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Transgenic expression of *MYB15* confers enhanced sensitivity to abscisic acid and improved drought tolerance in *Arabidopsis thaliana*

Zhenhua Ding [a, b], Shiming Li [a, b], Xueli An [a], Xin Liu [a], Huanju Qin [a], Daowen Wang [a, *]

[a] *State Key Laboratory of Plant Cell and Chromosome Engineering, Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing 100101, China*

[b] *Graduate School of Chinese Academy of Sciences, Beijing 100039, China*

Abstract

Abiotic stresses cause serious crop losses. Knowledge on genes functioning in plant responses to adverse growth conditions is essential for developing stress tolerant crops. Here we report that transgenic expression of *MYB15*, encoding a R2R3 MYB transcription factor in *Arabidopsis thaliana*, conferred hypersensitivity to exogenous abscisic acid (ABA) and improved tolerance to drought and salt stresses. The promoter of *MYB15* was active in not only vegetative and reproductive organs but also the guard cells of stomata. Its transcript level was substantially upregulated by ABA, drought or salt treatments. Compared with wild type (WT) control, *MYB15* overexpression lines were hypersensitive to ABA in germination assays, more susceptible to ABA-elicited inhibition of root elongation, and more sensitive to ABA-induced stomatal closure. In line with the above findings, the transcript levels of ABA biosynthesis (*ABA1*, *ABA2*), signaling (*ABI3*), and responsive genes (*AtADH1*, *RD22*, *RD29B*, *AtEM6*) were generally higher in *MYB15* overexpression seedlings than in WT controls after treatment with ABA. *MYB15* overexpression lines displayed improved survival and reduced water loss rates than WT control under water deficiency conditions. These overexpression lines also displayed higher tolerance to NaCl stress. Collectively, our data suggest that overexpression of *MYB15* improves drought and salt tolerance in *Arabidopsis* possibly by enhancing the expression levels of the genes involved in ABA biosynthesis and signaling, and those encoding the stress-protective proteins.

Keywords: ABA; abiotic stress; *Arabidopsis thaliana*; drought; *MYB15*; MYB transcription factor

Introduction

Abiotic stresses, including drought and salinity, are among the most serious constraints that limit the productivity of agricultural crops (Boyer, 1982). Among the measures that are able to ameliorate the damages caused by abiotic stresses, the cultivation of stress tolerant crops has been considered to be the most promising (Nakashima and Yamaguchi-Shinozaki, 2005; Valliyodan and Nguyen, 2006). For developing stress tolerant crop varieties, a thorough understanding of the molecular principles gov-

erning plant responses to various adverse environmental factors (e.g., drought, high salinity, cold) is essential. To this end, a variety of molecular and biotechnological approaches have been applied to study the genes that are important in plant responses to abiotic stresses. From the knowledge accumulated so far, which is mainly obtained from the molecular genetic studies conducted using the model species *Arabidopsis thaliana*, it appears that numerous genes are involved in plant responses to abiotic stresses, and there is ample evidence that the genes involved in the biosynthesis, signal transduction and function of abscisic acid (ABA) are crucial players in plant adaptations to drought, salinity and cold stresses (Kreps et al., 2002; Xiong et al., 2002; Zhu, 2002; Jiang

and Deyholos, 2006; Shinozaki and Yamaguchi-Shinozaki, 2007).

ABA plays essential roles in many physiological and developmental processes in higher plants, including seed maturation, dormancy and germination, seedling growth, stomatal closure, and the control of flowering time (Zeevaart and Creelman, 1988; Finkelstein et al., 2002; Razem et al., 2006). Molecular analysis has uncovered many genes controlling ABA biosynthesis, signaling and function. For example, three different ABA receptors have been identified (reviewed in Wang and Zhang, 2008). The function of *ABA1*, *ABA2* and *ABA3* genes in ABA biosynthesis has been well established (Zeevaart and Creelman, 1988; Xiong and Zhu, 2003). The most intensively investigated regulators of ABA signaling include several *ABI* genes. While *ABI1* and *ABI2* are negative regulators (Leung et al., 1997; Gosti et al., 1999; Saez et al., 2006), *ABI3*, *ABI4* and *ABI5* regulate ABA responses positively (Giraudat et al., 1992; Finkelstein et al., 1998; Finkelstein and Lynch, 2000). In addition to the *ABI* genes, a growing number of new regulatory loci are being found to participate in ABA signaling (reviewed in Hirayama and Shinozaki, 2007). Under drought, salt or cold conditions, ABA is often recruited as the primary signal for increasing the transcription levels of the stress responsive genes that may confer protection to the assaulted plants (Xiong et al., 2002; Zhu, 2002; Yamaguchi-Shinozaki and Shinozaki, 2006; Shinozaki and Yamaguchi-Shinozaki, 2007). The most intensively investigated *cis*-element functioning in the ABA dependent transcriptions of stress responsive genes (i.e., *RD20A*, *RD29B*) is ABRE (ABA-responsive element), with AREB/ABF proteins being the main *trans*-acting factors that bind to ABRE (Yamaguchi-Shinozaki and Shinozaki, 2006; Shinozaki and Yamaguchi-Shinozaki, 2007). Apart from the regulon consisting of ABRE *cis*-element and AREB/ABF transcription factors, alternative regulatory mechanisms, required for the expression of additional stress responsive genes (i.e., *RD22*, *AtADH1*, *GLY*) in an ABA dependent manner, have also been discovered (Abe et al., 2003; Fujita et al., 2004). For example, AtMYB2 and AtMYC2 transcription factors are required for the transcriptional upregulation of *RD22* and *AtADH1* genes under osmotic stress (Abe et al., 2003), and the NAC protein RD26 is crucial for the increased transcription of *GLY* (encoding a glyoxalase) upon drought, salt or ABA treatments (Fujita et al., 2004).

Consistent with the important role of ABA in plant adaptations to abiotic stresses, the expression levels of many of the genes involved in ABA biosynthesis and signal transduction are affected by adverse environmental factors. Furthermore, manipulations of the expression levels of ABA biosynthesis and signaling genes or those encoding the ABA-regulated stress-protective proteins often alter plant responses to drought, salinity and/or cold stresses. For example, the transcription of *ABA1*, *ABA2* and *ABA3* have all been shown to be upregulated by drought and salt stresses, which contribute to the elevation of ABA level in the plants affected by the two stresses (Xiong et al., 2002). The recessive loss-of-function mutants of *ABI1* (*abi1-2*, *abi1-3*) display enhanced ABA response and drought tolerance (Saez et al., 2006). The loss-of-function mutants of *ERA1*, *ABH1* or *GCR1*, which are all negative regulators of ABA signal transduction pathway, exhibit increased response to ABA-induced stomatal closure and reduced water loss under drought conditions (Pei et al., 1998; Hugouvieux et al., 2001; Pandey and Assmann, 2004). Overexpression of the positive regulators of ABA signaling, such as *ABI3*, *ABI4* or *ABI5*, also confers improved tolerance to several abiotic stresses (reviewed in Nakashima and Yamaguchi-Shinozaki, 2005). Overexpression of ABF proteins (ABF3, ABF4) results in enhanced ABA sensitivity and improved tolerance to chilling, freezing, high temperature, oxidation and drought stresses (Kang et al., 2002; Kim et al., 2004). Raising the expression levels of AtMYB2, AtMYC2 or both augments ABA sensitivity and improved osmotic tolerance (Abe et al., 2003). Finally, several studies have shown that overexpression of the ABA inducible and stress responsive genes result in improved stress tolerance (Xiong et al., 2002; Houde et al., 2004; Srivastava et al., 2006), which is in line with their proposed function in protecting the afflicted plants.

The MYB transcription factors are characterized by the possession of a conserved MYB DNA binding domain in their proteins. The MYB DNA binding domain can contain up to three imperfect repeats (named sequentially as R1, R2 and R3, respectively), with each consisting of around 53 amino acids and forming a helix-turn-helix motif (Stracke et al., 2001; Chen et al., 2006). About 200 genes encoding MYB transcription factors have been found in *Arabidopsis*, 126 of which encode R2R3 MYB proteins (Chen et al., 2006). MYB transcription factors have been found to play important roles in many physiological processes under normal or adverse growth conditions (Jin and Martin, 1999; Stracke et al., 2001; Chen et al., 2006). MYB15 is a member of the R2R3 MYB family of transcription factors in *Arabidopsis*. Recent studies have shown that MYB15 expression is detectable in both vege-