Plant Physiology and Biochemistry 112 (2017) 302–311

Contents lists available at ScienceDirect

Plant Physiology and Biochemistry

journal homepage: www.elsevier.com/locate/plaphy

Research article

PacMYBA, a sweet cherry R2R3-MYB transcription factor, is a positive regulator of salt stress tolerance and pathogen resistance

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ARTICLE INFO

Article history:
Received 29 September 2016
Received in revised form
12 January 2017
Accepted 14 January 2017
Available online 17 January 2017

Keywords:
Sweet cherry
Arabidopsis thaliana
R2R3-MYB transcription factor
Salt stress
Pathogen infection

ABSTRACT

Plant *R2R3-MYB* transcription factors play crucial roles in stress responses. We previously isolated a *R2R3-MYB* homolog from sweet cherry cv. Hong Deng, designated *PacMYBA* (GenBank accession No. KF974774). To explore the role of *PacMYBA* in the plant stress response, we heterologously expressed *PacMYBA* in transgenic *Arabidopsis thaliana* plants. In a previous study, we demonstrated that PacMYBA is mainly localized to the nucleus and could be induced by abscisic acid (ABA). Analysis of the promoter sequence of *PacMYBA* revealed that it contains several stress-related *cis*-elements. QPCR results showed that *PacMYBA* is induced by salt, salicylic (SA), and jasmonic acid (JA) in sweet cherry leaves. Transgenic *Arabidopsis* plants heterologously expressing *PacMYBA* exhibited enhanced salt-tolerance and increased resistance to *Pseudomonas syringe pv.* tomato (*Pst*) DC3000 infection. Overexpression of *PacMYBA* decreased the osmotic potential (OP), increased the free proline content, and increased the peroxidase content in transgenic *Arabidopsis* plants. Furthermore, overexpression of *PacMYBA* also affected the expression levels of salt stress- and pathogen defense-related genes in the transgenic plants. These results indicate that PacMYBA is a positive regulator of salt stress tolerance and pathogen resistance.

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1. Introduction

Plants have evolved extraordinary regulatory mechanisms that enable them to respond and adapt to various stress conditions (Apel and Hirt, 2004; Fujita et al., 2006; Atkinson and Urwin, 2012). Recent studies suggested that the ability of plants to withstand environmental stress and disease infection are controlled at the transcriptional level by complex signal transduction pathways (Chen and Zhu, 2004; Hirayama and Shinozaki, 2010; Leng et al., 2014). When a plant perceives stress, a signal is transmitted from the cell membrane to the cytoplasm and then to the nucleus, where it regulates gene expression and protein translation, and finally brings about physiological and metabolic changes that lead to stress tolerance (Atkinson and Urwin, 2012). Therefore, the regulatory genes in the nucleus and the encoded proteins are critical for the response to stress and their functions can be amplified through signal transduction cascades (von Koskull-Döring et al., 2007; Seo et al., 2008a; Jacobo-Velázquez et al., 2015).

The plant hormones ABA, JA, SA, ethylene (ET), and brassinosteroids (BRs) are endogenous, low molecular weight molecules that adjust plant growth in response to stress conditions (Zhao et al., 2015). ABA is associated both with the plant's response to abiotic stresses, such as drought, high temperature, salinity, and chilling (Finkelstein et al., 2002; Qin and Zeevaart, 2002; Seki et al., 2007; Wang et al., 2014), and to biotic stresses (Mauch-Mani and Mauch, 2005; Melotto et al., 2006; Kaliff et al., 2007; Seo et al., 2008b). Whereas the abiotic stress response is largely controlled by ABA, biotic stress signaling is mediated by JA, SA, ET, and BRs (Fujita et al., 2006; Thimmappa et al., 2014; Leng et al., 2014). JA plays a central role in regulating defense responses to tissue

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http://dx.doi.org/10.1016/j.plaphy.2017.01.015
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damage caused by herbivores and microbial pathogens (Devoto and Turner, 2005; Li et al., 2015). SA mainly functions in plant–microbe interactions. The exogenous application of SA can induce systemic acquired resistance (SAR) to a broad range of pathogens through the induction of pathogen-related genes (Raffaele et al., 2006; Vlot et al., 2009). Recent studies suggested that these hormones act together to govern plant defense signaling networks. Adie et al. (2007) reported that ABA contributes to plant defense responses in *Arabidopsis* through activating JA biosynthesis. The exogenous application of ABA can suppress the induction of SAR by inhibiting the JA biosynthesis pathway both upstream and downstream of the SA biosynthesis pathway, independently of the JA/ET signaling pathway (Yasuda et al., 2008). The ABA signaling pathway can also interact with the JA and SA signaling pathways during basal and induced resistance against *Pseudomonas syringae* and *Alternaria brassicicola* (Flors et al., 2008).

Transcription factors (TFs) are important regulators of gene expression in response to developmental and environmental cues (Jung et al., 2008; Schütze et al., 2008). TFs are classified into different families based on differences in their DNA-binding domains and overall protein structures. The plant MYB TF family regulates numerous response pathways, such as light signaling, pathogen defense, seed maturation, flower development, and fruit pigmentation (Zhang, 2003; Allan et al., 2008; Shen et al., 2014). MYB TFs are divided into four subfamilies, R1, R2R3, R1R2R3, and 4R (Dubos et al., 2010). Increasing evidence indicates that the *R2R3-MYB* genes are involved in the response to diverse abiotic and biotic stresses. AtMYB2, AtMYB15, and AtMYB44 are reported to regulate the ABA-mediated drought stress response (Abe et al., 2003; Jung et al., 2008; Ding et al., 2009). TaMYB56-B in wheat and MdSI-MYB1 in apple are involved in the salt stress response (Zhang et al., 2012; Wang et al., 2014) and AtMYB96 mediates ABA signaling in response to pathogen attack in *Arabidopsis* (Seo and Park, 2010). Overexpression of *OsMYB4* and *OsMYB3R-2* in rice enhances tolerance to cold and freezing (Ma et al., 2009; Laura et al., 2010), whereas overexpression of *AtMYB75* in *Arabidopsis* enhances resistance to the specialist insect herbivore *Pieris brassicae* (Onkokesung et al., 2014).

Members of the R2R3-MYB TF family, which are involved in the stress response, have been widely reported in many plants. In our previous study, we demonstrated that PacMYBA is localized to the nucleus and lacks transcriptional activation activity (Shen et al., 2014). We also showed that PacMYBA from sweet cherry (*Prunus avium* L.) plays crucial roles in ABA-regulated anthocyanin biosynthesis in the fruit (Shen et al., 2014). In the present study, we describe the function of *PacMYBA* in salt stress tolerance and disease resistance in transgenic *Arabidopsis* plants.

2. Materials and methods

2.1. Cherry plants and treatments

Two-year-old shoots with leaves were cut from adult sweet cherry trees (12 years old) during spring, 2011. Trees were grown at the Beijing Institute of Forestry and Pomology under field conditions. After cutting, the shoots were placed under a 16 h light/8 h dark regimen. For salt, SA and MeJA treatment, shoots were placed in a 250 mM NaCl, 10/50/100/200/300 μM SA, or 10/30/50/100/150 μM MeJA solution, respectively. For these treatments, the six leaves from each shoot were collected after 0, 1, 3, 6, or 12 h. Subsequently, leaves were immediately frozen in liquid nitrogen and stored at −80 °C for further analysis. Analyses were performed in biological triplicate.

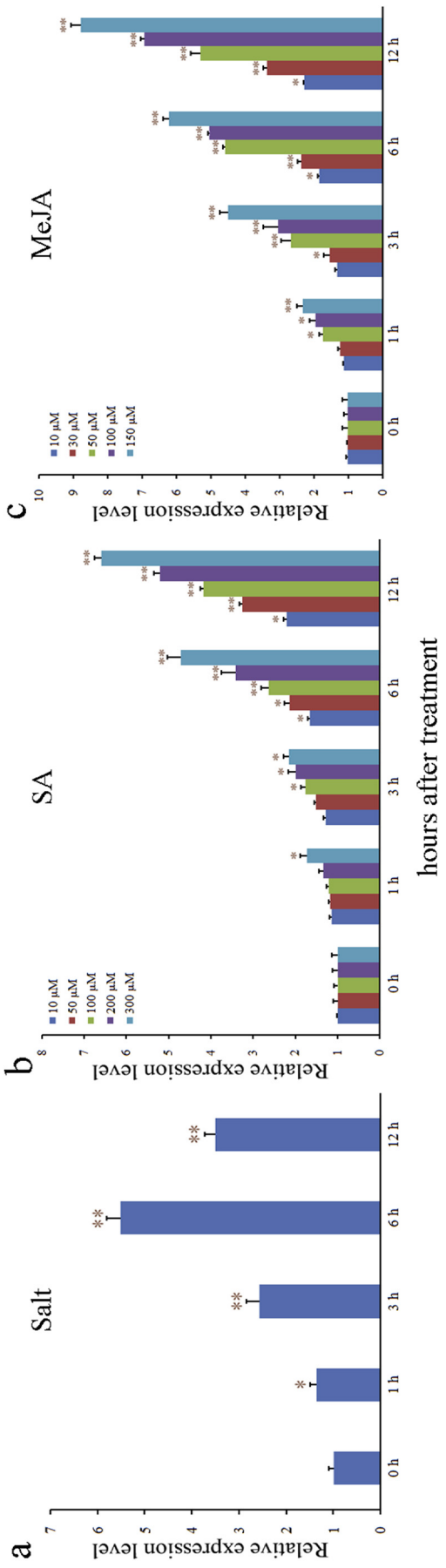

Fig. 1. Expression patterns of *PacMYBA* in sweet cherry cv. Hong Deng (*Prunus avium L.*) leaves in response to salt, SA, and MeJA treatments. The shoots were placed in 250 mM NaCl, 200 μM SA, or 50 μM MeJA solution. Six leaves were collected from the shoots at the indicated time points. The relative expression was calculated using actin mRNA as an internal control. The level of transcript before treatment (unstressed conditions) was assigned a value of 1. Error bars on each column represent the SE of three replicates. Statistically significant differences were assessed using Student's *t*-test (**P < 0.01, *P < 0.05).

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