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Research article

A salt-stress-regulator from the Poplar R2R3 MYB family integrates the regulation of lateral root emergence and ABA signaling to mediate salt stress tolerance in Arabidopsis

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ABSTRACT

The roles of most MYB transcription factors (TFs) in the poplar remain unclear. Here, we demonstrate that *PtrSSR1*, a **s**alt-**s**tress-**r**egulator in the *Populus trichocarpa* R2R3 MYB gene family, mediates the tolerance of transgenic Arabidopsis plants to salt stress. The transcripts of *PtrSSR1* could be induced by salt stress rapidly in poplar. Subcellular localization and yeast assays indicated that *PtrSSR1* encoded a nuclear protein with transactivation activity. The Arabidopsis transformants overexpressing *PtrSSR1* clearly displayed lateral root emergence (LRE) inhibition compared with wild-type (Wt) under normal conditions; while upon NaCl treatment, the transformants showed improved tolerance, and the LRs emerged faster from salt-induced inhibition. A strong correlation could exist between the LRE mediated by *PtrSSR1* and abscisic acid (ABA), mainly because the transformants displayed more sensitivity to exogenous ABA during both seed germination and LRE, and had a distinctly increased level of endogenous ABA. Furthermore, several ABA- and salt-related genes, such as *NCED3*, *ABI1* and *CBL1*, were up-regulated. Thus, our results suggest that elevation in the endogenous ABA content bring alteration of plant LR development, and that the poplar R2R3 MYB TF *PtrSSR1* vitally improve salt stress tolerance by integrating the regulation of LRE and ABA signaling in Arabidopsis.

1. Introduction

In a way, plant growth and development can be determined by the root organs, which directly contact the soil, assimilate most of the nutrients and water, and even interact with several soil microbes in the surrounding environment (Devaiah et al., 2007; Balestrini et al., 2012; Hill et al., 2013; Ramireddy et al., 2014). For most dicots, the root organs include at a minimum the primary root (PR) and lateral roots (LRs), and the LRs initiate later than the PR and link tightly along the PR, e.g., in Arabidopsis the LRs are specified as founder cells within the internal pericycle cell layer of the PR (Lucas et al., 2011; Bao et al., 2014). In the last decade, LR has been intensively explored using model plants, especially *Arabidopsis thaliana*, and hence the impact of plant hormones and

Abbreviations: TF, transcription factor; ABA, abscisic acid; LR, lateral root; LRE, lateral root emergence; PR, primary root; Wt, wild-type; OE-*PtrSSR1*, overexpression of *PtrSSR1*; NCED3, the cleavage enzyme 9-cis-epoxycarotenoid dioxygenase 3; CBL1, calcineurin B-like (CBL) protein 1; ABI1, ABA insensitive 1; P5CS, pyrroline-5-carboxylate synthetase; ZEP, zeaxanthin epoxidase; ELISA, enzyme-linked immuno sorbent assay; BLAST, basic local alignment search tool; EMBL, European molecular biology laboratory; MDB-d, MYB DNA binding-domain; DOI, days of induction; NoT., nontreatment.

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environmental conditions on LRs growth and development are well known (Dubrovsky et al., 2008; Laskowski et al., 2008; Moreno-Risueno et al., 2010; Overvoorde et al., 2010; Geng et al., 2013).

Under drought and salinity conditions, the growth and development of LRs could be suppressed (He et al., 2005; Duan et al., 2013; Zhan et al., 2015). The growth of both the PR and LRs can be inhibited by a mechanism that inhibits cell cycles progression and suppresses the size of the root apical meristem under a relatively higher concentration of salt conditions; in contrast, a relative-lower concentration of salt conditions could increase the growth of both types of root organs (Duan et al., 2013). The environmental impact is closely linked to auxin and ABA, which coordinate responses to the stresses regulating LR growth and development. In contrast to auxin, the stress-related hormone ABA is demonstrated to be involved in the process recently. In general, a relative lower ABA content is required for PR growth under water or salinity stress conditions, whereas a high concentration of ABA leads to inhibition in both the PR and LRs (De Smet et al., 2003; Duan et al., 2013; Geng et al., 2013). Additionally, recent evidence indicates that the inhibition of LRs requires LR-specific ABA signaling, which involves ABA receptor interaction with several MYB transcription factors (TFs), such as *MYB77* in Arabidopsis (Zhao et al., 2014).

Numerous research reports have indicated that TFs mediate response to abiotic stress and LR growth and development, e.g., a regulator of the osmotic stress response and stomatal movement in Arabidopsis, *WRKY46*, can modulate plant LR development by coordinating ABA and auxin signaling under salinity stress conditions (Ding et al., 2015). The MYB family has many members with diverse regulatory functions (Yanhui et al., 2006; Katiyar et al., 2012; Aoyagi et al., 2014; Cho et al., 2016), including the regulation of growth and development of root organs. For example, *AtMYB96* plays roles in drought stress and ABA signaling; and functions as a molecular linker that integrates ABA and auxin signals to modulate auxin homeostasis during LR development (Seo and Park, 2009; Seo et al., 2009). In contrast, *AtMYB93* functions as a negative regulator of LR development, for the mutant showing accelerated LR developmental progression and enhanced LR density (Gibbs and Coates, 2014; Gibbs et al., 2014).

However, the MYB TFs of poplar involved in the regulation of LR growth and development under abiotic stress conditions are largely unknown. In this study, we demonstrate the roles of a poplar R2R3 MYB TF *PtrSSR1* involved in the process. Our results indicated that overexpression of *PtrSSR1* (OE-*PtrSSR1*) improved the tolerance of transgenic Arabidopsis plants to salt stress and led to changes of LRE and that the LRE correlated with the level of endogenous ABA and salt stress condition.

2. Materials and methods

2.1. Plant materials

P. trichocarpa and *Arabidopsis thaliana* (Col-0) were used for initial gene cloning and transgenic plant material. The growth conditions for the poplar seedlings were described in previous report by Duan et al. (2015), and the Arabidopsis seedlings were cultured in a light-controlled incubator with a phase of 16-h light, 8-h dark at 22 °C; the humidity level was set at 65± 10%.

For expression profile analysis, three-week-old poplar seedlings of similar size in the culture bottle with MS agar medium were chosen to next undergo stress treatment and RNA extraction. For the seedlings cultured on MS agar medium, sterilized seeds were placed on the agar medium pre-cultured for 72-h and then transferred to the light incubators for culturing.

2.2. Constructs for transformation

Full-length *PtrSSR1* cDNA was amplified using gene specific primers (Supplementary Table 1) and inserted into the pCX-SN vector (Chen et al., 2009); *PtrSSR1* was driven by the cauliflower mosaic virus 35S (CaMV35S) promoter. The sequenced construct was introduced into *Agrobacterium tumefaciens* strain EHA105, which was then used for the Wt Arabidopsis transformation; the floral dip method was used described in a previous report (Clough and Bent, 1998). Homozygous T3 progeny were screened for transformants with hygromycin (30 μg mL^{-1}) added to the 1/2 MS agar medium and primer-specific PCR.

2.3. RNA preparation and RT-PCR

For the tissue expression profile analysis, the method described in a previous report by Jang et al. was used with the following slight modifications (Jiang et al., 2014): three-week-old poplar seedlings were cultured in 1/2 MS agar medium in bottles without lids for 72-h in the incubator, after which the leaves, shoots, roots, and petioles were separately harvested using liquid nitrogen and then used for RNA extraction with Trizol reagent. After treatment to remove genomic DNA with DNase I enzyme, total RNA from different tissues were subjected to reverse transcription into first strand cDNA, and then semi-qRT-PCR was performed. The PCR products were subjected to agarose gel electrophoresis.

For the real-time RT-PCR, the SYBR PrimeScript™ RT-PCR Kit (Takara, Dalian, China) was used according to the manual. For each PCR, 100–200 ng cDNA was used for each 20 μL PCR reaction. Gene specific-primers were designed and *Actin* was used as a control.

2.4. Transient expression and yeast assays

The cDNA of *PtrSSR1* was amplified using gene specific primer (supplementary table 2) and fused with *GFP* and BD in plasmids of pCX-DG and pGBKT7, respectively. And the constructs sequenced were then expressed in onion epidermal cells and yeast cells. For the transient expression, a bombarded-mediated transformation using gene gun was performed (Duan et al., 2015), the transformed cells were cultured for 36-h in MS medium at 25 °C, and then the fluorescence was examined with Carl Zeiss fluorescence microscopy and the nuclei stained with solution of DAPI (4',6-diamidino-2-phenylindole) were for further determination.

For the transactivation activity of *PtrSSR1* analysis, the empty plasmid pGBKT7 used as a negative control and fused vector pGBKT7-*PtrSSR1* were both transformed into yeast cells with LiAc mediated methods (Duan et al., 2015), and the screened clones were grown in SD/-Trp and SD/-Trp-Leu-His respectively at 28 °C for 3–5 days. After the considered periods, the photographs were taken.

2.5. Seed germination assay

We referred to the report by Jung et al. (2008). for performing seed germination procedure. Transgenic and Wt mature seeds were harvested and stored at 4 °C for more than one month and then were plated on MS agar (1.0%) medium after being surface sterilized. After pre-culturing at 4°Cfor 72-h, different concentrations of ABA (0.25, 0.5, 1.0 μM) were added to the plates, which were then transported into the light-controlled incubator for germination, and plates without added ABA were set as the control. Then, every 12-/24-h, the number of seeds germinating, which was considered when the primary roots were just breaking through the seed coats, was counted. For each assay, no fewer than 40 seeds were used for each condition, and three repeats under the same condition were