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Short communication

Increased *STM* expression is associated with drought tolerance in *Arabidopsis*

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ABSTRACT

In higher plants, shoot apical meristem (SAM) maintains cell division activity in order to give rise to aerial plant organs. Several lines of evidence have suggested that plants ensure stem cell proliferation activity in response to various external stimuli, thereby contributing to plant adaptation and fitness. Here, we report that the abscisic acid (ABA)-inducible R2R3-type MYB96 transcription factor regulates transcript accumulation of *SHOOT MERISTEMLESS* (*STM*) possibly to contribute to plant adaptation to environmental stress. *STM* was up-regulated in *MYB96*-overexpressing activation-tagging *myb96-ox* plants, but down-regulated in *MYB96*-deficient *myb96-1* mutant plants, even in the presence of ABA. Notably, the MYB96 transcription factor bound directly to the *STM* promoter. In addition, consistent with the role of MYB96 in drought tolerance, transgenic plants overexpressing *STM* (35S:*STM-MYC*) were more tolerant to drought stress. These observations suggest that the MYB96-STM module contributes to enhancing plant tolerance to drought stress.

1. Introduction

The SAM retains an indeterminate pluripotent nature in order to give rise to the aerial parts of plants. New organs are differentiated in the peripheral zone (PZ) (Dodsworth, 2009). The cell population in the PZ is maintained by continuous recruitment of stem cells from the central zone (CZ) (Gallois et al., 2002). The balance between the rate of cell proliferation and differentiation is crucial for the ordered transition of cells.

A well-known signaling mechanism in the stem cell niche is the negative feedback regulation between CLAVATA3 (CLV3) and WUSCHEL (WUS). The homeodomain transcription factor WUS activates *CLV3* though direct binding to its promoter and promotes stem cell division (Yadav et al., 2011). The mature 12-amino-acid CLV3 peptide perceived by CLV receptor complexes, including CLV1, CLV2, SUPPRESSOR OF LLP1-2 (SOL2)/CORYNE (CRN) and RECEPTOR LIKE PROTEIN KINASE 2 (RPK2)/TOADSTOOL 2 (TOAD2), represses *WUS* expression. The CLV3-WUS module is necessary to maintain the pool of stem cells in the CZ of the meristem (Brand et al., 2000; Schoof et al., 2000).

In addition, the class I KNOTTED1-like homeobox (KNOX) genes constitute another important genetic pathway that determines stem cell establishment and maintenance, as well as prevents organ differentiation. In particular, the *STM* gene is mainly expressed in both the CZ and PZ of the meristem and maintains the cells in an undifferentiated state (Barton and Poethig, 1993; Clark et al., 1996; Gallois et al., 2002; Long et al., 1996). The other SAM-expressed class I KNOX members, including BREVIPEDICELLUS (BP)/KNOTTED-LIKE GENE FROM ARABIDOPSIS THALIANA 1 (KNAT1), KNAT2, and KNAT6, have functions partially redundant with STM in maintaining indeterminate stem cell fate by modulating hormone metabolism (Hay et al., 2002; Jasinski et al., 2005; Yanai et al., 2005).

Plants have evolved elaborate protective mechanisms to deal with environmental challenges, such as temperature extremes, water deficit, and high salinity. Upon exposure to abiotic stresses, they stimulate altered shoot and root development, biosynthesis of osmoprotectants, reprogramming of primary and secondary metabolite accumulation, and reestablishment of hormone homeostasis (Kido et al., 2013; Peleg and Blumwald, 2011; Ramakrishna and Ravishankar, 2011). In addition, several recent studies have supported the idea that plants also require stem cell proliferation activity to attain developmental plasticity, and thus to overcome

Abbreviations: ABA, abscisic acid; CaMV, Cauliflower mosaic virus; ChIP, chromatin immunoprecipitation; CK, cytokinin; CLV, CLAVATA; CZ, central zone; IPT, isopentenyltransferase; KNAT, KNOTTED-LIKE GENE FROM ARABIDOPSIS THALIANA; KNOX, KNOTTED1-LIKE HOMEOBOX; PZ, peripheral zone; RT-qPCR, quantitative real-time RT-PCR; SAM, shoot apical meristem; STM, SHOOT MERISTEMLESS; WUS, WUSCHEL.

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their sessile nature under environmentally unfavorable conditions. In support of this, abiotic stress-experienced cells show a stem cell-like state (Grafi et al., 2011a, 2011b). Furthermore, the transcriptome of stress-experienced cells largely overlaps with that of stem cells or dedifferentiated cells (Grafi et al., 2011b). Maintenance of the undifferentiated state might enable plants to acquire a new cell fate with better fitness to environmental disadvantages (Shoshani and Zipori, 2011). However, it is unclear how environmental fluctuation affects meristem proliferative activity in plants.

In this study, we report that the R2R3-type MYB96 transcription factor, a central ABA signaling mediator, establishes possible links between the *STM* expression and drought tolerance. The MYB96 transcription factor binds to the *STM* promoter and activates its expression under drought conditions. Consistent with this, *STM*-overexpressing transgenic plants showed enhanced tolerance to drought stress. These observations provide novel insight into how plants ensure indeterminate cell division to enhance their adaptation capability under stressful conditions.

2. Materials and methods

2.1. Plant material and growth conditions

Arabidopsis thaliana (Columbia-0 ecotype) was used for all experiments described, unless specified otherwise. Plants were grown under long day conditions (LDs; a 16-h light/8-h dark cycle) with cool white fluorescent light (100 μmol photons m^{-2} s^{-1}) at 22 °C. The *myb96-ox* and *myb96-1* mutants were previously reported (Seo et al., 2009). To produce transgenic plants overexpressing the *STM* and *WUS* genes, the full-length cDNAs were subcloned into the binary pBA002 vector under the control of the Cauliflower mosaic virus (CaMV) 35 S promoter. *Agrobacterium tumefaciens*-mediated *Arabidopsis* transformation was performed using a modified floral dip method (Clough and Bent, 1998). At least 30 transformants were obtained for each construct, and we selected representative transgenic lines exhibiting severe and mild phenotypes for subsequent in-depth studies.

2.2. Quantitative real-time RT-PCR analysis

Total RNA was extracted using TRI agent (TAKARA Bio, Singa, Japan), according to the manufacturer's recommendations. Reverse transcription (RT) was performed using Moloney Murine Leukemia Virus (M-MLV) reverse transcriptase (Dr. Protein, Seoul, South Korea) with oligo (dT18) for the synthesis of first-strand cDNA from 2 μg of total RNA. Total RNA samples were pretreated with an RNAse-free DNAse. cDNAs were diluted to 100 μL with TE buffer, and 1 μL of diluted cDNA was used for PCR amplification.

Quantitative RT-PCR reactions were performed in 96-well blocks using the Step-One Plus Real-Time PCR System (Applied Biosystems). The PCR primers used are listed in Table S1. The values for each set of primers were normalized relative to the *EUKARYOTIC TRANSLATION INITIATION FACTOR 4A1* (*eIF4A*) gene (At3g13920). All RT-qPCR reactions were performed in biological triplicates using total RNA samples extracted from three independent replicate samples. The comparative $\Delta\Delta C_T$ method was employed to evaluate the relative quantities of each amplified product in the samples. The threshold cycle (C_T) was automatically determined for each reaction by the system set with default parameters. The specificities of the RT-qPCR reactions were determined by melt curve analysis of the amplified products using the standard method installed in the system.

2.3. Treatment with ABA and drought stress

For treatment with ABA, two-week-old seedlings grown under LDs were transferred to half-strength Murashige and Skoog (MS) liquid-medium supplemented with 20 μM (+)-*cis*,*trans*-ABA (L06278) (Alfa Aesar, Ward Hill, MA, USA).

Drought stress was induced in 2-week-old plants grown in soil under LD conditions by halting watering. To prevent direct air drying of seedlings, small pores were made in the plastic cover 7 d following the start of drought, and the cover was removed 7 d later. Survival rates were calculated for each group of plants. Three independent biological measurements of at least 30 plants were averaged to obtain the results.

2.4. Chromatin immunoprecipitation

Chromatin immunoprecipitation (ChIP) assay was performed as previously described (Schoppee-Bortz and Wamhoff, 2011). The *pMYB96:MYB96-MYC* transgenic plants, anti-MYC antibodies (Millipore, Billerica, USA) and salmon sperm DNA/protein A agarose beads (Millipore, Billerica, USA) were used for ChIP. DNA was purified using phenol/chloroform/isoamyl alcohol and sodium acetate (pH 5.2). The amount of precipitated DNA fragments was quantified by quantitative real-time PCR using specific primer sets (Table S2). The values were normalized with the input DNA level. The values in control plants were set to 1 after normalization against *eIF4a* for quantitative PCR analysis.

2.5. Transient gene expression assays

For transient expression assays using *Arabidopsis* protoplasts, reporter and effector plasmids were constructed. The reporter plasmid contains a minimal 35 S promoter sequence and the *GUS* gene. The core element on the *STM* promoter was inserted into the reporter plasmid. To construct the p35S-MYB96 effector plasmid, the MYB96 cDNA was inserted into the effector vector containing the CaMV 35 S promoter. Recombinant reporter and effector plasmids were cotransformed into *Arabidopsis* protoplasts by polyethylene glycol (PEG)-mediated transformation. The GUS activities were measured by a fluorometric method. A CaMV 35 S promoter-luciferase construct was also cotransformed as an internal control. The luciferase assay was performed using the Luciferase Assay System kit (Promega, Madison, WI).

3. Results

3.1. Regulation of STM expression by MYB96

We previously reported that MYB96 regulates a variety of physiological processes under drought conditions, and consistent with its diverse roles, the *MYB96*-overexpressing activation-tagging *myb96-ox* mutant show pleiotropic developmental phenotypes, including dwarfism, reduced height, smaller and curled leaves, and delayed flowering (Seo et al., 2009, 2011; Seo and Park, 2010). In particular, what we focused on in this study was that *myb96-ox* plants have altered phyllotaxis and a disordered leaf arrangement (Fig. S1). Given that the disruption of phyllotaxis is frequently observed in genetic mutants with altered stem cell homeostasis (Pinon et al., 2013), we hypothesized that MYB96 would be implicated in the control of stem cell proliferation probably under environmental stress conditions.

To obtain clues as to the role of MYB96 in stem cell activity regulation, we evaluated the expression profiles of genes involved in plant meristem development, including *STM*, *WUS*, *CLV1*, *CLV2*, *CLV3*, *PHABULOSA* (*PHB*), *PHAVOLUTA* (*PHV*),