AtMybL-O modulates abscisic acid biosynthesis to optimize plant growth and ABA signaling in response to drought stress

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Abstract To combat constant stress from the external environment throughout their life cycle, plants have evolved their own defense mechanisms. Through robust and complicated defense mechanisms, plants have increased their productivity and adaptability under harsh conditions. In this study, we demonstrated the function of the *AtMybL-O* gene by using knockout (ko) mutants to expand the existing research field. The *atmybl-o ko* mutant seedlings grew similarly to the wild type (WT) in response to osmotic stress, while the *AtMybL-O* overexpression lines exhibit growth suppression in the same growth condition. Further, we attempted to understand the functional mechanism of *AtMybL-O* with respect to stress response toward drought stress. Firstly, we determined the changes in gene expression of the mutants in response to mannitol treatment and identified a strong increase in the expression of *COR15b*, *DREB1A*, and *NCED3* gene in the mutant. Finally, through abscisic acid (ABA) measurement

experiments, we observed that the ABA content of mannitol-treated mutants was higher than that of the WT. Therefore, our results indicate that *AtMybL-O* modulates ABA biosynthesis and ABA signaling in response to drought stress.

Keywords Abscisic acid · *Arabidopsis* · *AtMybL-O* · MYB transcription factor · NCED3

Introduction

As plants grow, plants in particular suffer from abiotic stress due to changes in their natural environment [1]. Some of these abiotic stresses are high temperature, cold, drought, salinity, and heavy metals. These stresses damage plants in many ways. As plants are unable to avoid these stresses by moving, plants have evolved defense mechanisms and survived successfully [2–6].

Plants activate or repress diverse genes and respond to a variety of stresses depending on the situation. In these conditions, transcription factors (TFs) directly or indirectly interact with DNA to activate or suppress the expression of specific genes. Thus, changes in the expression of TFs strongly influence the expression of downstream genes [7]. There are many different types of these TFs, which are known as “super families.” Among the TF super families, the MYB family is a group of highly developed TFs in plants, with more than 120 MYB genes [8, 9]. As well known, the MYB gene is involved in the expression of a variety of genes and plays an important role in the response to stress. For example, *AtMyb96* is involved in abscisic acid (ABA) signaling under drought stress in *Arabidopsis* [10]. Moreover, in response to high salinity, the loss of

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AtMyb73 induces *SOS1* and *SOS3* which have crucial roles in salt stress signaling. [11]. The *AtMyb15*-overexpressing plant shows enhanced sensitivity to ABA and has tolerance to drought stress in *Arabidopsis* [12]. Even in rice, AtMyb44 of *Arabidopsis* confers tolerance to drought stress [13].

The function of the *AtMybL-O* gene was indirectly identified by using overexpression in previous studies [14]. The overexpression of *AtMybL-O* was weak against osmotic stress caused by salt or mannitol and also changes the expression of the marker genes involved in osmotic stress, which had a significant negative effect on growth and survival rate under stress conditions [14]. Because we did not include the characterization results of the *atmybl-o* knockout mutant in our previous report, in this study, we attempted to further our research using these knockout mutants (*SALK_208938* and *SALK_057292* from the ABRC). By studying *atmybl-o* mutants, the function of the *AtMybL-O* gene should become more definitive. Although the *atmybl-o* mutants have similar phenotypes compared with wild type, the mutants responded significantly to mannitol on the expression of major marker genes involved in osmotic stress. Therefore, present study shows that the function of *AtMybL-O* in osmotic stress is regulation in expression of abiotic stress-related genes.

Materials and methods

Plant materials and growth conditions

In the present study, *Arabidopsis thaliana* Columbia-0 (Col-0) was used as the wild-type plant. The knockout mutants *atmybl-o ko#1* (*SALK_208938*) and *atmybl-o ko#2* (*SALK_057292*) were obtained from the *Arabidopsis* Biological Resource Center (ABRC). The *AtMybL-O*-overexpression (*AtMybL-O-OE*) lines were generated by Jeong et al. [14]. Normally, seedlings were cultivated under the following conditions: 23 °C, white fluorescent light (16 h light/8 h dark), and 60% relative humidity. Phenotypes of the plants were determined on 1/2 Murashige and Skoog medium (2% sucrose, pH 5.8) supplemented with 300 mM mannitol.

RNA isolation, reverse transcriptase PCR, and quantitative real-time PCR

Total RNA was extracted from 8-day-old seedlings supplemented with 0, 300, or 400 mM mannitol for 6 h. cDNA was synthesized using the total RNA via a cDNA synthesis kit (RevertAid First Strand cDNA Synthesis Kit, Thermo Fisher Scientific, Waltham, USA). Reverse transcriptase PCR (RT-PCR) was amplified by IP-pro Taq polymerase

(CMT2002, Cosmogenetech, Seoul, South Korea). Quantitative real-time PCR (qRT-PCR) was conducted as described by Lee et al. [15]. Specific transcripts of the cDNA were analyzed via qRT-PCR using EvaGreen MasterMix (BrightGreen qPCR MasterMix, Applied Biological Materials, Richmond, Canada). *ACTIN2* and *ACTIN8* served as the control genes, and the gene primers for RT and qRT-PCR are listed in Table S1.

Determination of ABA contents

The amount of ABA content was determined as described by Liu et al. [16]. ABA was extracted from 12-day-old seedlings following treatment with 300 mM mannitol for 24 h. The amount of ABA content was analyzed using Phytodetek Elisa kit (Agdia, Inc., Elkhart, US) following the manufacturer's instructions.

Statistical analyses

Each experiment was carried out in triplicate. Statistical analyses were performed via one-way ANOVA, and Tukey's test was used at a 95% confidence value.

Gene accession number

Gene accession numbers for genes in this study are as follows: ABI3 (AT3G24650), ABI4 (AT2G40220), COR15B (AT2G42530), DREB1A (AT4G25480), NCED3 (AT3G14440), ZEP (AT5G67030), *AtMybL-O* (AT5G04760).

Results and discussion

AtMybL-O-OE plants are sensitive to drought stress

In our earlier studies, the *AtMybL-O* overexpression (OE) lines exhibited enhanced sensitivity toward salt stress [14]. Thus, we were curious whether the *atmybl-o* mutants would exhibit enhanced tolerance to osmotic stress. To address this question, root length was measured first to check any differences in growth performance in the mutants compared with the *AtMybL-O-OE* lines in response to osmotic stress. As shown in Fig. 1, the root growth of *AtMybL-O-OE* lines was more constrained than that of the wild type (WT), as we observed previously [14]. In the case of the WT, the root length growth was about 30% when treated with mannitol compared to the results under the control condition. The *AtMybL-O-OE* lines showed a further decrease of about 20%. However, *atmybl-o* mutants exhibit no significant difference with WT in the growth of root when treated with mannitol (Fig. 1B). Using RT-PCR, we

Fig. 1 Phenotypes and primary root lengths of Col-0, *atmybl-o* mutants, and *AtMybL-O*-overexpression lines under drought stress. (A) Phenotypes of Col-0, *atmybl-o* mutants, and *AtMybL-O*-overexpression lines under drought stress. The seeds were sown on normal media. After 2 days, the seedlings were transferred to medium supplemented with 300 mM mannitol. The figure shows seedlings 14 days after the transfer. (B) Primary root lengths of Col-0, *atmybl-o* mutants, and *AtMybL-O*-overexpression lines under drought stress. The values in this graph show the results of the mannitol treatment for the control as a rate. The experiments included 40 seedlings per sample, and error bars imply the standard error (SE) of the three replicates. The different letters indicate significant differences ($P < 0.05$). (C) Transcripts of Col-0, *atmybl-o* mutants, and *AtMybL-O*-OE lines by RT-PCR. Seedlings grew under normal condition before sampling

confirmed that two independent lines of the *atmybl-o* mutants do not produce any transcript (Fig. 1C). There could be another homolog of *AtMybL-O* gene in *Ara-bidopsis* genome that is not identified yet.

Alterations of gene expression in the *atmybl-o* and *AtMybL-O*-OE plants in response to osmotic stress

To determine whether the *AtMybL-O* function is related with the osmotic stress signaling, the expression levels of the transcripts involved in osmotic stress with the mannitol treatments were analyzed using qRT-PCR in WT, *atmybl-o* and *AtMybL-O*-OE plants. We observed that the *AtMybL-O*-OE lines showed a total decline in marker genes of osmotic stress [14]. In the present study, on the contrary, the *atmybl-o* mutants increased the expression of various genes involved in response to drought. In particular, there was a noticeable increase in the major abiotic stress marker genes; *COR15b* [17] and *DREB1A* [18] (Fig. 2). This result indicates a significant increase in the sensitivity to drought of the *atmybl-o* mutants. In the case of *COR15b*, its gene expression in the *atmybl-o* mutants increased even under normal conditions. This observation suggests that *AtMybL-O* may act a negative regulator of *COR15b* expression. Altered transcript accumulation of *COR15b* and *DREB1A* led us to determine the ABA contents, as ABA is very well known the regulation of abiotic stress tolerance [6, 19]. Before measuring the ABA contents, we decided to examine the transcript levels of several genes including *NCED3* and *ZEP*, which are the key genes involved in ABA hormone biosynthesis. We observed a significant increase only in *NCED3* not *ZEP* in the *atmybl-o* mutants under osmotic stress, when compared to the WT (Fig. 2).

Modulation of ABA biosynthesis by *AtMybL-O*

Because the *NCED3* transcript level was higher in the *atmybl-o* mutants under drought stress, it was of interest to determine their ABA contents in comparison with the WT and *AtMybL-O*-OE plants. We reasoned that the increased expression of major abiotic stress marker genes under osmotic stress in the *atmybl-o* mutants resulted from the enhanced ABA contents which were caused by the increased expression of *NCED3*. To confirm this question, we decided to determine whether the *atmybl-o* mutants synthesize more ABA than the WT do. As we expected, the *atmybl-o* had more ABA contents than the WT did. Moreover, the ABA contents were much lower in the *AtMybL-O*-OE plants than in the WT under mannitol-treated conditions as well as in the control (Fig. 3).

Taken together, our results demonstrate that the reduced resistance to osmotic stress in the *AtMybL-O*-OE plants may be caused by the decrease in the ABA amount, whereas the opposite is true in part for the case of the *atmybl-o* ko lines. ABA plays a crucial role in plant adaptation to the severe environment for plants [20, 21]. However, like other plant hormones, ABA also suppresses

Fig. 2 Transcript levels of osmotic stress-related genes in Col-0 and mutants in response to mannitol treatments. Relative transcript levels of *ABI3*, *ABI4*, *ZEP*, *NCED3*, *COR15b*, and *DREB1A* in the Col-0 and *atmybl-o* mutants were determined via qRT-PCR under treatment

with 300 or 400 mM mannitol for 6 h. Error bars imply the standard error (SE) of the three replicates. The different letters indicate significant differences ($P < 0.05$)

Fig. 3 ABA contents of Col-0, *atmybl-o* mutant, and *AtMybl-O*-overexpression lines in response to osmotic stress. ABA contents of Col-0, *atmybl-o* mutant, and *AtMybl-O*-overexpression lines under drought stress. ABA was extracted using 12-day-old seedlings after treatment with 300 mM mannitol for 24 h. Error bars imply the standard error (SE) of the three replicates. The different letters indicate significant differences ($P < 0.05$)

the overall growth, especially when available in large amounts [22–24], indicating that the modulation of ABA content to its optimal level is of priority for the plant to survive. In this context, it seems that *AtMybl-O* reduces the expression of *NCED3*, which is a major gene in ABA biosynthesis, conferring a mechanism to precisely regulate

Fig. 4 Working model of *AtMybl-O*, negatively regulating *NCED3* in ABA biosynthesis. Drought stress induces expression of *NCED3*, resulting in ABA biosynthesis. ABA enhances tolerance to abiotic stress in plants, but has a negative effect on growth in plants. *AtMybl-O* represses expression of *NCED3*, precisely regulating ABA by decreasing the biosynthesis of ABA that is overproduced for drought stress

the ABA amount by limiting the biosynthesis of ABA that is overproduced under stress conditions (Fig. 4).

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