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Enhanced oxidative stress in the jasmonic acid-deficient tomato mutant *def-1* exposed to NaCl stress

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ABSTRACT

Jasmonic acid (JA) has been mostly studied in responses to biotic stresses, such as herbivore attack and pathogenic infection. More recently, the involvement of JA in abiotic stresses including salinity was highlighted; yet, its role in salt stress remained unclear. In the current study, we compared the physiological and biochemical responses of wild-type (WT) tomato (*Solanum lycopersicum*) cv Castlemart and its JA-deficient mutant *defenseless-1* (*def-1*) under salt stress to investigate the role of JA. Plant growth, photosynthetic pigment content, ion accumulation, oxidative stress-related parameters, proline accumulation and total phenolic compounds, in addition to both enzymatic and non-enzymatic antioxidant activities, were measured in both genotypes after 14 days of 100 mM NaCl treatment. Although we observed in both genotypes similar growth pattern and sodium, calcium and potassium levels in leaves under salt stress, *def-1* plants exhibited a more pronounced decrease of nitrogen content in both leaves and roots and a slightly higher level of sodium in roots compared to WT plants. In addition, *def-1* plants exposed to salt stress showed reactive oxygen species (ROS)-associated injury phenotypes. These oxidative stress symptoms in *def-1* were associated with lower activity of both enzymatic antioxidants and non-enzymatic antioxidants. Furthermore, the levels of the non-enzymatic ROS scavengers proline and total phenolic compounds increased in both genotypes exposed to salt stress, with a higher amount of proline in the WT plants. Overall the results of this study suggest that endogenous JA mainly enhanced tomato salt tolerance by maintaining ROS homeostasis.

1. Introduction

Salinity is considered to be a global threat that decreases the yield of commercial crops worldwide (Parida and Das, 2005). Tomato (*Solanum lycopersicum*), as a glycophyte, is relatively sensitive to salinity stress mainly at the seedling stage (Sun et al., 2010). Salt stress induces both osmotic and ionic stresses resulting in the disturbance of multiple physiological processes such as ionic homeostasis, water relations and gas exchanges (Parida and Das, 2005). As a result of limited CO_2 fixation in salt-stressed plants, the over-production of reactive oxygen species (ROS), such as superoxide ($O_2^{\cdot -}$), hydroxyl radicals ($OH^{\cdot}$) and hydrogen peroxide (H_2O_2) can be induced in the chloroplasts and other organelles (Parida and Das, 2005; Miller et al., 2010). The resulting ROS-associated injury typically includes oxidative damage to proteins, lipids, DNA and photosynthetic pigments (Parida and Das, 2005; Miller et al., 2010). To survive under saline environments, plants have evolved mechanisms of salt tolerance such as exclusion or selective accumulation of toxic ions, compatible solute synthesis and ROS homeostasis by the activation of both enzymatic and non-enzymatic antioxidants (Parida and Das, 2005).

Plant hormones play a crucial role in plant defense against both biotic and abiotic stresses (Wasternack, 2007; Peleg and Blumwald, 2011). Jasmonic acid (JA) is one of the hormones that acts as a vital signaling molecule in biotic stress responses (herbivores and necrotrophic pathogens) and development (root growth and fertility) (Wasternack, 2015). Furthermore, increasing evidence has shown that JA is also involved in the plant responses to many abiotic stresses such as UV and cold (Dar et al., 2015). The biosynthesis of JA begins with the peroxidation of α-linolenic acid by lipoxygenase (LOX) leading to the formation of JA and its derivatives, including (+)-7-iso-Jasmonoyl-L-isoleucine (JA-Ile) and methyl jasmonate (MeJA), collectively known as jasmonates (JAs) (Wasternack, 2015). JA-Ile is synthesized by conjugation of JA and isoleucine in the presence of the JA amino acid synthetase enzyme JAR1 (Jasmonate-Resistant 1) (Wasternack, 2015). JA-Ile can promote the SCF^{COI1}- mediated ubiquitination of JAZs (repressor for jasmonate insensitive1, MYC2/JIN1), allowing degradation of JAZ proteins by the 26S proteasome and release of the transcription factors such as MYC2. The released MYC2 can then promote the

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expression of early JA-responsive genes (Chung et al., 2009; Mira et al., 2016).

While JA has been mostly studied during biotic stress such as herbivore attack (wounding) and pathogenic infection (Wasternack, 2007), a growing body of evidence suggests that JA is also involved in salt stress. Salinity stress activates the expression of genes involved in the JA synthesis branch of α-linolenic acid metabolism (Pedranzani et al., 2003). In addition, JA has been shown to accumulate in response to salt stress in different plant species (Moons et al., 1997; Pedranzani et al., 2003). For example, in *Solanum lycopersicum*, a correlation between salt tolerance and high levels of JA has been reported (Pedranzani et al., 2003). Moreover, exogenous JA treatments alleviated salt-induced injury in a variety of plants by increasing the photosynthesis rate (Walia et al., 2007), proline content (Fedina and Tsonev, 1997), ABA level (Kang et al., 2005), activity of antioxidant enzymes (Qiu et al., 2014) and/or decreasing Na^+ accumulation in the shoots (Fedina and Tsonev, 1997). Hence, it is likely that exogenous JA does play a positive role in salinity tolerance. However, it is difficult to draw similar conclusions for the role of endogenous JA in salt tolerance, as while exogenous JA is present continuously for the plant tissues, endogenous JA signaling is tightly controlled, resulting in fluctuations in endogenous JA level under stress conditions (Ismail et al., 2014).

A few recent studies have focused on the use of mutants and transgenic *Arabidopsis thaliana* plants altered in the JA pathway to study the role of JA in salt-stressed plants (Zhao et al., 2014; Ding et al., 2016; Lim et al., 2015). The over expression of two genes of the JA biosynthesis pathway, *TaAOC1* (gene encoding the enzyme allene oxide cyclase, Zhao et al., 2014) and *CaLOX1* (Lim et al., 2015) resulted in improved salt tolerance. These plants exhibited either a high survival rate, elevated superoxide dismutase (SOD) activity and an increased level of JA (Zhao et al., 2014) or an improved germination rate, biomass and chlorophyll content (Lim et al., 2015) during salt stress. In addition, a *lox3* (*lipoxygenase3*) mutant (impaired in JA and JA-Il accumulation) showed hypersensitivity to salinity (associated with a reduction in survival rate, germination and lateral root number). This salt sensitive phenotype was rescued by exogenous treatment with MeJA (Ding et al., 2016). Although these studies suggest that endogenous JA can positively regulate salt tolerance; the mechanisms by which JA regulates salt tolerance still need to be further investigated. Studies using plants bearing mutations within a hormone-biosynthetic pathway have been instrumental in advancing our understanding of plant responses to unfavorable environmental conditions (Peleg and Blumwald, 2011). The JA-deficient tomato mutant *def-1* (*defenseless-1*) (Howe et al., 1996) has been used extensively in many studies to illustrate how JA influences tomato development and seed germination (Wu and Bradford, 2003), defense against herbivorous insects (Grinberg-Yaari et al., 2015), defense against fungal pathogens (Mehari et al., 2015) and lycopene biosynthesis in tomato fruits (Liu et al., 2012). Therefore, the *def-1* mutant could also be a useful tool to determine the role of JA in salt tolerance. The objective of our study was to determine the involvement of JA in both ROS and ion homeostasis by comparatively analyzing the stress responses of JA-deficient mutant *def-1* (with a defective JA synthesis pathway, Howe et al., 1996) and its wild-type. We hypothesized that salt tolerance of tomato JA-deficient mutant *def-1* will be lower than that in the wild- type plants, exhibiting oxidative stress symptoms and ionic imbalance.

2. Materials and methods

2.1. Plant growth and salt treatment

Tomato (*Solanum lycopersicum*) seeds of the *def-1* mutant and wild-type (WT) cv Castlemart were kindly provided by Dr. Gregg Howe (Michigan State University, USA). Seeds of both genotypes were surface sterilized for 10 min with 5% sodium hypochlorite. The seeds were germinated in Petri dishes containing two filter papers (Whatman No.1) moistened with 5 mL of half strength modified Hoagland nutrient solution (see composition below). The Petri dishes were incubated at 24 °C for 2 days in the dark and then transferred for 5 days in the following greenhouse conditions: 25/19 °C day/night temperatures, 18 h/6 h photoperiod with natural light supplemented by sodium lamps (P.L. light systems, Beamsville, ON, Canada). The well-grown seedling were transferred for 12 days to an aerated hydroponic solution (half strength modified Hoagland solution containing 2 mM NH_4NO_3; 1 mM KH_2PO_4; 1.5 mM $CaCl_2$; 0.5 mM KCl_2; 1 mM $MgSO_4$; 23 μM H_3BO_3; 5 μM $MnCl_2$; 0.4 μM $ZnSO_4$; 0.2 μM $CuSO_4$; 0.07 μM H_2MoO_4 in addition to 7 μM Fe-EDTA).

The nutrient solution was changed every 4 days to avoid nutrient deficiency. Five plants from each genotype were placed in 10-L containers (each container was considered as one replicate), in a completely random block design with four replicates. Seedlings from both genotypes were exposed to salinity stress at the stage of two-three true leaves by adding 100 mM NaCl to the nutrient solution for 14 days. To avoid NaCl-induced osmotic shock, NaCl was added to the nutrient solution in a stepwise method by 25 mM increments a day until reaching the final concentration (100 mM). After 14 days of salt treatment, leaves were collected, immediately frozen in liquid nitrogen and stored at −80 °C for subsequent determination of lipid peroxidation, H_2O_2 level, proline content and enzymatic antioxidants analysis. The remaining plants were harvested, washed three times with distilled water and the tissues were lyophilized to determine dry weights, total phenolic content, DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical scavenging activity as well as ion accumulation in shoots and roots.

2.2. Photosynthetic pigments, elemental analysis and free proline content

To determine leaf chlorophyll a (Chla), chlorophyll b (Chlb), and carotenoids, 30 mg of freeze-dried tissues were incubated with 10 mL of acetone (80%) and kept in dark for 24 h. The absorbance was measured at 480, 645, and 663 nm. Quantifications of Chla, Chlb and carotenoids in the extracts were calculated using MacKinney equations (Sestak et al., 1971).

Lyophilized shoots and roots were ground separately using a coffee grinder (Black & Decker, Brockville, ON Canada). The sodium, potassium and calcium contents of plant tissues were analyzed using a Direct Current Plasma (DCP) spectrophotometer. For N analysis, a CHNOS elemental analyser 'vario Micro' (Elementar, Hanau, Germany) was used. The elemental analysis was performed by Stratford Agri. Analysis (Stratford, ON, Canada). In the leaf tissue, free proline content was determined according to the method described by Bates et al. (1973). Proline content was expressed as μmol proline g^{-1} FW using a calibration curve (0–0.250 μmol/ml proline).

2.3. Total phenolic content

Total phenolic content was measured using Folin-Ciocalteu's reagent (Sigma-Aldrich, Canada) according to Singleton and Rossi (1965). The total phenolic content of leaves was expressed in mg of gallic acid equivalents (GAE)/g dry weight.

2.4. Determination of lipid peroxidation and hydrogen peroxide (H_2O_2)

Lipid peroxidation of leaves was measured as the amount of malondialdehyde (MDA) produced in leaves using the 2-thiobarbituric acid (TBA) method as described by Heath and Packer (1968). The levels of MDA–TBA complex (red pigment) was calculated from the extinction coefficient equal to 155 mM^{-1} cm^{-1}. The level of MDA–TBA was expressed as μmol g^{-1} fresh weight. The level of H_2O_2 was measured in the leaves of both genotypes using the FOX-1 method (ferrous oxidation with Xylenol Orange) described by Wolf (1994). The levels of H_2O_2 were expressed as μmol g^{-1} fresh weight using a standard curve.