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Hardening with salicylic acid induces concentration-dependent changes in abscisic acid biosynthesis of tomato under salt stress

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ABSTRACT

The role of salicylic acid (SA) in the control of abscisic acid (ABA) biosynthesis is controversial although both plant growth regulators may accumulate in tissues under abiotic and biotic stress conditions. Hardening of tomato plants to salinity stress with 10^{-4} M SA ("high SA") resulted in an up-regulation of ABA biosynthesis genes, zeaxanthin epoxidase (*SlZEP1*), 9-*cis*-epoxycarotenoid dioxygenase (*SlNCED1*) and aldehyde oxidases (*SlAO1* and *SlAO2*) in the roots and led to ABA accumulation both in root and leaf tissues. In plants pre-treated with lower concentration of SA (10^{-7} M, "low SA"), the up-regulation of *SlNCED1* in the roots promoted ABA accumulation in the root tissues but the hormone concentration remained at control level in the leaves. Salt stress induced by 100 mM NaCl reduced the transcript abundance of ABA biosynthetic genes and inhibited SlAO activity in plants hardened with "high SA", but the tissues maintained root ABA level over the untreated control. The combined effect of "high SA" and ABA under salt stress led to partially recovered photosynthetic activity, reduced ethylene production in root apices, and restored root growth, which is one of the main features of salt tolerance. Unlike "high SA", hardening with "low SA" had no influence on ethylene production, and led to reduced elongation of roots in plants exposed to 100 mM NaCl. The up-regulation of carotenoid cleavage dioxygenases *SlCCD1A* and *SlCCD1B* by SA, which produce apocarotenoids, may open new pathways in SA sensing and signalling processes.

Introduction

Salinity is one of the major abiotic stress factors, which adversely influence crop production, thus understanding the mechanisms of plant response to salt stress is of great importance. High salinity causes osmotic stress, ion disequilibrium as well as oxidative stress in tissues (Zhu, 2001) and activates the expression of a large number of genes in tomato at an early stage after salt exposure (Ouyang et al., 2007).

The exogenous application of salicylic acid (SA) has been reported to induce tolerance to salt stress (Tari et al., 2002; Jayakannan et al., 2015). SA alleviated the adverse effect of high salinity by decreasing K^+ leakage from *Arabidopsis* root tissues and by enhancing the H^+-ATPase activity (Jayakannan et al., 2013), which provides a driving force for Na^+/H^+ exchanger at the plasma membrane and leads to reduced sodium accumulation in the cytosol (Shi et al., 2000).

Our previous studies demonstrated that hardening of tomato to salt stress with exogenous SA depended on SA concentration and on the duration of treatments. Sublethal, 10^{-4} M SA induced halophyte traits in cultivated tomato plants and facilitated the accumulation of Na^+ in leaf vacuole, resulting in an osmotic adjustment and growth in saline environment (Szepesi et al., 2009). High SA concentration induced also the accumulation of compatible osmolytes (Poór et al., 2011), decreased the accumulation of H_2O_2 in the leaves, the generation of reactive oxygen species (ROS) and nitric oxide (NO) in root apices after salt exposure (Gémes et al., 2011) and activated the expression of specific glutathione S-transferase genes, which participated in the detoxification processes under salt stress (Csiszár et al., 2014).

SA regulates various aspects of plant responses to abiotic and biotic stress through extensive signalling cross-talk with other plant hormones such as abscisic acid (ABA) and ethylene. SA can increase the concentration of ABA and ABA may promote the

Abbreviations: ABA, abscisic acid; AB, Aldabscisic aldehyde; AO, aldehyde oxydase; BIT, 1,2-benzisothiazol-3(2H)-one1,1-dioxide; CCD, carotenoid cleavage dioxygenase; Chl *a*, chlorophyll *a*; DW, dry weight; DTT, dithio-DL-threitol; FW, fresh weight; F_v/F_m, maximal quantum yield of PSII; NAld, 2-naphthaldehyde; NCED, 9-*cis*-Epoxycarotenoid dioxygenase; NPQ, non photochemical quenching; PMSF, phenazine methosulfate; PSII, photochemical system II; Φ_{PSII}, actual quantum yield of PSII electron transport in the light; qP, photochemical quenching parameter; SA, salicylic acid; SAR, systemic acquired resistance; ZEP, zeaxanthin epoxidase.

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biosynthesis of SA in developmental phase- and tissue-specific manner (Seo and Park, 2010), furthermore, both SA and ABA may mutually inhibit each other's signalling (Yasuda et al., 2008; Kanno et al., 2010; de Torres Zabala et al., 2009). SA at high concentration proved to be a potent inhibitor of ethylene biosynthesis (Leslie and Romani, 1986), thus it may control plant growth and development via modulation of ethylene production. Interaction between SA and indoleacetic acid signalling has also been demonstrated (Tamás et al., 2015).

Maintenance of an optimal ABA concentration plays a pivotal role in the acclimation to high salinity. ABA up-regulates the genes, which contribute to ion homeostasis, such as plasma membrane and vacuolar ATP-ases (Janicka-Russak et al., 2012) as well as Na^+/H^+ antiporters (Yarra et al., 2012). Furthermore, ABA up-regulates several genes that are responsible for osmotic adjustment (Szegletes et al., 2000; Rhodes et al., 2004) and for the mitigation of oxidative stress (Ashraf, 2009). Reallocation of photosynthetic products to the roots (Albacete et al., 2008) and the inhibition of ethylene production (Ghanem et al., 2008) are also mediated by ABA signalling. It was demonstrated that ABA was sufficient to prevent ethylene-induced growth inhibition in maize seminal roots exposed to osmotic stress (Sharp and LeNoble, 2002). However, excessive ABA accumulation may reduce the relative growth rate or biomass production and may prevent the dilution of Na^+ concentration in the cytoplasm by the growth of tissues under salt stress.

The synthesis of ABA from C_{40} carotenoids is induced by up-regulation of ABA biosynthetic genes in plants exposed to abiotic stress (Xiong and Zhu, 2003). The first reaction, the conversion of zeaxanthin to all-*trans*-violaxanthin is catalysed by zeaxanthin epoxidase (ZEP) (Seo and Koshiba, 2002) that is encoded by *ABA1* in *Arabidopsis* (Xiong et al., 2002) and *SlZEP1* gene in tomato (Thompson et al., 2000). The rate-limiting step of ABA biosynthesis is catalysed by 9-*cis*-epoxycarotenoid dioxygenase (SlNCED), which produces a C-15 intermedier, xanthoxin from 9-*cis*-isomers of violaxanthin and neoxanthin (Thompson et al., 2000; Ahrazem et al., 2012). Xanthoxin is converted into abscisic aldehyde (ABAld) by a short chain dehydrogenase/reductase (Gonzáles-Guzman et al., 2002) and the oxidation of ABAld to ABA is catalysed by aldehyde oxydases (AOs) (Schwartz and Zeevaart, 2010). Mutations in a single AO gene may have limited effects because alternative pathways for conversion of ABAld to ABA exist.

Although the oxidative cleavage of neoxanthin catalysed by NCED is rate limiting in the leaves, the activity of ZEP might limit ABA synthesis in non-photosynthetic tissues, such as seeds and roots (Xiong and Zhu, 2003). Water deficit and salt treatment increased *ZEP* (Xiong et al., 2002; Mulholland et al., 2003) and *NCED* expressions in various species (Mulholland et al., 2003; Gallé et al., 2013). Working with ABA-deficient mutants of *Arabidopsis*, Barrero et al. (2006) have shown that besides the ABA-dependent regulation of ABA biosynthesis an ABA-independent pathway also exists in plants. They confirmed that *NCED3* is the ABA biosynthetic gene that is most responsive to 300 mM NaCl treatment in severe ABA-deficient *aba1*, *aba2*, *aba3*, *aao3* mutants, thus high salinity can induce *ABA1*, *NCED3* and *AAO3* genes in an ABA-independent pathway.

Based on sequence similarity to NCEDs, a second class of carotenoide cleavage dioxygenases (CCDs) were also characterised. This class of CCDs is involved in the synthesis of apocarotenoids such as β-cyclocitral, β-ionone, geranial, theaspirone and damascenones (Auldridge et al., 2006). CCDs and their products play a role in plant development, plant and fungi interactions and they are components of floral scent and fruit flavour (Camara and Bouvier, 2004).

The oxidation of ABAld to ABA is another putative control point of ABA synthesis; however, its regulatory role is still debated. The three known *Pisum sativum* AOs exhibited different expression profile under salt treatment in young, fully expanded and senescing leaves of plants (Zdunek-Zastocka, 2008). It is also known that AOδ encoded by *AO3* gene is responsible for the last step in ABA biosynthesis in *Arabidopsis* seeds and leaves (Seo et al., 2000, 2004). Among the aldehyde oxidases characterised so far, *AO3* in *Arabidopsis* (Seo et al., 2000) and two other isoforms, *AO2* and *AO3* in barley roots (Omarov et al., 2003) use ABAld efficiently as a substrate. In tomato, five AO coding sequences, three functional AO genes and two pseudogenes (*TAO1*–*TAO5*) have been described (Ori et al., 1997), which are expressed both in shoots and roots, but they may play different roles in the physiological processes of tomato (Min et al., 2000).

Based on the earlier results the application of SA causes overproduction of various forms of reactive oxygen and nitrogen species leading to the activation or repression of various signalling pathways (Gémes et al., 2011; Jayakannan et al., 2015). Moreover, Li et al. (2015) have recently reported that SA induces the accumulation of hydrogen sulphide (H_2S), a gaseous transmitter, which may act as a novel downstream signal of SA in maize plants exposed to high temperature.

The interaction of SA, ABA and ethylene are implicated in stress acclimation and in accordance with other stress responses, these growth regulators may elicit specific signalling by the activation of specific receptors. However, due to ROS, NO and H_2S production, at higher doses they may activate unspecific signalling pathways. During the past two decades, several aspects of chemical hardening was investigated, but the interaction of SA, ABA and ethylene in SA-induced hardening to salt stress has not been elucidated in detail. The aim of our work was to determine how exogenous SA affects the accumulation of ABA and the expression of ABA biosynthesis genes after a long-term hardening period and subsequent salt stress in tomato plants. Accumulating evidence demonstrates that finely tuned SA and ABA balance is critical for plant survival under high salinity. The question is how ABA accumulation is regulated when plants remain salt sensitive (hardened with "low SA") or when they can tolerate high salinity (hardened with "high SA").

Materials and methods

Solanum lycopersicum Mill. L. cvar. Rio Fuego was used in the experiments. Seeds were germinated on 26 °C, for 3 days in the dark. Then the seedlings were grown in a greenhouse in perlite and after 7 days they were cultivated in hydroponic culture for 2 weeks as described by Poór et al. (2011). The nutrient solution contained 2 mM $Ca(NO_3)_2$, 1 mM $MgSO_4$, 0.5 mM KH_2PO_4, 0.5 mM Na_2HPO_4, 0.5 mM KCl, micronutrients (10^{-6} M $MnSO_4$, $5{\cdot}10^{-7}$ M $ZnSO_4$, 10^{-7} M $CuSO_4$, 10^{-7} M $(NH_4)_6Mo_7O_{24}$, 10^{-5} M H_3BO_4) and $2{\cdot}10^{-5}$ M Fe-EDTA at pH = 5.8 and was changed three times a week. The plants were grown in 12/12 h light/dark cycle at 22 °C and the light intensity and relative humidity were 300 μmol m^{-2} s^{-1} and 55–60%, respectively.

Three-week-old plants were pre-treated with 10^{-7} M and 10^{-4} M SA ("low SA" and "high SA", respectively) for three weeks, then the plants were treated with 100 mM NaCl to induce salt stress. SA was present in the nutrient solution when the plants were exposed to high salinity. The pH value was adjusted to 5.8 in control and 10^{-7} M, 10^{-4} M SA and 100 mM NaCl containing culture solutions. Samples were taken for the analytical procedures and the determination of growth parameters, the shoot and root length, fresh and dry weight (FW and DW, respectively) occurred after 1 week of salt treatment.

Screening of databases, phylogenetic analyses

Tomato ZEP, NCED, CCD and AO sequences were searched at the SOL Genomics Network (SGN, http://solgenomics.net) database.