and II for Fe uptake (Cheng *et al.*, 2007).

Several genes involved in Fe uptake have been identified in rice. Loss-of-function mutants of *OsNAAT* (*Nicotianamine Aminotransferase*) were found to have a low assimilation capacity for Fe(III), but a normal assimilation capacity for Fe(II) (Cheng *et al.*, 2007). OsYSL15 and OsIRT1 were reported to be Fe(III)–MA and Fe(II) transporters in rice,

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Analyzing circadian expression data by harmonic regression based on autoregressive spectral estimation

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ABSTRACT

Motivation: Circadian rhythms are prevalent in most organisms. Identification of circadian-regulated genes is a crucial step in discovering underlying pathways and processes that are clock-controlled. Such genes are largely detected by searching periodic patterns in microarray data. However, temporal gene expression profiles usually have a short time-series with low sampling frequency and high levels of noise. This makes circadian rhythmic analysis of temporal microarray data very challenging.

Results: We propose an algorithm named ARSER, which combines time domain and frequency domain analysis for extracting and characterizing rhythmic expression profiles from temporal microarray data. ARSER employs autoregressive spectral estimation to predict an expression profile's periodicity from the frequency spectrum and then models the rhythmic patterns by using a harmonic regression model to fit the time-series. ARSER describes the rhythmic patterns by four parameters: period, phase, amplitude and mean level, and measures the multiple testing significance by false discovery rate *q*-value. When tested on well defined periodic and non-periodic short time-series data, ARSER was superior to two existing and widely-used methods, COSOPT and Fisher's *G*-test, during identification of sinusoidal and non-sinusoidal periodic patterns in short, noisy and non-stationary time-series. Finally, analysis of *Arabidopsis* microarray data using ARSER led to identification of a novel set of previously undetected non-sinusoidal periodic transcripts, which may lead to new insights into molecular mechanisms of circadian rhythms.

Availability: ARSER is implemented by Python and R. All source codes are available from http://bioinformatics.cau.edu.cn/ARSER

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1 INTRODUCTION

Circadian rhythm is one of the most well-studied periodic processes in living organisms. DNA microarray technologies have often been applied in circadian rhythm studies (Duffield, 2003). Thus, we can monitor the mRNA expression of the whole-genome level, which is an effective way to simultaneously identify many hundreds or thousands of periodic transcripts. The matter to be addressed is which genes are rhythmically expressed based on their gene expression profiles. This can be classified as a periodicity identification problem. However, there are computational challenges when dealing with this issue: sparse determination of sampling rate, and short periods of data collection for microarray experiments (Bar-Joseph, 2004). Circadian microarray experiments are usually designed to collect data every 4 h over a course of 48 h, generating expression profiles with 12 or 13 time-points (Yamada and Ueda, 2007). There are two main factors that limit the number of data points that can be feasibly obtained: budget constraints and dampening of the circadian rhythm (Ceriani *et al.*, 2002). Such short time-series data render many methods of classical time-series analysis inappropriate, since they generally require much larger samples to generate statistically significant results.

A variety of algorithms have been developed and applied to microarray time-series analysis; Chudova *et al.* (2009) indicated that the existing technologies fall into two major categories: time-domain and frequency-domain analyses. Typical time-domain methods rely on sinusoid-based pattern matching technology, while frequency-domain methods are based on spectral analysis methods. Of the time-domain methods, COSOPT (Straume, 2004) is a well-known algorithm frequently used to analyze circadian microarray data in *Arabidopsis* (Edwards *et al.*, 2006), *Drosophila* (Ceriani *et al.*, 2002) and mammalian systems (Panda *et al.*, 2002). COSOPT measures the goodness-of-fit between experimental data and a series of cosine curves of varying phases and period lengths. The advantages of pattern-matching methods are simplicity and computational efficiency, while they are not effective at finding periodic signals that are not perfectly sinusoidal (Chudova *et al.*, 2009).

Of the frequency-domain methods, Fisher's *G*-test was proposed to detect periodic gene expression profiles by Wichert *et al.* (2004) and has been used to analyze circadian microarray data of *Arabidopsis* (Blasing *et al.*, 2005) and mammalian systems (Hughes *et al.*, 2009; Ptitsyn *et al.*, 2006). Fisher's *G*-test searches periodicity by computing the periodogram of experimental data and tests the significance of the dominant frequency using Fisher's *G*-statistic; however, it is limited by low frequency resolution for short time-series generated by circadian microarray experiments, which means it is often not adequate to resolve the periodicity of interest (Langmead *et al.*, 2003). Time-domain and frequency-domain methods are two different ways to analyze the time-series, each with advantages and disadvantages. Frequency-domain methods are noise-tolerant and model-independent but their results are difficult for biologists to understand. Time-domain methods can give comprehensive and easily-understood descriptions for rhythms but are noise-sensitive and model-dependent (e.g. sinusoid).

Considering the above limitations, we propose an algorithm named ARSER that combines time-domain and frequency-domain analyses to identify periodic transcripts in large-scale time-course gene expression profiles. ARSER employs autoregressive (AR) spectral analysis (Takalo *et al.*, 2005) to estimate the period length of a circadian rhythm from the frequency spectrum. It is well-suited to analyze short time-series since it can generate smooth and high-resolution spectra from gene expression profiles. It is related (but not identical) to a method called maximum entropy spectral analysis,

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Gene Expression Profiles Deciphering Rice Phenotypic Variation between Nipponbare (Japonica) and 93-11 (Indica) during Oxidative Stress

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Abstract

Rice is a very important food staple that feeds more than half the world's population. Two major Asian cultivated rice (*Oryza sativa L.*) subspecies, *japonica* and *indica*, show significant phenotypic variation in their stress responses. However, the molecular mechanisms underlying this phenotypic variation are still largely unknown. A common link among different stresses is that they produce an oxidative burst and result in an increase of reactive oxygen species (ROS). In this study, methyl viologen (MV) as a ROS agent was applied to investigate the rice oxidative stress response. We observed that 93-11 (*indica*) seedlings exhibited leaf senescence with severe lesions under MV treatment compared to Nipponbare (*japonica*). Whole-genome microarray experiments were conducted, and 1,062 probe sets were identified with gene expression level polymorphisms between the two rice cultivars in addition to differential expression under MV treatment, which were assigned as Core Intersectional Probesets (CIPs). These CIPs were analyzed by gene ontology (GO) and highlighted with enrichment GO terms related to toxin and oxidative stress responses as well as other responses. These GO term-enriched genes of the CIPs include glutathine S-transferases (GSTs), P450, plant defense genes, and secondary metabolism related genes such as chalcone synthase (CHS). Further insertion/deletion (InDel) and regulatory element analyses for these identified CIPs suggested that there may be some eQTL hotspots related to oxidative stress in the rice genome, such as GST genes encoded on chromosome 10. In addition, we identified a group of marker genes individuating the *japonica* and *indica* subspecies. In summary, we developed a new strategy combining biological experiments and data mining to study the possible molecular mechanism of phenotypic variation during oxidative stress between Nipponbare and 93-11. This study will aid in the analysis of the molecular basis of quantitative traits.

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Introduction

Rice (*Oryza sativa*) is the major food staple for about half of the world's population, and it also is a model monocot plant for molecular and genetic studies. *Oryza sativa L. ssp indica* (Hsien) and *Oryza sativa L. ssp japonica* (Keng) are two major Asian cultivated rice (*Oryza sativa L.*) subspecies [1,2,3]. These two subspecies have been distinguished based on morphological characters and geographical distribution for 2,000 years. Indica and Japonica rice originated from different ancestors and they diverged about 0.2~0.44 million years ago [4,5]. From Khush's report, *indica* was probably domesticated in eastern India and *japonica* somewhere in South China [6]. These variations affect genomic structure and may cause intra-specific phenotypic adaptations. For example, there exists variance of seed maturity, seed quality, stress and defense tolerance between the two subspecies. Genome-wide comparative analyses were conducted on DNA sequences derived from *indica* and *japonica* rice [5,6,7,8,9,10,11,12,13]. In recent years, sequence variance analysis between the two rice subspecies have become well-established due to the publicly available rice genome, including the genome sequences of the *japonica* variety Nipponbare and *indica* variety 93-11 [14,15,16,17], and a genetic map for 150 rice recombinant inbred lines constructed by the recently introduced next-generation sequencing technology [18]. In order to further elucidate genetic differences between rice subspecies, an approach using Gene Ontology (GO) analysis together with genomic variation analysis was conducted by different research groups [19,20]. Several GO terms were highlighted with significant enrichment, including production of defense-related compounds, cell wall components, cell signaling proteins, and transcription factors. The GO analysis results indicated that there was positive selection either by natural means

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Nicotianamine, a Novel Enhancer of Rice Iron Bioavailability to Humans

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Abstract

Background: Polished rice is a staple food for over 50% of the world's population, but contains little bioavailable iron (Fe) to meet human needs. Thus, biofortifying the rice grain with novel promoters or enhancers of Fe utilization would be one of the most effective strategies to prevent the high prevalence of Fe deficiency and iron deficiency anemia in the developing world.

Methodology/Principal Findings: We transformed an elite rice line cultivated in Southern China with the rice nicotianamine synthase gene (*OsNAS1*) fused to a rice glutelin promoter. Endosperm overexpression of *OsNAS1* resulted in a significant increase in nicotianamine (NA) concentrations in both unpolished and polished grain. Bioavailability of Fe from the high NA grain, as measured by ferritin synthesis in an *in vitro* Caco-2 cell model that simulates the human digestive system, was twice as much as that of the control line. When added at 1:1 molar ratio to ferrous Fe in the cell system, NA was twice as effective when compared to ascorbic acid (one of the most potent known enhancers of Fe bioavailability) in promoting more ferritin synthesis.

Conclusions: Our data demonstrated that NA is a novel and effective promoter of iron utilization. Biofortifying polished rice with this compound has great potential in combating global human iron deficiency in people dependent on rice for their sustenance.

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Introduction

Iron (Fe) deficiency is the most prevalent nutrient deficiency in the world afflicting over 50% of the world population[1,2]. Inadequate intake of iron and consumption of foods low in bioavailable iron are the major causes of this problem. Compared with heme-iron derived from animal foods, non-heme iron, the major form of iron in plant foods, is much less bioavailable (2 to 10%) from the diet[3,4]. The low bioavailability of non-heme iron in these foods is attributed to the high amounts of inhibitors of iron absorption (i.e., phytate and polyphenolics)[5]. Although promoter compounds of iron utilization such as ascorbic acid (AA)[6,7] and ethylenediaminetetraacetic acid (EDTA)[8] have been used as dietary fortificants to improve human iron nutritional status[6], this approach has limited accessibility or sustainability to resource-poor people afflicted with iron deficiency in the Global South. Alternatively, biofortifying staple crops with enhancers of iron absorption would be a more effective and sustainable solution. However, past efforts have focused mainly on increasing the total iron concentration in edible portions of food crops[9,10,11]. Little effort or progress has been made in exploring new plant compounds that promote bioavailability of iron from food staples.

Nicotianamine (NA) is biosynthesized from three molecules of *S*-adenosylmethionine (SAM) by NA synthase (NAS)[12,13]. As a transition metal-chelator, NA facilitates the intra- and intercellular transport of essential trace metal cations, including Fe^{2+}, Fe^{3+} and Zn^{2+}, in plants[14]. Ectopic expression of the *Arabidopsis NAS* gene in tobacco resulted in a six-fold increase in NA level and a significant increase of Fe, Zn and manganese concentrations in leaves of adult plants[15]. A recent study showed that activation of *OsNAS3* led to increase of Fe, Zn in both green tissue and mature seed. Anemic mice fed with the *OsNAS3* activated transgenic rice seeds recovered to normal levels of hemoglobin and hematocrit within 2 weeks[16]. Because of these positive effects of NA on iron uptake and accumulation in plant roots and seeds[15,17], we postulated that elevating NA in the edible portions of rice grain might improve iron bioavailability to animal or humans by chelating iron to form a soluble NA-ferrous complex. Therefore, we over expressed the *OsNAS1* gene in rice-grain endosperm, and obtained a significant increase of NA concentrations in the

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Tobacco microtubule-associated protein, MAP65-1c, bundles and stabilizes microtubules

Qiutao Meng · Jizhou Du · Jiejie Li · Xiaomei Lü · Xian Zeng · Ming Yuan · Tonglin Mao

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Abstract Three genes that encode MAP65-1 family proteins have been identified in the *Nicotiana tabacum* genome. In this study, NtMAP65-1c fusion protein was shown to bind and bundle microtubules (MTs). Further in vitro investigations demonstrated that NtMAP65-1c not only alters MT assembly and nucleation, but also exhibits high MT stabilizing activity against cold or katanin-induced destabilization. Analysis of NtMAP65-1c-GFP expressing BY-2 cells clearly demonstrated that NtMAP65-1c was able to bind to MTs during specific stages of the cell cycle. Furthermore, in vivo, NtMAP65-1c-GFP-bound cortical MTs displayed an increase in resistance against the MT-disrupting drug, propyzamide, as well as against cold temperatures. Taken together, these results strongly suggest that NtMAP65-1c stabilizes MTs and is involved in the regulation of MT organization and cellular dynamics.

Keywords NtMAP65-1c · Microtubules-associated protein · Microtubule stabilization · Katanin

Qiutao Meng and Jizhou Du have contributed equally to this paper and are considered as joint first authors.

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Abbreviations

MAPs Microtubule-associated proteins
PIPES Piperazine-N,N′-bis (2-ethanesulfonic acid 1,4-piperazinediethanesulfonic acid)
EGTA Ethylene glycol-bis-(*β*-aminoethyl ether)-N,N,N′,N′-tetraacetic acid

Introduction

Microtubules (MTs) play an important role in the regulation of cell elongation, expansion and division, as well as plant morphogenesis (Wasteneys and Galway 2003; Ehrhardt and Shaw 2006; Ishida et al. 2007). Microtubule-associated proteins (MAPs) control the organization and dynamics of MTs (Hamada 2007). The MAP65/PROTEIN REGULATING CYTOKINESIS 1 (PRC1)/Anaphase spindle elongation 1 (Ase1) consists of a conserved protein family found in nearly all eukaryotic groups (Hussey et al. 2002). Nine MAP65 genes have been identified in the *Arabidopsis* genome, which share between 22 and 82% amino acid identity (Hussey et al. 2002). Biochemical studies have shown that AtMAP65 family members exhibit varied functions in regulating MT organization, stabilization and dynamics, in vitro. For example, AtMAP65-1 and -5 have been shown to promote the rate of MT polymerization (Mao et al. 2005a; Smertenko et al. 2008), while AtMAP65-2 dramatically increases MT stabilization against cold-induced depolymerization (Li et al. 2009). Previous studies have also shown that various AtMAP65 proteins exhibit different cellular localizations. For example, AtMAP65-1 and -2 are mainly associated with interphasic and mitotic MT arrays (Smertenko et al. 2004; Li et al. 2009). The MT-binding activity of AtMAP65-1 is

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The putative *Arabidopsis* zinc transporter ZTP29 is involved in the response to salt stress

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Abstract Salt stress leads to a stress response, called the unfolded protein response (UPR), in the endoplasmic reticulum (ER). UPR is also induced in a wide range of organisms by zinc deficiency. However, it is not clear whether regulation of zinc levels is involved in the initiation of the UPR in plant response to salt stress. In this study, a putative zinc transporter, ZTP29, was identified in *Arabidopsis thaliana*. ZTP29 localizes to the ER membrane and is expressed primarily in hypocotyl and cotyledon tissues, but its expression can be induced in root tissue by salt stress. T-DNA insertion into the *ZTP29* gene led to NaCl hypersensitivity in seed germination and seedling growth, leaf etiolation, and widening of cells in the root elongation zone. In addition, in *ztp29* mutant plants, salt stress-induced upregulation of the UPR pathway genes *BiP2* and *bZIP60* was inhibited. Furthermore, under conditions of salt stress, upregulation of *BiP2* and *bZIP60* was inhibited by treatment with high concentrations of zinc in both control and *ztp29* plants. However, zinc chelation restored salt stress-induced *BiP2* and *bZIP60* upregulation in *ztp29* mutant plants. These experimental results suggest that ZTP29 is involved in the response to salt stress, perhaps through regulation of zinc levels required to induce the UPR pathway.

Keywords Zinc · Transporter · Unfolded protein response · Salt stress · Endoplasmic reticulum · Arabidopsis

Miaoying Wang and Qiangyi Xu have equally contributed to this work.

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Introduction

A great deal of research effort has been devoted to understanding the mechanisms of salt tolerance in plants because salt stress has a major impact on plant growth and crop production. In saline environments, plants must sense and transduce stress signals in order to activate response pathways leading to adaptation to, or tolerance of, the abiotic stress (Serrano and Rodriguez 2001).

Salt activates a stress response pathway in the endoplasmic reticulum (ER) in *Arabidopsis thaliana* (Liu et al. 2007b). Inhibition of normal protein folding or secretion causes unfolded or misfolded proteins to accumulate in the ER, leading to ER stress (Urade 2007), which activates a signaling pathway termed the "unfolded protein response" or UPR. Plants undergoing the UPR mitigate accumulation of unfolded or misfolded proteins via upregulated expression of genes coding for ER-folding proteins (Rutkowski and Kaufman 2004). Two membrane-associated basic domain/leucine zipper (bZIP) factors, bZIP60 and bZIP28, are likely candidates for these ER stress sensors/transducers (Liu et al. 2007a). The UPR also results in an increase in the activity of chaperone proteins, such as BiP (Binding Protein), which play crucial roles in facilitating protein folding during ER stress (Wilson

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Characterization of *DUF724* gene family in *Arabidopsis thaliana*

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Abstract Eighteen genes that encode the proteins with highly conserved Domain of Unknown Function 724 (DUF724) and Agenet domains were identified in plant taxa but not in animals and fungi. They are actively expressed in many different plant tissues, implying that they may play important roles in plants. Here we report the characterization of their structural organizations, expression patterns and protein–protein interactions. In *Arabidopsis,* the *DUF724* genes were expressed in roots, leaves, shoot apical meristems, anthers and pollen grains. At least seven of the ten *Arabidopsis* DUF724 proteins (AtDuf1 to AtDuf10) were localized in nucleus. Three of them (AtDuf3, AtDuf5 and AtDuf7) may form homodimers or homopolymers, but did not interact with other members of the same family. Together with the significant similarity between DUF724 proteins and FMRP in the fundamental and characteristic molecular architecture, the results implies the *DUF724* gene family may be involved in the polar growth of plant cells via transportation of RNAs.

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Keywords DUF724 · Nuclear protein · FMRP · RNA transport · *Arabidopsis*

Introduction

The *Arabidopsis* Genome Initiative reported that about 30% of *Arabidopsis* genes encode plant-specific proteins and proteins with unknown function (The *Arabidopsis* Genome Initiative 2000). In addition, approximately 1,004 proteins were found to have domains of unknown function (DUFs) or belong to the uncharacterized protein families (UPFs), amount to about 16% of the protein families in Pfam database (Bateman et al. 2004). Studies have shown that different DUF proteins function diversely and play crucial roles in plant growth and development. For example, a centrally positioned domain of DUF299 (Hulo et al. 2006) has been confirmed to be a dominating structural element in the *Arabidopsis* regulatory protein (RP) polypeptides (Chastain et al. 2008). Several *DUF784* genes, which were transcribed in synergid cells of the embryo sac, have been demonstrated to be important for embryo sac development at the late developmental stages and mediate the interaction of female gametophyte with pollen tubes (Jones-Rhoades et al. 2007).

The researches for pollen-specific expressed genes from published microarray databases showed that a number of DUF724 proteins are highly expressed in pollen and shoot apex (www.arabidopsis.org), indicating that they may be involved in pollen development. To study the functions of DUF724 proteins on pollen development, we searched the *DUF724* genes in different plant species. Ten *DUF724* genes in *Arabidopsis* genome (*AtDuf1* to *AtDuf10*), five in rice genome (*OsDuf1* to *OsDuf5*), one in *Vitis vinifera* genome (*ViDuf1*) and other two in poplar genome (*PtDuf1*

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DATABASE Open Access

ProFITS of maize: a database of protein families involved in the transduction of signalling in the maize genome

Yi Ling[†], Zhou Du[†], Zhenhai Zhang, Zhen Su[*]

Abstract

Background: Maize (*Zea mays* ssp. *mays* L.) is an important model for plant basic and applied research. In 2009, the B73 maize genome sequencing made a great step forward, using clone by clone strategy; however, functional annotation and gene classification of the maize genome are still limited. Thus, a well-annotated datasets and informative database will be important for further research discoveries. Signal transduction is a fundamental biological process in living cells, and many protein families participate in this process in sensing, amplifying and responding to various extracellular or internal stimuli. Therefore, it is a good starting point to integrate information on the maize functional genes involved in signal transduction.

Results: Here we introduce a comprehensive database 'ProFITS' (Protein Families Involved in the Transduction of Signalling), which endeavours to identify and classify protein kinases/phosphatases, transcription factors and ubiquitin-proteasome-system related genes in the B73 maize genome. Users can explore gene models, corresponding transcripts and FLcDNAs using the three abovementioned protein hierarchical categories, and visualize them using an AJAX-based genome browser (JBrowse) or Generic Genome Browser (GBrowse). Functional annotations such as GO annotation, protein signatures, protein best-hits in the *Arabidopsis* and rice genome are provided. In addition, pre-calculated transcription factor binding sites of each gene are generated and mutant information is incorporated into ProFITS. In short, ProFITS provides a user-friendly web interface for studies in signal transduction process in maize.

Conclusion: ProFITS, which utilizes both the B73 maize genome and full length cDNA (FLcDNA) datasets, provides users a comprehensive platform of maize annotation with specific focus on the categorization of families involved in the signal transduction process. ProFITS is designed as a user-friendly web interface and it is valuable for experimental researchers. It is freely available now to all users at http://bioinfo.cau.edu.cn/ProFITS.

Background

Maize (*Zea mays* ssp. *mays* L.) is an important economic crop, and has served as a model organism for plant genetic research for several decades. The B73 maize genome was sequenced in 2009 [1-3], providing unprecedented opportunities for genome-wide annotation, classification and comparative genomics research. However, the comprehensive maize genome sequence repositories, MaizeSequence http://www.maizesequence.org [1] and maizeGDB http://www.maizegdb.org/ [4] provide limited information concerning gene families' categorization. The thriving of research discoveries may be hampered under these circumstances.

Signal transduction is a fundamental biological process in living cells for sensing, amplifying and responding to various extracellular or internal stimuli [5]. Many gene products (proteins) are involved in this process. During the signal transduction process, the status of protein-protein interaction, protein three-dimensional architecture, and the localization of proteins could be altered by rapid changes in protein activities or stabilities. Protein phosphorylation and ubiquitination are two major donators of these changes through post-translation covalent modification. Furthermore, when they are associated

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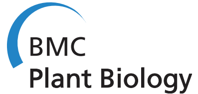

RESEARCH ARTICLE **Open Access**

Identification of a novel iron regulated basic helix-loop-helix protein involved in Fe homeostasis in *Oryza sativa*

Luqing Zheng,[1,2], Yinghui Ying[1,2], Lu Wang[1,2], Fang Wang[1], James Whelan[1,3], Huixia Shou[1,2*]

Abstract

Background: Iron (Fe) is the most limiting micronutrient element for crop production in alkaline soils. A number of transcription factors involved in regulating Fe uptake from soil and transport in plants have been identified. Analysis of transcriptome data from *Oryza sativa* grown under limiting Fe conditions reveals that transcript abundances of several genes encoding transcription factors are altered by Fe availability. These transcription factors are putative regulators of Fe deficiency responses.

Results: Transcript abundance of one nuclear located basic helix-loop-helix family transcription factor, *OsIRO3*, is up-regulated from 25- to 90-fold under Fe deficiency in both root and shoot respectively. The expression of *OsIRO3* is specifically induced by Fe deficiency, and not by other micronutrient deficiencies. Transgenic rice plants over-expressing *OsIRO3* were hypersensitive to Fe deficiency, indicating that the Fe deficiency response was compromised. Furthermore, the Fe concentration in shoots of transgenic rice plants over-expressing *OsIRO3* was less than that in wild-type plants. Analysis of the transcript abundances of genes normally induced by Fe deficiency in *OsIRO3* over-expressing plants indicated their induction was markedly suppressed.

Conclusion: A novel Fe regulated bHLH transcription factor (OsIRO3) that plays an important role for Fe homeostasis in rice was identified. The inhibitory effect of *OsIRO3* over-expression on Fe deficiency response gene expression combined with hypersensitivity of *OsIRO3* over-expression lines to low Fe suggest that OsIRO3 is a negative regulator of the Fe deficiency response in rice.

Background

Iron (Fe) is an essential micronutrient for plant growth and production. This is due to the fact that it is an essential co-factor in a variety of enzymes that play critical roles in photosynthesis, respiration and nitrogen fixation [1]. Although Fe is the second most abundant metal element in the earth crust, its bio-availability is limited, especially in alkaline soils where Fe largely exists as insoluble hydroxides or oxides [2]. While the optimal Fe concentration for plant growth is in the range of 10^{-9} to 10^{-4}M , the bio-available Fe in most soils is approximately 10^{-17} M [2,3]. Plants have two distinct uptake strategies to increase the efficiency of Fe uptake from soil [4]. The reduction strategy employed by non-grass plant species uses a Fe deficiency induced reductase to convert insoluble Fe(III) to Fe(II), the latter being transported into plant cells by the Fe(II) transporter IRT1 [4-6]. In contrast, grass species use a chelating strategy to obtain Fe from soil. The chelating strategy consists of Fe deficiency induced biosynthesis and secretion of phytosiderophore(s) and cognate high affinity transporters, Fe(III)-phytosiderophore Yellow Stripe Transporter, ZmYS1, HvYS1 and OsYSL15 [7-9].

Many of the components involved in Fe uptake for both of the strategies outlined above have been identified at a molecular level. In Arabidopsis H^+-ATPase 2 (AHA2) that mediates acidification of the rhizosphere [10], a ferric reductase FRO2 [6] and a ferrous Fe transporter IRT1 [5], represent the three key components required for a strategy I Fe uptake system. Identification of the molecular components involved in strategy II Fe uptake system has focused on the biosynthesis of the Fe

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Regular Paper

MALE GAMETOPHYTE DEFECTIVE 1, Encoding the F_Ad Subunit of Mitochondrial F_1F_0-ATP Synthase, is Essential for Pollen Formation in *Arabidopsis thaliana*

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In flowering plants, pollen formation is a complex process and strictly controlled by genetic factors. Although thousands of genes have been identified to be highly or specifically expressed in pollen grains, little is known about the functions and regulatory mechanisms of the genes in pollen formation. Here we report the characterization of a novel gene, *MALE GAMETOPHYTE DEFECTIVE 1* (*MGP1*), that is essential for pollen formation in *Arabidopsis thaliana*. *MGP1* encodes the F_Ad subunit of mitochondrial F_1F_0-ATP synthase in Arabidopsis. It was highly expressed in pollen grains at the later developmental stage. Mutation in *MGP1* led to destruction of the mitochondria in pollen grains at the dehydration stage and subsequently death of the pollen grains. These results suggested that *MGP1* plays important roles in pollen formation, possibly by regulating the activity of mitochondrial F_1F_0-ATP synthase in Arabidopsis pollen grains.

Keywords: Arabidopsis • ATP synthase • Gametogenesis • MGP1 • Mitochondria • Pollen.

Abbreviations: CaMV, cauliflower mosaic virus; DAPI, 4′,6-diamidino-2-phenylindole; *Ds*, *dissociation*; GFP, green fluorescent protein; GUS, β-glucuronidase; IF_1 (INH1), F_1-ATPase inhibitor; MS, Murashige and Skoog; PI, propidium iodide; RT–PCR, reverse transcription–PCR; SEM, scanning electron microscopy; STF1, F_1-ATPase inhibitor stabilizer 1; TEM, transmission electron microscopy; TAIL-PCR, thermal asymmetric interlaced PCR.

Introduction

In flowering plants, the male gametophyte or pollen grain is a multicelled life unit. It is produced in the male sexual organ, the anther. The process comprises several important steps. First, the reproductive cells in an anther primordium divide and differentiate into microspore mother cells. Then, the microspore mother cells undergo meiosis to give rise to haploid microspores. In the angiosperms including *Arabidopsis thaliana*, before anthesis, the individual microspore further undergoes two rounds of mitosis to form a three-celled pollen grain that consists of a larger vegetative cell and two sperm cells. The three-celled pollen grain further undergoes dehydration to form a mature pollen grain (McCormick 2004). The dehydration of pollen grains is crucial for maximum maintenance of pollen viability and then the pollen grains can tolerate various environmental stresses after they are released from the anther (Twell 2002, Swanson et al. 2004). A defect in any of the steps above will interrupt pollen formation or affect male gametophytic function (Scott et al. 1991, McCormick 1993, McCormick 2004).

Pollen formation is a highly energy-consuming process (Lee and Warmke 1979). As in other non-photosynthetic tissues with undifferentiated plastids and amyloplasts, the energy is supplied by mitochondria exclusively in the developing pollen grains (De Paepe et al. 1993). Dysfunction of mitochondria in pollen grains will drastically affect pollen development (Hanson and Bentolila 2004). In mitochondria, the energy-bearing compound, ATP, is synthesized by F_1F_0-ATP synthase (Pedersen and Carafoli 1987). Furthermore, as well as ATP synthesis, the mitochondrial F_1F_0-ATP synthase also engages in ATP hydrolysis, depending on the cell's physiological pH (Cabezón et al. 2000, Cabezón et al. 2002). The purpose of ATP hydrolysis is to restore the impaired mitochondrial membrane potential. In yeast, the ATP hydrolysis activity of the mitochondrial F_1F_0-ATP synthase is regulated by F_1-ATPase inhibitor (INH1/IF_1) and F_1-ATPase inhibitor stabiliser 1 (STF1) proteins. They inhibit the ATP hydrolysis activity of mitochondrial F_1F_0-ATP synthase when the impaired membrane potential is rectified and ATP hydrolysis is not needed to ensure an adequate supply of ATP for cell physiological activity, which plays important roles in cell development (Hashimoto et al. 1987, Ichikawa et al. 1990, Cabezón et al. 2001, Venard et al. 2003). To date, however, little is known about the roles of the related components of mitochondrial F_1F_0-ATP synthase in pollen grains of higher plants although it is important for sexual plant reproduction.

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Regular Paper

Mutations in the Arabidopsis Nuclear-Encoded Mitochondrial Phage-Type RNA Polymerase Gene *RPOTm* Led to Defects in Pollen Tube Growth, Female Gametogenesis and Embryogenesis

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The mitochondrial genes in *Arabidopsis thaliana* are transcribed by a small family of nuclear-encoded T3/T7 phage-type RNA polymerases (RPOTs). At least two nuclear-encoded RPOTs (RPOTm and RPOTmp) are located in mitochondria in *A. thaliana*. Their genetic roles are largely unknown. Here we report the characterization of novel mutations in the *A. thaliana RPOTm* gene. The mutations did not affect pollen formation, but significantly retarded the growth of the *rpoTm* mutant pollen tubes and had an impact on the fusion of the polar nuclei in the *rpoTm* mutant embryo sacs. Moreover, development of the *rpoTm/–* mutant embryo was arrested at the globular stage. The *rpoTm rpoTmp* double mutation could enhance the *rpoTm* mutant phenotype. Expression of *RPOTmp* under control of the *RPOTm* promoter could not complement the phenotype of the *rpoTm* mutations. All these data indicate that *RPOTm* is important for normal pollen tube growth, female gametogenesis and embryo development, and has distinct genetic and molecular roles in plant development, which cannot be replaced by *RPOTmp*.

Key words: Arabidopsis • Embryogenesis • Gametogenesis • Mitochondria • Phage-type RNA polymerase • Pollen.

Abbreviations: ABRC, Arabidopsis Biological Resource Center; CLSM, confocal laser scanning microscopy; DAPI, 4′,6-diamidino-2-phenylindole; DIC, differential interference contrast microscopy; GUS, β-glucuronidase; Kan, kanamycin; RPOT, RNA polymerase of T3/T7 type; RNAP, RNA polymerase; RT–PCR, reverse transcriptase–PCR; SEM, scanning electron microscopy; TAIL-PCR, thermal asymmetric interlaced PCR; TEM, transmission electron microscopy.

Introduction

The plant life cycle alternates between a diploid sporophytic phase and a haploid gametophytic phase. The diploid male and female sporophytes produce haploid microspores and megaspores that give rise to male and female gametophytes, respectively. Specifically, in the anther, the diploid pollen mother cell (PMC) undergoes meiosis to generate haploid microspores. Then, each individual microspore undergoes two rounds of mitosis to form a three-celled pollen grain (male gametophyte) that comprises two sperm cells and a vegetative cell. When the mature pollen grain lands on the female stigma, shortly thereafter it germinates and produces a pollen tube that invades the stigmatic tissue and then elongates in the female transmitting tract to deliver the two sperm cells into an embryo sac (McCormick 1993, McCormick 2004).

In the ovule, the megaspore mother cell (MMC) undergoes meiosis to give rise to four haploid megaspores, three of which undergo programmed cell death and only one survives and differentiates into a functional megaspore. The functional megaspore then undergoes three rounds of mitotic nuclear division to generate an eight-nucleus coenocytic embryo sac. The coenocytic embryo sac then goes through the fusion of polar nuclei and cytokinesis to form a seven-celled embryo sac (female gametophyte) that consists of four different cell types: three antipodal cells (n), one central cell (2n), one egg cell (n) and two synergid cells (n). Then, the antipodal cells undergo programmed cell death. When a pollen tube enters the embryo sac by penetrating one of the two synergid cells through the filiform apparatus, the synergid cell also undergoes cell death. Subsequently, the two sperm cells are discharged from the pollen tube and migrate to the egg cell and central cell nucleus to achieve double fertilization. Thereafter, embryogenesis takes place (Drews et al. 1998, Yadegari and Drews, 2004, Shi et al. 2005).

Several lines of evidences have shown that the mitochondrion is deeply involved in gametophyte development. First, physiological data showed that during pollen formation, the number of mitochondria per cell increased in maize (*Zea mays*)

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Regular Paper

A Rice Stromal Processing Peptidase Regulates Chloroplast and Root Development

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The stromal processing peptidase (SPP) is a metalloendopeptidase that cleaves a broad range of precursor substrates. In this study, we isolated a rice mutant showing leaf chlorosis at the early seedling stage but inhibition of root growth during the whole growth period. Genetic analysis demonstrates that the phenotypes of the mutant were caused by a recessive single gene mutation. The mutated gene was cloned by map-based cloning, and was shown to encode an SPP. Sequence analysis showed a glutamate deletion in the highly conserved C-terminus of SPP in the mutant. The mutation of SPP in the mutant was verified by transgenic complementation. *SPP* is constitutively expressed in all tissues. Subcellular localization analysis indicates that SPP is targeted to the chloroplast. The expression of some genes associated with chloroplast development was decreased in young seedlings of the *spp* mutant, but not in 14-day-old seedlings. Western blot analysis revealed that the Rubisco small subunit is not precisely processed in the *spp* mutant in 7-day-old seedlings, but the processing activity in the *spp* mutant is restored in 14-day-old seedlings. Moreover, the expression levels of *Cab1R* and *Cab2R* for the light-harvesting chlorophyll *a*/*b*-binding protein (LHCP) were highly up-regulated in the transgenic plants with overexpression of *SPP*. The present results reveal that SPP is essential for chloroplast biogenesis at the early growth stage and for rice root development; this is the first report on the function of SPP in monocot plants.

Keywords: Chloroplast development • *Oryza sativa* L. • Root • Stromal processing peptidase.

Abbreviations: AR, adventitious root; CaMV, cauliflower mosaic virus; EMS, ethylmethane sulfonate; GFP, green fluorescent protein; GUS, β-glucuronidase; LHCP, light-harvesting chlorophyll *a*/*b*-binding protein; LR, lateral root; ORF, open reading frame; RbcS, ribulose-1,5-bisphosphate carboxylase/oxygenase small subunit; RT–PCR, reverse transcription–PCR; SPP, stromal processing peptidase; SSR, simple sequence repeat; TEM, transmission electron microscopy; TP, transit peptide; WT, wild type.

Introduction

The chloroplast, a multifunctional organelle, is the site of photosynthesis and also houses an amazing array of biosynthetic pathways needed for normal plant growth and development. Chloroplast development is tightly regulated by the coordinated expression of plastid-encoded and nuclear-encoded genes during leaf development. The precise coordination of gene expression through two-way signaling between plastids and the nucleus is essential for chloroplast biogenesis in plant cells (Mandel et al. 1996, Koussevitzky et al. 2007, Yoo et al. 2009). The protein products of nuclear-encoded genes involved in chloroplast development are generally synthesized as precursor proteins with cleavable N-terminal chloroplast transit peptides, which direct the transport of the precursor across the chloroplast envelope into the stroma, where it is cleaved off by a stromal processing peptidase (SPP) (Su et al. 1999, Chew et al. 2004).

The general SPP from pea has been characterized (Oblong and Lamppa 1992, VanderVere et al. 1995, Richter and Lamppa 1998, Richter and Lamppa 1999, Richter and Lamppa 2002). It is a member of a class of metallopeptidases that possess a signature zinc-binding motif (HXXEH) at the catalytic site. Although these members most probably share a common catalytic mechanism that depends on the zinc-binding domain (Becker and Roth 1992, Perlman et al. 1993, Kitada et al. 1995, Striebel et al. 1996), they have different substrate specificities (Rawlings and Barrett 1995). The general SPP cleaves not only pre-LHCP (the major light harvesting chlorophyll *a*/*b*-binding protein) and pre-RbcS (ribulose-1,5-bisphosphate carboxylase/oxygenase small subunit), but also a broad range of imported precursors destined for different compartments and biosynthetic pathways of the chloroplast (VanderVere et al. 1995, Richter and Lamppa 1998). Although most of the precursors (the substrates of SPP) are targeted to the chloroplast stroma, there are also precursors which are targeted to other cell organelles, including the precursors of pyruvate kinase isozyme A which are found only in the stroma of leucoplasts, and glutathione reductase which is dually targeted to the chloroplast stroma and mitochondrial matrix (Creissen et al. 1995, Wan et al. 1995).

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ORIGINAL ARTICLE

The AtTudor2, a protein with SN-Tudor domains, is involved in control of seed germination in *Arabidopsis*

Shijie Liu · Jianheng Jia · Yang Gao · Bangyue Zhang · Yuzhen Han

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Abstract The 4SN-Tudor domain protein is an almost ubiquitous eukaryotic protein with four Staphylococcal nuclease domains at the N terminus and a Tudor domain towards the C terminus. It has been found that Tudor-SN protein has multiple roles in governing gene expression during cell growth and development in animals. In plant, although Tudor-SN orthologs have been found in rice, pea and *Arabidopsis*, and are associated with cytoskeleton, their roles in growth and development are poorly understood. In this study, we investigated the function of *Arabidopsis* Tudor-SN protein, AtTudor. Our results indicated that the expression of *AtTudor2* in seeds was evidently higher than in other tissues. Furthermore, we found that the expression of a key enzyme for GA biosynthesis, *AtGA20ox3*, was downregulated obviously in *AtTudor2* T-DNA insertion mutant and *AtTudor1*/*AtTudor2* RNAi transgenic lines. Together, our results suggest that AtTudor2 is involved in GA biosynthesis and seed germination of *Arabidopsis*.

Keywords *Arabidopsis* · AtTudor2 · AtGA20ox3 · Seed germination

Abbreviations

SN	Staphylococcal nuclease
GA20ox	GA 20-oxidase
GA3ox	GA 3-oxidase
WT	Wild type
DEX	Dexamethazone

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Introduction

Tudor-SN proteins containing four Staphylococcal nuclease (SN) domains and a C-terminal Tudor-SN domain have been found in various eukaryotes from fungi to plants. It was first discovered as a coactivator for Epstein-Barr virus nuclear antigen 2 (Tong et al. 1995) and later characterized as a bridge between transcription factors (such as STAT5 and STAT6) and basal transcriptional machinery (Yang et al. 2002; Paukku et al. 2003). Subsequently, it was identified as a component of RNA-induced silencing complex and to be involved in posttranscriptional regulation (Scadden 2005). In addition, recent studies indicated that it also interacted with U5 snRNP and functioned in spliceosome assembly and pre-mRNA splicing (Yang et al. 2007). In rice, OsTudor-SN (Rp120) was found to be a component of RNase-sensitive complex that was related to RNA transport. It is co-localized with storage protein RNAs, and decline in the OsTudor-SN expression leads to the reduction of seed storage proteins both at RNA levels and polypeptide levels (Sami-Subbu et al. 2001; Wang et al. 2008). Tudor-SN orthologs also exist in pea and *Arabidopsis* and are cytoskeleton-associated (Abe et al. 2003; Chuong et al. 2004). However, its function in growth and development is still unclear so far.

In *Arabidopsis*, seed dormancy and germination are complex physiological processes. Freshly harvested mature seeds are in the state of dormancy, which prevents seeds from germinating under unsuitable conditions. Dormancy

the FEBS Journal

Expression profile of PIN, AUX/LAX and PGP auxin transporter gene families in *Sorghum bicolor* under phytohormone and abiotic stress

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Keywords
abiotic stresses; AUX/LAX; PGP; PIN; *Sorghum bicolor*

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Note
Proteins are shown in uppercase, genes are shown in uppercase italics and mutants are shown in lowercase italics

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Auxin is transported by the influx carriers auxin resistant 1/like aux1 (AUX/LAX), and the efflux carriers pin-formed (PIN) and P-glycoprotein (PGP), which play a major role in polar auxin transport. Several auxin transporter genes have been characterized in dicotyledonous *Arabidopsis*, but most are unknown in monocotyledons, especially in sorghum. Here, we analyze the chromosome distribution, gene duplication and intron/exon of *SbPIN*, *SbLAX* and *SbPGP* gene families, and examine their phylogenic relationships in *Arabidopsis*, rice and sorghum. Real-time PCR analysis demonstrated that most of these genes were differently expressed in the organs of sorghum. *SbPIN3* and *SbPIN9* were highly expressed in flowers, *SbLAX2* and *SbPGP17* were mainly expressed in stems, and *SbPGP7* was strongly expressed in roots. This suggests that individual genes might participate in specific organ development. The expression profiles of these gene families were analyzed after treatment with: (a) the phytohormones indole-3-acetic acid and brassinosteroid; (b) the polar auxin transport inhibitors 1-naphthoxyacetic acids, 1-naphthylphthalamic acid and 2,3,5-triiodobenzoic acid; and (c) abscissic acid and the abiotic stresses of high salinity and drought. Most of the auxin transporter genes were strongly induced by indole-3-acetic acid and brassinosteroid, providing new evidence for the synergism of these phytohormones. Interestingly, most genes showed similar trends in expression under polar auxin transport inhibitors and each also responded to abscissic acid, salt and drought. This study provides new insights into the auxin transporters of sorghum.

Introduction

Auxin plays a critical role in the spatiotemporal coordination of plant growth and development, through polar auxin transport [1–5]. Auxin transport proteins in *Arabidopsis* are grouped into three families: auxin resistant 1/like aux1 (AUX1/LAX) influx carriers, pin-formed (PIN) efflux carriers and P-glycoprotein

Abbreviations
ABA, abscissic acid; ABC, ATP-binding cassette; AUX1/LAX, auxin resistant 1/like aux1; BR, brassinosteroid; HMM, hidden Markov model; IAA, indole-3-acetic acid; 1-NOA, 1-naphthoxyacetic acid; NPA, 1-naphthylphthalamic acid; PATI, polar auxin transport inhibitor; PGP, P-glycoprotein; PIN, pin-formed; TIBA, 2,3,5-triiodobenzoic acid.

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Molecular Plant • Volume 3 • Number 2 • Pages 280–287 • March 2010 REVIEW ARTICLE

Plant Sensing and Signaling in Response to K^+-Deficiency

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ABSTRACT Potassium (K^+) is one of the essential macronutrients for plant growth and development. However, K^+ content in soils is usually limited so that the crop yields are restricted. Plants may adapt to K^+-deficient environment by adjusting their physiological and morphological status, indicating that plants may have evolved their sensing and signaling mechanisms in response to K^+-deficiency. This short review particularly discusses some components as possible sensors or signal transducers involved in plant sensing and signaling in response to K^+-deficiency, such as K^+ channels and transporters, H^+-ATPase, some cytoplasmic enzymes, etc. Possible involvement of Ca^{2+} and ROS signals in plant responses to K^+-deficiency is also discussed.

Key words: K^+-deficiency; K^+ channel; K^+ transporter; Ca^{2+} signaling.

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INTRODUCTION

As an essential macronutrient for plant growth and development, potassium (K^+) plays crucial roles in many fundamental metabolic processes (Clarkson and Hanson, 1980). K^+ is the most abundant cation in living plant cells, whose content could reach up to 10% of the plant dry weight (Leigh and Wyn Jones, 1984). The cytoplasmic K^+ concentration ($[K^+]_{cyt}$) in living plant cells is estimated around 100 mM (from 40 to 200 mM) and appears to be relatively stable, which seems to meet the optimal K^+ concentration for cytoplasmic enzyme activities (Leigh and Wyn Jones, 1984; Walker et al., 1996; Britto and Kronzucker, 2008). In contrast with the high K^+ concentration in living plant cells, the typical K^+ concentration at the interface of roots and soils is usually within micromolar range from 0.1 to 1 mM (Schroeder et al., 1994; Maathuis, 2009). Obviously, K^+ is taken up by plants against its concentration gradient under most circumstances. It is known that K^+ transport from external environment into a living plant cell is accomplished through K^+ transporters and channels located at the plasma membrane (PM) of root cells (Véry and Sentenac, 2003; Ashley et al., 2006; Ward et al., 2009). A number of genes encoding K^+ channels and transporters have been identified in many plant species (Mäser et al., 2001; Véry and Sentenac, 2003; Lebaudy et al., 2007; Gierth and Mäser, 2007; Chen et al., 2008). For example, a total of 71 K^+ channels and transporters have been identified in *Arabidopsis* so far, and they are mainly divided into six gene families, including three channel families (Shaker, TPK, and Kir-like families) and three transporter families (KUP/HAK/KT, HKT, and CPA families). AKT1 has been confirmed as the most important inward-rectifying Shaker K^+ channel for K^+ acquisition by plants, which primarily expresses in epidermis of *Arabidopsis* roots and participates in K^+ uptake from soils into root cells (Sentenac et al., 1992; Lagarde et al., 1996; Hirsch et al., 1998). Some K^+ transporters from KUP/HAK/KT family such as AtKUP1 (Kim et al., 1998), AtKUP4 (Rigas et al., 2001), and AtHAK5 (Gierth et al., 2005) have been identified as high-affinity K^+ transporters, but their physiological functions *in planta* remain unclear.

Plant K^+ acquisition shows typical dual (high and low) affinity mechanisms (Epstein et al., 1963). The 'Mechanism 1', so-called high-affinity uptake mechanism, operates at low external K^+ concentrations ($[K^+]_{ext}$ usually within 0.2 mM K^+), which is mediated by K^+ transporters, usually K^+–H^+ symport with 1:1 stoichiometry (Epstein et al., 1963; Maathuis and Sanders, 1994; Britto and Kronzucker, 2008). The H^+ electrical gradients across the PM generated by transmembrane proton pumps provide the motive force for the K^+ transport into plant cells (Cheeseman et al., 1980; Palmgren, 2001). The 'Mechanism 2' dominantly works when the $[K^+]_{ext}$ is higher than 1 mM. This mechanism is also termed as low-affinity uptake mechanism

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Two Rubisco activase isoforms may play different roles in photosynthetic heat acclimation in the rice plant

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Studies on some plant species have shown that increasing the growth temperature gradually or pretreating with high temperature can lead to obvious photosynthetic acclimation to high temperature. To test whether this acclimation arises from heat adaptation of ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco, EC 4.1.1.39) activation mediated by Rubisco activase (RCA), gene expression of RCA large isoform (RCA_L) and RCA small isoform (RCA_S) in rice was determined using a 4-day heat stress treatment [40/30°C (day/night)] followed by a 3-day recovery under control conditions [30/22°C (day/night)]. The heat stress significantly induced the expression of RCA_L as determined by both mRNA and protein levels. Correlative analysis indicated that RCA_S protein content was extremely significantly related to Rubisco initial activity and net photosynthetic rate (Pn) under both heat stress and normal conditions. Immunoblot analysis of the Rubisco–RCA complex revealed that the ratio of RCA_L to Rubisco increased markedly in heat-acclimated rice leaves. Furthermore, transgenic rice plants expressing enhanced amounts of RCA_L exhibited higher thermotolerance in Pn and Rubisco initial activity and grew better at high temperature than wild-type (WT) plants and transgenic rice plants expressing enhanced amounts of RCA_S. Under normal conditions, the transgenic rice plants expressing enhanced amounts of RCA_S showed higher Pn and produced more biomass than transgenic rice plants expressing enhanced amounts of RCA_L and wild-type plants. Together, these suggest that the heat-induced RCA_L may play an important role in photosynthetic acclimation to moderate heat stress in vivo, while RCA_S plays a major role in maintaining Rubisco initial activity under normal conditions.

Introduction

Photosynthesis is one among the physiological processes that are the most sensitive to high temperature stress (Berry and Björkman 1980). High temperature damages the permeability of thylakoid membranes resulting in proton leakage and reduced electron flow (Bukhov et al. 1999, Schrader et al. 2004), followed by a reduction in ribulose 1,5-bisphosphate (RuBP) regeneration (Kubien and Sage 2008). Additionally, high temperature reduces the activation state of ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco, EC 4.1.1.39), which

Abbreviations – BSA, bovine serum albumin; ELISA, enzyme-linked immunosorbent assay; PBST, phosphate-buffered saline with 0.05% Tween; Pn, net photosynthetic rate; PPFD, photosynthetic photon flux density; PS II, photosystem II; PVDF, polyvinylidene fluoride; RCA, Rubisco activase; RCA_L, Rubisco activase large isoform; RCA_S, Rubisco activase small isoform; RLS, Rubisco large subunit; RSS, Rubisco small subunit; Rubisco, ribulose 1,5-bisphosphate carboxylase/oxygenase; RT-PCR, reverse transcriptase polymerase chain reaction; RuBP, ribulose 1,5-bisphosphate; SDS–PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis; WT, wild-type.

Photochemistry and Photobiology, 2010, 86: 110–116

Increased UV-B Radiation Affects the Viability, Reactive Oxygen Species Accumulation and Antioxidant Enzyme Activities in Maize (*Zea mays* L.) Pollen

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ABSTRACT

The increase in UV-B radiation reaching the earth's surface has prompted extensive studies on the effects of UV-B on plants. However, most of these studies have not addressed the close characteristics related to future survival of plant populations. The purpose of this study was to investigate the effects of UV-B radiation on reactive oxygen species (ROS) accumulation and antioxidant defense system in relation to germination, tube length and viability of maize pollen. Our results indicate that increased UV-B radiation decreased the pollen germination rate and tube length *in vitro* and also its fertilization ability in the field. Production of $O_2^{\bullet-}$ and H_2O_2 increased by UV-B radiation treatment, and their continuous accumulation resulted in lipid peroxidization. The activities of superoxide dismutase, catalase, peroxidase and DPPH-radical scavenging were decreased by increased UV-B radiation. The increased ROS and lipid peroxidization, and decreased activities of the antioxidants may be attributed to the effects of UV-B radiation on pollen germination, tube growth and fertilization ability.

INTRODUCTION

Human activities, such as the release of chlorofluorocarbons and nitrogen oxides in the stratosphere, have significantly decreased the ozone concentration in the last 30 years (1). The stratospheric ozone layer is the key factor in reducing solar UV-B radiation reaching the earth's surface. It has been reported that UV-B radiation at the earth's surface has increased by 6–14% since the 1980s accompanied by the depletion of ozone (2). At present, the release of 95% ozone-depleting substances has been reduced due to the Montreal Protocol; however, many of the ozone-depleting substances already in the atmosphere are long-lived, recovery cannot be immediate and present projections estimate a return to pre-1980 levels by 2050–2075 (3). In addition, stratospheric ozone recovery may possibly be delayed due to a number of uncertainties, including interaction with other projected changes in global climate such as global warming (3,4). Therefore, it still remains interesting to investigate the effects of elevated UV-B radiation on various aspects of plant growth, continuously (1,5,6).

Reproduction plays an important role in the survival and succession of seed-bearing plants. Previous results indicated that the reproductive function was influenced by increased UV-B radiation *via* pollination (7,8). It was shown that pollen vitality was one of the important factors for the success of pollination. Although the pollen of an open flower appears to be well shielded from solar UV-B when still within the anther sacs as the anther wall attenuates UV-B radiation by at least 98% (9), it may still be exposed to natural UV-B radiation following dehiscence and prior to successful germination and stigma penetration.

The pollen grain wall contains UV-B-absorbing pigments that screen UV-B radiation (10). Nevertheless, pollen germination and tube elongation in most species or cultivars are inhibited by elevated UV-B radiation *in vitro* and pollen viability of some species that growth under increased UV-B radiation was also reduced. Flint and Caldwell (11) reported partial inhibition of pollen germination in *Scrophularia peregrina*, *Geranium viscosissimum*, *Papaver rhoeas* and *Cleome lutea* under increased UV-B radiation *in vitro*. Musil (12) investigated four dicotyledonous *Asteraceae* and four monocotyledonous *Iridaceae* species. In two of eight species, pollen germination, tube length and the number of seed set were inhibited by increased UV-B radiation. Torabinejad *et al.* (13) investigated 34 taxa of pollen grains exposed to two levels of UV-B radiation. The pollen germination was inhibited in five tested species and pollen tube length in more than 50% of these species under high level UV-B radiation. Feng *et al.* (14) showed that pollen germination and tube growth in more than 60% of 19 higher plants were reduced under high-level UV-B radiation *in vitro*.

Although information on the responses of pollen to UV-B radiation is widely available, the underlying mechanism has not been sufficiently explored. It was shown that induction of reactive oxygen species (ROS) production was an early effect of UV-B radiation in plants (15). Recently, He *et al.* (7) reported that hydrogen peroxide (H_2O_2) was involved in the UV-B-inhibited pollen germination and tube growth of

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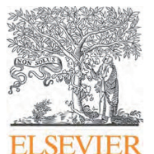

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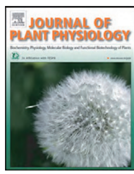

Silicon effects on photosynthesis and antioxidant parameters of soybean seedlings under drought and ultraviolet-B radiation

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ABSTRACT

Silicon (Si) may be involved in metabolic, physiological, and/or structural activity in higher plants exposed to abiotic and biotic stresses. This has not yet been determined due to the absence of direct evidence that it is part of the molecule of an essential plant constituent or metabolite. The aim of this study was to investigate the effect of silicon on soybean seedlings under drought and ultraviolet-B (UV-B) radiation stresses. The relative leaf water content (RWC), which was the main factor resulting in reduced growth in response to drought, increased 19.0% and 30.0% with Si application under drought and drought + UV-B stresses, respectively. Under UV-B radiation, the anthocyanin and phenol levels decreased 91.5% and 10.0% in the treatment of Si. Ultraviolet-B radiation and drought stress caused great membrane damage, as assessed by lipid peroxidation and osmolyte leakage, but Si application significantly reduced the membrane damage. Catalase (CAT), peroxidase (POD), superoxide dismutase (SOD) and hydrogen peroxide were observed under stress conditions. Proline increased primarily in drought-stressed seedlings and may be the drought-induced factor with a protective role in response to UV-B and silicon. Photosynthesis (P_N) increased following Si application by 21.0%, 18.3% and 21.5% under UV-B radiation, drought and the combination, respectively. The physiological and biochemical parameters measured indicated that the UV-B light had more adverse effects on growth of soybean seedlings than drought, but the data also showed that Si could alleviate seedling damage under these stress conditions.

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Introduction

The depletion of the stratospheric ozone layer is leading to an increase in solar ultraviolet-B (UV-B: 280–320 nm) radiation reaching the Earth's surface (Madronich et al., 1998). All living organisms of the biosphere are exposed to UV-B at intensities that vary with the solar angle and thickness of the stratospheric ozone layer. In plants, wide inter- and intra-specific differences have been reported in response to UV-B radiation; some plant species were unaffected and several species apparently had stimulated growth, but most species were sensitive to the extent that damage resulted (Kakani et al., 2003). Numerous environmental factors have also been shown to weaken or enhance the responses of plants to UV radiation. The extent of growth inhibition of wheat and pea seedlings irradiated with UV-B was affected by drought (Alexieva et al., 2001). Sensitivity of crops to UV-B was also influenced by water regime, ambient levels of visible radiation and nutrient status (Balakumar et al., 1993).

Drought is an important environmental stress that adversely affects plant growth and causes a reduction in growth rate, stem elongation, leaf expansion and stomatal movements (Engelbrecht et al., 2007). Given that current climate change models predict drought to increase in frequency and severity in several regions around the world (IPCC, 2007), there is an increasingly urgent need to better understand the impact of drought on plant function, and particularly the physiological mechanisms underlying plant responses during and in recovery from water stress.

Silicon (Si) has been regarded as an essential element in a number of species of the Poaceae and Cyperaceae, but it has not been possible to demonstrate that it is essential to all higher plants because direct evidence is still lacking that it is part of the molecule of an essential plant constituent or metabolite (Epstein, 1999). Recently, the role of Si in plant metabolism has received increasing attention. Liang et al. (2003) suggested that Si may be involved in metabolic or physiological and/or structural activity in higher plants exposed to abiotic and biotic stresses. It has been reported that Si increases plant tolerance to drought (Gong et al., 2005),

Abbreviations: CAT, catalase; MDA, malondialdehyde; POD, peroxidase; P_N, net photosynthetic rate; RWC, relative leaf water content; Si, silicon; SOD, superoxide dismutase; TCA, trichloroacetic acid; UV-B, ultraviolet-B radiation.

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Engineering the K⁺ uptake regulatory pathway by MultiRound Gateway

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MultiRound Gateway

ABSTRACT

In a previous study, we described improved versions of MultiRound Gateway vectors. Here, we engineered a calcineurin B-like (CBL) pathway for potassium (K^+) nutrition to demonstrate their effectiveness. Using the two improved entry vectors pL12R34H-Ap and pL34R12-Cm, and through 2–4 rounds of Gateway recombination reactions, we generated five pMDC99-derived binary vectors [pK21 (CIPK23 + CBL1), pK29 (CIPK23 + CBL9), pK31 (CIPK23 + CBL1 + AKT1), pK39 (CIPK23 + CBL9 + AKT1), and pK4 (CIPK23 + CBL1 + AKT1 + CBL9)], in which all four genes have the same pSuper promoter and tNos terminator. pK31, pK39 and pK4 were transformed into *Arabidopsis*. PCR analysis confirmed that all transgenes usually co-existed in the K31, K39 or K4 transgenic plants, and qRT-PCR analysis indicated that the transgenes were expressed at reasonably high levels. The eight overexpression lines, except K31-1, displayed significantly tolerant phenotypes to low-K^+ and low-K^+ combined with low-Ca^{2+} compared to the wild type. Significant differences between the K31, K39 and K4 lines were not observed. These results indicate that the improved MultiRound Gateway vectors efficiently assembled multiple transgenes, which were stably inherited and expressed in transformed plants, even with the same promoter and terminator.

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1. Introduction

Polygenes can control some crop production traits, metabolic pathways (e.g. carotenoid biosynthesis), signaling pathways (e.g. ABA signal transduction), and multimeric proteins such as vacuolar H^+-ATPase. Characterization of genetic regulatory and metabolic pathways progresses alongside functional genomics efforts. Genetic engineering of these polygenic traits, pathways or protein complexes is frequently required for both basic and applied research (Daniell and Dhingra, 2002; Halpin, 2005; Dafny-Yelin and Tzfira, 2007). Transgenic golden rice (Ye et al., 2000), purple tomatoes (Butelli et al., 2008), and red corn (Zhu et al., 2008) demonstrate the bright prospects for plant multigene transformation. The linking of multiple expression cassettes into a single binary plasmid has strong advantages over other approaches, though co-transformation, re-transformation, and sexual crosses can be applied to the delivery of multiple genes into plant cells (Dafny-Yelin and Tzfira, 2007). Therefore, researchers developed several systems to assemble multiple genes, such as the homing endonuclease-based pRCS/pAUX and pSAT vector systems (Goderis et al., 2002; Tzfira et al., 2005; Dafny-Yelin et al., 2007; Dafny-Yelin and Tzfira, 2007), Cre-lox P recombination (Lin et al., 2003), MultiSite Gateway (Cheo et al., 2004; Sasaki et al., 2004; Karimi et al., 2007a,b), and MultiRound Gateway (Chen et al., 2006). The MultiRound Gateway approach we developed is simple, efficient and more flexible than existing methods. DNA fragments of interest are cloned into two entry vectors via traditional cut-and-ligate methods, and then introduced into a destination vector in a defined order and orientation through multiple rounds of two-component Gateway recombination reactions. Transferring only desired DNA fragments from entry clones to destination vectors eliminates the steps to remove redundant recombination sites or the vector backbone.

We previously developed two sets of MultiRound Gateway entry vectors, with one set (pL12R34-sacB/pL34R12-ccdB) based on sacB and ccdB negative selection markers, and the other (pL12R34-Ap/pL34R12-Cm) based on ApR and CmR positive selection markers (Chen et al., 2006). However, both sets had a high rate of false background clones. Correct clones were rarely acquired using positive selection vectors derived from pL12R34-Ap/pL34R12-Cm due to co-transformation of the entry clones and destination vectors (unpublished data). We performed four rounds of LR recombination reactions using negative selection vectors derived from pL12R34-sacB/pL34R12-ccdB, but the sacB negative selection was sometimes not reliable (unpublished data). In order to enhance LR

Abbreviations: AKT, *Arabidopsis* K^+ transporter; Ap, ampicillin; ApR, ampicillin-resistant; CBL, calcineurin B-like; CIPK, CBL-interacting protein kinase; Cm, chloramphenicol; CmR, chloramphenicol-resistant; Gm, gentamycin; Kn, kanamycin; MS, Murashige and Skoog; qRT-PCR, quantitative real-time PCR.

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Molecular Plant • Pages 1–11, 2010 RESEARCH ARTICLE

OsCAND1 Is Required for Crown Root Emergence in Rice

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ABSTRACT **Crown roots are main components of the fibrous root system and important for crops to anchor and absorb water and nutrition. To understand the molecular mechanisms of crown root formation, we isolated a rice mutant defective in crown root emergence designated as *Oscand1* (named after the *Arabidopsis* homologous gene *AtCAND1*). The defect of visible crown root in the *Oscand1* mutant is the result of cessation of the G2/M cell cycle transition in the crown root meristem. Map-based cloning revealed that *OsCAND1* is a homolog of *Arabidopsis CAND1*. During crown root primordium development, the expression of *OsCAND1* is confined to the root cap after the establishment of fundamental organization. The transgenic plants harboring *DR5::GUS* showed that auxin signaling in crown root tip is abnormal in the mutant. Exogenous auxin application can partially rescue the defect of crown root development in *Oscand1*. Taken together, these data show that *OsCAND1* is involved in auxin signaling to maintain the G2/M cell cycle transition in crown root meristem and, consequently, the emergence of crown root. Our findings provide new information about the molecular regulation of the emergence of crown root in rice.**

Key words: ***Oryza sativa* L.; emergence of crown root; *OsCAND1*; cell cycle.**

INTRODUCTION

The crown roots are a major component of the fibrous root architecture of most cereal crops, including rice. The anatomy of different stages of crown root primordium in rice has been characterized in detail (Itoh et al., 2005), and crown root formation can be divided into seven stages. First, the initial cells are formed in a few layers by one or two periclinal divisions of the innermost ground meristem cells (pericycle cells). Then, the epidermis–endodermis, central cylinder, and root cap initial cells are established from the primordia; at this point, the epidermis–endodermis initial cells differentiate into epidermis and endodermis, and endodermal cells form cortical cells, defining the second, third, and fourth stages, respectively. In later developmental stages, the fundamental organization of root is formed with establishment of columella and metaxylem vessel and elongated and vacuolated cells at the fifth and sixth stages. Finally, the crown roots emerge from the stem.

Recently, a few mutants affecting crown root primordia initiation have been identified and characterized in rice, contributing to our understanding of the genetic mechanisms underlying crown root development. The first periclinal division is suppressed in *crl1* (*crown rootless 1*)/*arl1* (*adventitious rootless 1*) (Inukai et al., 2005; Liu et al., 2005). *CRL1/ARL1* encodes an AS2/LOB-domain protein. The maize-related gene *RTCS* is also involved in postembryonic shoot-borne root formation (Taramino et al., 2007). In addition, primordia initiation is impaired in *crl4* (*crown rootless 4*)/*Osgnom1* mutants (Kitomi et al., 2008b; Liu et al., 2009). CRL4/OsGNOM1 is highly homologous to the GNOM1 protein in *Arabidopsis thaliana* (*Arabidopsis*). Furthermore, initiation of crown root is delayed in *wox11* (*WUSCHEL-related Homeobox 11*) mutants, which produce fewer crown roots (Zhao et al., 2009). During crown root primordia development, cell elongation and vacuolation of the primordia are suppressed in *crl2* and *crl3* mutants (Inukai et al., 2001; Kitomi et al., 2008a). In spite of these findings, however, knowledge about the molecular mechanisms of crown root primordium emergence remains limited.

A phytohormone critical for plant root development is auxin (De Smet and Jurgens, 2007). The regulatory role of auxin

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Invited Expert Review

The Actin Cytoskeleton and Signaling Network during Pollen Tube Tip Growth

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Abstract

The organization and dynamics of the actin cytoskeleton play key roles in many aspects of plant cell development. The actin cytoskeleton responds to internal developmental cues and environmental signals and is involved in cell division, subcellular organelle movement, cell polarity and polar cell growth. The tip-growing pollen tubes provide an ideal model system to investigate fundamental mechanisms of underlying polarized cell growth. In this system, most signaling cascades required for tip growth, such as Ca^{2+}-, small GTPases- and lipid-mediated signaling have been found to be involved in transmitting signals to a large group of actin-binding proteins. These actin-binding proteins subsequently regulate the structure of the actin network, as well as the rapid turnover of actin filaments (F-actin), thereby eventually controlling tip growth. The actin cytoskeleton acts as an integrator in which multiple signaling pathways converge, providing a general growth and regulatory mechanism that applies not only for tip growth but also for polarized diffuse growth in plants.

Fu Y (2010) The actin cytoskeleton and signaling network during pollen tube tip growth. *J. Integr. Plant Biol.* **52**(2), 131–137.

Introduction

Plant cells not being motile means that cell morphogenesis relies strictly on polar growth and cell expansion occurs at specific sites of the cell surface. Tip growth is an extreme form of polar growth that is used by some specialized cell types, such as pollen tubes and root hairs in plants. The growth of pollen tubes and root hairs is restricted to the apex to form the tube-like or the hair-like structure. This is important for specific cell functions. For example, pollen tubes in flowering plants are germinated by pollen grains; tip-growing pollen tubes penetrate the stigma and the style rapidly *in vivo* to deliver sperm cells to ovules and to accomplish double fertilization. *In vitro*, pollen tubes from many species can also grow rapidly and can be easily manipulated, thus providing an ideal experimental model to investigate the regulatory mechanisms of the establishment of polarity and polarized cell growth. There has been a wealth of evidence indicating that the precise spatial and temporal regulation of tip growth requires an active role of the cytoskeleton.

In pollen tubes, MTs were found to be involved in the response to self-incompatibility signals (Poulter et al. 2008), also in organizing the generative cell and vegetative nucleus into a co-migrating unit (Astrom et al. 1995). However, it is believed that MTs have little role in regulating the tip growth of angiosperm pollen tubes, since treatment with MT-disruption drugs oryzalin or colchicine did not affect pollen tube growth (Raudaskoski et al. 2001; Laitiainen et al. 2002; Gossot and Geitmann 2007).

By contrast, pharmacologically disrupting F-actin organization and dynamics caused growth inhibition in both root

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Research Article

MALE GAMETOPHYTE DEFECTIVE 2, Encoding a Sialyltransferase-like Protein, is Required for Normal Pollen Germination and Pollen Tube Growth in *Arabidopsis*

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Abstract

Sialyltransferases (SiaTs) exist widely in vertebrates and play important roles in a variety of biological processes. In plants, several genes have also been identified to encode the proteins that share homology with the vertebrate SiaTs. However, very little is known about their functions in plants. Here we report the identification and characterization of a novel *Arabidopsis* gene, *MALE GAMETOPHYTE DEFECTIVE 2* (*MGP2*) that encodes a sialyltransferase-like protein. *MGP2* was expressed in all tissues including pollen grains and pollen tubes. The MGP2 protein was targeted to Golgi apparatus. Knockout of *MGP2* significantly inhibited the pollen germination and retarded pollen tube growth *in vitro* and *in vivo*, but did not affect female gametophytic functions. These results suggest that the sialyltransferase-like protein MGP2 is important for normal pollen germination and pollen tube growth, giving a novel insight into the biological roles of the sialyltransferase-like proteins in plants.

Deng Y, Wang W, Li WQ, Xia C, Liao HZ, Zhang XQ, Ye D (2010) *MALE GAMETOPHYTE DEFECTIVE 2* (*MGP2*), encoding a sialyltransferase-like protein, is required for normal pollen germination and pollen tube growth in *Arabidopsis*. *J. Integr. Plant Biol*. **52**(9), 829–843.

Introduction

Sialic acids (Sias) are negatively charged nine-carbon acidic sugars, and are typically located at the non-reductive terminal position of carbohydrate groups of glycoconjugates. The sialylation of sialylglycoconjugate mediates a variety of biological processes, such as cell-cell communication, cell-substrate interaction, adhesion, and protein targeting (Varki and Schauer 2008). In sialylglycoconjugate synthesis, transfer of a sialic acid base from CMP-Sia, a sugar-nucleotide donor, to an acceptor carbohydrate is catalyzed by sialyltransferase (SiaT). SiaTs exist widely in vertebrates. The CAZy (carbohydrate-active enzymes) data shows that *Homo sapiens*, *Mus musculus*, and *Rattus norvegicus* genomes all contain at least 20 genes that encode sialyltransferases involved in the biosynthesis of sialylglycoconjugates (http://www.cazy.org/fam/acc_GT.html).

In plants, currently, very little is known about the presence and functions of sialic acids. Solid experimental evidence is still lacking to show that the biosynthesis of sialic acids and sialylation of glycoconjugates actually occur in plants (Seveno et al. 2004; Zeleny et al. 2006). Recently, *in vitro* assays indicated that two *Oryza sativa* sialyltransferase-like proteins (designated OsSTLP1 and OsSTLP3) had sialyltransferase-like activity, and the Sia-specific lectin could interact with the constituents of the total glycoconjugates extracted from *Arabidopsis* plants, indicating the potential for the sialyltransferase activity in plants (Shah et al. 2003; Takashima et al. 2006). In addition, the mammalian α-2,6-sialyltransferase expressed in *Arabidopsis* is targeted specifically to the Golgi apparatus and the protein has significant α-2,6-sialyltransferase activity when supplied with the appropriate substrates (Wee et al. 1998). These results show that the plant cells have the biochemical potential to

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Research Article

Coronatine Alleviates Water Deficiency Stress on Winter Wheat Seedlings

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Abstract

With the aim to determine whether coronatine (COR) alleviates drought stress on wheat, two winter wheat (*Triticum aestivum* L.) cultivars, ChangWu134 (drought-tolerant) and Shan253 (drought-sensitive) were studied under hydroponic conditions. Seedlings at the three-leaf stage were cultured in a Hoagland solution containing COR at 0.1 μM for 24 h, and then exposed to 20% polyethylene glycol 6000 (PEG-6000). Under simulated drought (SD), COR increased the dry weight of shoots and roots of the two cultivars significantly; the root/shoot ratio also increased by 30% for Shan253 and 40% for ChangWu134. Both cultivars treated with COR under SD (0.1COR+PEG) maintained significantly higher relative water content, photosynthesis, transpiration, intercellular concentration of CO_2 and stomatal conductance in leaves than those not treated with PEG. Under drought, COR significantly decreased the relative conductivity and malondialdehyde production, and the loss of 1,1-diphenyl-2-picrylhydrazyl scavenging activity in leaves was significantly alleviated in COR-treated plants. The activity of peroxidase, catalase, glutathione reductase and ascorbate peroxidase were adversely affected by drought. Leaves of plants treated with COR under drought produced less abscisic acid (ABA) than those not treated. Thus, COR might alleviate drought effects on wheat by reducing active oxygen species production, activating antioxidant enzymes and changing the ABA level.

Li X, Shen X, Li J, Eneji AE, Li Z, Tian X, Duan L (2010) Coronatine alleviates water deficiency stress on winter wheat seedlings. *J. Integr. Plant Biol*. **52**(7), 616–625.

Introduction

Wheat is widely cultivated as staple crop throughout the world, and water deficiency is one of the most important limitations to its growth and grain yield (Xiong et al. 2007). Although many studies have focused on the effects and physiological mechanism of drought stress, simple and practical techniques for improving drought resistance are still elusive (Reynolds et al. 2006).

Biochemical and physiological changes have been widely studied in wheat, maize and rice, exposed to drought and osmotic stress, which was acknowledged as a crucial damage factor; osmotic stress caused by drought leads to dehydration of plant cells, accumulation of activated oxygen species (AOS), imbalance of photosynthesis and respiration, and stunted growth (Peus et al. 1998; Lukáš et al. 2006). Plants evolved several enzymatic antioxidants to eliminate AOS (Parida et al. 2004), including catalase (CAT) and peroxidase (POD) catalyzing the decomposition of hydrogen peroxide (H_2O_2) and superoxide dismutase (SOD) transforming the superoxide radical (O_2^-) to H_2O_2. For plants without CAT in chloroplasts, H_2O_2 could be decomposed through the ascorbate-glutathione cycle with ascorbate peroxidase (APX) and glutathione reductase (GR) (Kang et al. 1998). Thus, when plants endured water

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Overexpression of the tonoplast aquaporin AtTIP5;1 conferred tolerance to boron toxicity in *Arabidopsis*

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Abstract

Boron (B) toxicity to plants is responsible for low crop productivity in many regions of the world. Here we report a novel and effective means to alleviate the B toxicity to plants under high B circumstance. Functional characterization of *AtTIP5;1*, an aquaporin gene, revealed that overexpression of *AtTIP5;1* (OxAtTIP5;1) in *Arabidopsis* significantly increased its tolerance to high B toxicity. Compared to wild-type plants, OxAtTIP5;1 plants exhibited longer hypocotyls, accelerated development, increased silique production under high B treatments. GUS staining and quantitative RT-PCR (qRT-PCR) results demonstrated that the expression of *AtTIP5;1* was induced by high B concentration treatment. Subcellular localization analysis revealed that the AtTIP5;1-GFP fusion protein was localized on the tonoplast membrane, which was consistent with the prediction based on bioinformatics. Taken together, our results suggest that AtTIP5;1 is involved in B transport pathway possibly *via* vacuolar compartmentation for B, and that overexpression of *AtTIP5;1* in plants may provide an effective way to overcome the problem resulting from high B concentration toxicity.

Keywords: aquaporin; boron; toxicity tolerance

Introduction

Boron (B) is an essential micronutrient for plants and plays important roles in plant growth and development. There is a narrow margin between B deficiency and toxicity in some plants and both conditions can reduce the quality and yield of many crop species (Camacho-Cristobal et al., 2008; Yau and Ryan, 2008). For better understanding of B utility and tolerance in the plants, the mechanisms that regulate the absorption and transportation of this micronutrient must be elucidated.

Many recent studies have provided insight into B regulation pathway (O'Neill et al., 2004; Kato et al., 2009; Koshiba et al., 2009), which includes several genes involved in B uptake and translocation (Takano et al., 2002, 2006; Miwa et al., 2007; Nakagawa et al., 2007; Sutton et al., 2007; Tanaka et al., 2008). A number of studies have revealed the effects of low B in plants (Takano et al., 2002, 2006; Nakagawa et al., 2007; Tanaka et al., 2008; Kato et al., 2009; Koshiba et al., 2009), whereas there are few reports about the high B toxicity and tolerance of plants (Nozawa et al., 2006; Miwa et al., 2007; Sutton et al., 2007). *Arabidopsis thaliana* BOR1, an efflux-type B transporter, is the first B transporter identified in a bio-

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Analysis of QTLs for yield-related traits in Yuanjiang common wild rice (*Oryza rufipogon* Griff.)

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Abstract

Using an accession of common wild rice (*Oryza rufipogon* Griff.) collected from Yuanjiang County, Yunnan Province, China, as the donor and an elite cultivar 93-11, widely used in two-line *indica* hybrid rice production in China, as the recurrent parent, an advanced backcross populations were developed. Through genotyping of 187 SSR markers and investigation of six yield-related traits of two generations (BC_4F_2 and BC_4F_4), a total of 26 QTLs were detected by employing single point analysis and interval mapping in both generations. Of the 26 QTLs, the alleles of 10 (38.5%) QTLs originating from *O. rufipogon* had shown a beneficial effect for yield-related traits in the 93-11 genetic background. In addition, five QTLs controlling yield and its components were newly identified, indicating that there are potentially novel alleles in Yuanjiang common wild rice. Three regions underling significant QTLs for several yield-related traits were detected on chromosome 1, 7 and 12. The QTL clusters were founded and corresponding agronomic traits of those QTLs showed highly significant correlation, suggesting the pleiotropism or tight linkage. Fine-mapping and cloning of these yield-related QTLs from wild rice would be helpful to elucidating molecular mechanism of rice domestication and rice breeding in the future.

Keywords: common wild rice; yield-related traits; advanced backcross population; QTL

Introduction

Rice (*Oryza sativa* L.) is one of the most important food crops in the world, and it is very important to increase the yield through broadening the genetic variation in the modern rice breeding. In the genus of *Oryza*, there are more than 20 wild species and two cultivated species (Vaughan, 1994), the wild species of rice has been well recognized as a primary gene pool that conserves a lot of specific genes which are presently not available for extinct in the cultivated rice, such as male sterility (Lin and Yuan, 1980) and several resistance genes, for example, resistance to grassy stunt virus from annual wild rice (*O. nivara*) (Khush et al., 1977), rice bacterial blight resistance gene X*a-21* from *O. longistaminata* (Khush et al., 1991; Song et al., 1995) and *Xa-23* from *O. rufipogon* (Zhang et al., 2000).

Although wild relatives of crops were important source of genetic variation for cultivated crops (Tanksley and McCouch, 1997; Zamir, 2001) and it have long been used

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• RESEARCH PAPERS •

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A membrane-tethered transcription factor ANAC089 negatively regulates floral initiation in *Arabidopsis thaliana*

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The plant-specific NAC (NAM, ATAF1/2, and CUC2) transcription factors have a regulatory function in developmental processes and stress responses. Notably a group of NAC members named NTLs (NTM1-Like) are membrane-tethered, ensuring plants rapidly respond to developmental changes and environmental stimuli. Our results indicated that ANAC089 was a membrane-tethered transcription factor and its truncated form was responsible for the physiological function in flowering time control.

***Arabidopsis thaliana*, ANAC089, floral initiation, membrane-tethered transcription factor**

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Transcription factors regulate many vital cellular pathways by controlling the expression of downstream genes. There are more than 110 NAC (NAM, ATAF1/2, and CUC2) members comprising one of the largest transcription factor families in *Arabidopsis* [1]. NAC proteins have a highly conserved NAC DNA-binding domain in their N-terminal regions and quite diverse C-terminal regions with transcriptional activities. Several NAC proteins have been functionally described in a variety of plant growth and developmental processes, such as apical meristem formation [2,3], cell cycle control [4], flower development [5], lateral root formation and development [6,7], secondary wall thickenings [8,9], and leaf senescence [10,11]. Additionally, A few NAC members have been reported to contribute to various stress responses [12–14].

A group of NAC members named NTLs (NTM1-Like) are membrane-tethered transcription factors (MTTFs) which ensure that plants rapidly respond to developmental or environmental stimuli [15–17]. MTTFs differ from cytosolic transcription factors in that they are innately inserted into a membrane and exist in a dormant state [18]. The structures of MTTFs are very similar, including a transmembrane domain (TMD) as the membrane anchor and a cytosolic domain containing the transcription factor (TF) motif. MTTFs are activated by proteolytic cleavage, which liberates the cytosolic domain from the TMD and enables the MTTFs to travel to the nucleus. MTTFs identified in *Arabidopsis* include four members of the bZIP family (AtbZIP17, AtbZIP28, AtbZIP49 and AtbZIP60) [19–21] and four of the NAC family (NTM1, NTL6, NTL8 and NTL9) [4,11,15].

Genome-scale analysis shows that over 10% of the NAC transcription factors are membrane-tethered transcription factors (MTTFs) in *Arabidopsis* [15]. Only a few plant MTTFs have been identified and functionally studied. Therefore, characterization of MTTFs is vital not only because these proteins are involved in development and stress response but also because doing so provides a model for studying the mechanism for selective proteolysis and the mo-

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Overexpression of the *Rap2.4f* transcriptional factor in *Arabidopsis* promotes leaf senescence

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Senescence is a complex and highly regulated process. Leaf senescence is influenced by endogenous developmental and external environmental signals. In this work, we found that expression of an Ap2/DREB-type transcription factor gene, *Arabidopsis Rap2.4f* (*At4g28140*), was upregulated by salt, mannitol, and dark treatments. Constitutively overexpressing *Rap2.4f* under the control of the CaMV 35S promoter led to an increased chlorophyll degradation rate and upregulation of many senescence-associated genes in the transgenic *Arabidopsis* lines. Our results show that *Rap2.4f* is a positive regulator of senescence, promoting both developmental and dark-induced leaf senescence.

Arabidopsis thaliana*, leaf senescence, AP2, *Rap2.4f

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Leaf senescence is the final phase of leaf development and is crucial for plant survival and environmental adaptation [1]. Senescence is a tightly regulated process and occurs in an age-dependent manner. During senescence, nutrients including nitrogen, phosphorus, and potassium are relocated from senescent leaves to young leaves and developing seeds [2]. The leaf senescence process includes the breakdown of chloroplasts, decreases in cellular metabolic activities, and the degeneration of mitochondria and nuclei. As senescence starts, carbon assimilation decreases drastically, and both chlorophylls and macromolecules accumulated during the growth phase degrade very rapidly [3,4].

Senescence requires differential expression of a series of specific genes. Many senescence-associated genes (SAGs) have been identified in *Arabidopsis* [3]. Recently, *Arabidopsis* Affymetrix GeneChip arrays were used to investigate changes in global expression patterns during natural and dark-induced leaf senescence [5,6]. 96 putative transcription factor genes were upregulated during leaf senescence, including NAC, WRKY, zinc-finger, AP2/EREBP, and MYB proteins. Leaf senescence is regulated by endogenous developmental and external environmental signals. The external environmental factors that regulate leaf senescence include salt, drought, extreme light or temperatures, UV-B, shading, pathogen attack, and nutrient stress [3]. Thus, some of the genes that regulate stress responses may also have an important role in regulating leaf senescence. Transcript profiling studies have revealed that many of the genes affected in senescing leaves are also induced by biotic and abiotic stresses, indicating that there is cross-talk between stress responses and leaf senescence [7,8]. For example, among 43 transcription factor genes upregulated during senescence, 28 were also induced by various stresses [3].

In *Arabidopsis*, the AP2/ERF proteins make up the largest family of transcription factors. Genes in the AP2/ERF family have roles in the regulation of developmental processes, hormonal signal transduction, and biotic and abiotic stress responses [9]. CBF1, CBF2, and CBF3 play a central

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Full Length Research Paper

Cloning and functional analysis in transgenic tobacco of a tapetum-specific promoter from *Arabidopsis*

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The 5'-flanking region of 1174 bp upstream of the translation start point (TSP) of a reported *Arabidopsis* anther-specific gene, *Anther7* gene (*ATA7)*, which putatively encodes a protein related to lipid transfer protein, was cloned and functionally analyzed in transgenic tobacco after been fused with β-glucuronidase (GUS) gene reporter. Histochemical GUS staining of the transgenic plants showed that the cloned fragment did drive GUS expression exclusively in the anther, not in any other parts of floral organs, including pollens and nor in any vegetative tissue. Transverse section of the GUS-blue anthers disclosed that the blue cells were present uniquely in the tapetum of the anther. A series of 5'-deletion of cloned fragment indicated that a short segment of 179 bp upstream of the TSP (-155 bp upstream of the transcription start site) retained not only the promoter's driving power, but also its tapetum-specificity. *Cis*-acting element search in this short segment revealed the presence of numbers of organ- and tissue-specific motifs, including pollen-specific LAT52 and SLG13. These results indicated that the tapetum-specificity of *ATA7* gene is mainly conferred by its promoter, and such a promoter, in particular, the core one should be useful both for identification of tapetum-involved genes and for biotechnological applications.

Key words: *Arabidopsis*, *Anther7* gene of *Arabidopsis theliana* (*ATA7*), anther-specific promoter, tapetum-specific promoter.

INTRODUCTION

Anther development is a complicated process and involves large number of gene expression, including anther-specific ones. The anther specific genes make anthers to become different from other plant organs, and play an important role in plant reproductive development. They are associated with anther cell division and differentiation (Nonomura et al., 2003), tapetum development (Jung et al., 2005; Luo et al., 2006; Xu et al., 2006; Wu et al., 2008), male meiosis (Yang et al., 2003; Kapoor and Takasuji 2006), pollen maturation (Park et al., 2005, 2006; Zhao et al., 2006; Gupta et al., 2007), anther dehiscence (Zhu et al., 2004), stamen filament development (Mariani et al., 1990), etc. The anther-specificity of these genes has been found to be regulated mainly by gene's promoter and in particular by the key *cis*-elements in the promoter at the transcription level (Mascarenhas, 1992; Ariizumi et al., 2002). Therefore, a large number of anther-specific promoters and their key *cis*-elements from different plant species have been isolated and functionally analyzed, such as TA29 (Koltunow et al., 1990), NeIF-4A8 (Brander and Kuhlemeier, 1995), Zm13 (Hamilton et al., 1998), LAT52 (Muschietti et al., 1994), Osg6B (Yokoi et al., 1997), PyD3 (Xiao et al., 2006), PsEND1 (Roque et al., 2007) and MdAGP3 (Choi et al., 2010). Some of these anther-specific promoters, such as TA29, LAT 52, PsEND1 and key *cis*-element, for example,

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Abbreviations: TSP, Translation start point; **GUS,** β-glucuronidase; ***ATA7*,** *anther7* gene of *Arabidopsis theliana*; **MS,** Murashige and Skoog; **CTAB,** cetyl trimethylammonium bromide; **PCR,** polymerase chain reaction; **Carb,** carbenicillin; **KanR,** kanamycin resistant; **KanS,** kanamycin -susceptible; **FAA,** formaldehyde : acetic acid : ethanol; **Str,** streptomycin; **TSS,** transcription start site; **PLACE,** plant cis-acting regulatory DNA elements; **ATA7F0,** activity of *anther7* gene of *Arabidopsis theliana* full-length promoter; **CK,** untransformed plants.