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Research article

Hormone profiling and transcription analysis reveal a major role of ABA in tomato salt tolerance

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ABSTRACT

The response and adaptation of plants to different environmental stresses are of great interest as they provide the key to understanding the mechanisms underlying stress tolerance. In this study, the changing patterns of four endogenous hormones and various physiological and biochemical parameters of both a salt-tolerant (LA2711) and a salt-sensitive (ZS-5) tomato cultivar were examined under salt stress and non-stress conditions. Additionally, the transcription of key genes in the abscisic acid (ABA) biosynthesis and metabolism were analyzed at different time points. The results indicated that gene expression responsible for ABA biosynthesis and metabolism coincided with the hormone level, and *SlNCED1* and *SlCYP707A3* may play major roles in the process. LA2711 performed superior to ZS-5 on various parameters, including seed germination, Na^+ compartmentation, selective absorption of K^+, and antioxidant enzymes activity. The difference in salt tolerance between the two genotypes could be attributed to the different levels of ABA due to differences in gene expression of key genes in ABA biosynthesis and metabolism. Although gibberellin, cytokinin and auxin were involved, our results indicated that ABA signaling plays a major role in tomato salt tolerance. As compared to ZS-5, LA2711 had a higher capability to selectively absorb and redistribute K^+ and a higher tolerance to Na^+ in young leaves, which may be the main physiological mechanisms of salt tolerance.

1. Introduction

High salinity is one of the most severe environmental stresses which cause crop yield loss and product quality deterioration. Plants have developed different mechanisms to adapt to salinity stress, involving complex physiological and biochemical changes. In the process of stress adaptation, hormones, especially abscisic acid (ABA), play important roles.

Abscisic acid is a phytohormone that regulates plant growth and development and abiotic stress response. In tomato, *SlNCED1* and *SlNCED2* encoding 9-cis-epoxycarotenoid dioxygenase are the primary genes responsible for ABA biosynthesis, whereas *SlCYP707A1*, *SlCYP707A2*, *SlCYP707A3* and *SlCYP707A4* encoding ABA 8′-hydroxylase are main genes for ABA metabolism (Sun et al., 2011; Nakaune et al., 2012). Under drought stress, a higher expression of *SlNCED1* is maintained in the roots and leaves of tomato. This increases the concentration of ABA in leaves, leading to reduction of stomatal conductance and improvement of water use efficiency (Thompson et al., 2007; Tung et al., 2008). *SlNCED2* is expressed mainly in vegetative tissues and young fruits, and its expression in fruits is not in step with the ABA level (Sun et al., 2012). There are still no reports of expression patterns of these tomato genes under salt stress.

ABA regulates several aspects of plant response to abiotic stress. Under stress, plants produce ABA which induces the expression of genes encoding ion transporters, thus enhancing the ion selective absorption and contributing to the transfer of Na^+ from the cytoplasm to vacuole or discharge it out of plant (Yu et al., 2007; Zhao

Abbreviations: APX, ascorbate peroxidase; ABA, abscisic acid; CTK, cytokinin; CAT, catalase; ETH, ethylene; GAs, Gibberellins; IAA, auxin; NCED, 9-cis-epoxycarotenoid dioxygenase; POD, peroxidase; PVPP, polyvinylpolypyrrolidone; SOD, superoxide dismutase; ZR, trans-zeatin-riboside.

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et al., 2009; Yarra et al., 2012). ABA is also involved in the modified process of the ATPase activities of the plasma membrane and tonoplast and improving proton pump activity, which will provide more power for the Na^+/H^+ antiport, thus simultaneously enhancing the selective absorption of K^+ (Janicka-Russak and Klobus, 2007; Olias et al., 2009). Under osmotic stress, ABA induces a rapid elevation of Ca^{2+} in apical cytoplasm, which improves the K^+ selective absorption and maintains the normal K^+/Na^+ ratio (Borsani et al., 2001; Dodd et al., 2010; Huertas et al., 2012). In addition, ABA activates the expression of genes encoding antioxidant enzymes, increasing the activity of antioxidant enzymes including superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX) (Jiang and Zhang, 2002). The increased capacity of reactive oxygen species (ROS) scavenging alleviates the damaging effect of ROS on fat/oil and protein (Hu et al., 2006). ABA also plays critical roles in reducing stomatal conductance and inducing the expression of genes responsible for various osmolytes synthesis such as proline and betaine (Jung et al., 2008; Antoni et al., 2011).

Gibberellin (GA) acts as an antagonist to ABA. GA enhances seed germination under salt stress through different mechanisms such as inducing synthesis of some enzymes and stimulating H^+-ATPase activity of the tonoplast (Maggio et al., 2010). At low salt stress, GA_3 reduces the stomatal resistance of leaf, accelerates transpiration and increases water use efficiency, thus improving the salt tolerance of plants. However, GA_3 does not reduce the inhibition of high salt stress on plant growth (Maggio et al., 2010; Achard et al., 2006). Other phytohormones promoting the growth of plants, such as cytokinin (CTK) and auxin (i.e. indole-3-acetic acid, IAA), can also increase the salt tolerance of plants (Wang et al., 2009; Park et al., 2011).

Homeostasis of the cell is disrupted at different levels under salt stress. Salinity causes ionic stress (mainly due to Na^+, Cl^- and SO_4^{2-}), osmotic stress, and secondary stresses including nutritional imbalance and oxidative stress for glycophytes (Zhu, 2002). High concentrations of Na^+ disturb the osmotic balance, causing "physiological drought", which prevents plant water uptake. Halophytic plants are tolerant to sodium toxicity, therefore osmotic stress might be the main cause of growth inhibition. To cope with the harmful effects of salt stress, plants have evolved a series of biochemical and molecular mechanisms, mainly including selective buildup or exclusion of salt ions, control of ion uptake by roots and transport into leaves, ion compartmentalization, synthesis of compatible osmolytes, and induction of antioxidative enzymes (Rodriguez-Rosales et al., 2008).

Understanding the mechanisms of plant salt tolerance will provide effective means to breed or genetically engineer salt-tolerant crops. Here, we report the profiling of various endogenous phytohormones (ABA, GA_3, ZR and IAA) in a salt-tolerant tomato cultivar (LA2711) and a sensitive one (ZS-5), and genes involved in the biosynthesis and metabolism of ABA at the transcriptional level. We also measured the growth and various physiological and biochemical parameters of the two genotypes tested. The results would further provide insights in the response and adaptation of tomato to salt stress.

2. Materials and methods

2.1. Plant materials

Tomato (*Solanum lycopersicum*) seeds of LA2711 and ZS-5 were kindly provided by the Tomato Genetic Resource Center (TGRC, Davis USA) and the Chinese Academy of Agricultural Science (CAAS, Beijing, China), respectively. LA2711 is found to be salt-tolerant (Mahmoud et al., 1986), while ZS-5 is sensitive to salt (Wang et al., 2005). Both accessions belong to cultivated tomato with large fruits, and their genetic backgrounds are relatively close.

2.2. Measurement of growth and physiological parameters

Salt-stress treatment of tomato was reported previously (Ouyang et al., 2007). Briefly, tomato seeds were surface-sterilized and sown on agar-solidified MS medium (Murashige and Skoog, 1962) in triplicate, supplemented without or with different concentrations of salt (50–150 mM NaCl). Seeds were germinated at 24 °C in the dark for 2 days and then were moved to an incubator at 24 °C and under a light–dark regime of 16 h light/8 h darkness. The germination was recorded at 12-h intervals for 27 consecutive days using the criteria of appearance of 0.2 cm-radicle protrusion. Additionally, seed germination rate and potential, seedling fresh weight, radical length, lateral root number, cotyledon length and hypocotyl length were recorded and analyzed.

Photosynthesis parameters, ions and antioxidant enzymes were measured on LA2711 and ZS-5 plants. Briefly, five-week-old plants of both genotypes grown hydroponically with one-fifth Johnson's solution supplemented with 10 μM Fe-EDDHA were transferred either to a new nutrient solution with 150 mM NaCl for salt stress or to a nutrient solution without salt as the control (Ouyang et al., 2007; Wang et al., 2001). Net photosynthetic rate per unit area, stomatal conductance and evaporation rate of the third leaves from the top were measured after 7 d treatment with a TPS-1 photosynthesis system (PP System, UK). Fifteen days after salt treatment, the third leaves from the top, the youngest fully expanded leaves and root tips were sampled for the determination of ions, and at the same time, the root tissue were used for determination of antioxidant enzymes. Na^+, K^+, Ca^{2+} and Mg^{2+} contents were determined by flame photometry as described in Asch et al. (2000). POD, SOD, CAT activities and proline content were determined according to previous work (Bates et al., 1973; Cavalcanti et al., 2004).

2.3. Observation of stomatal status for tomato leaves

Five-week-old tomato plants grown hydroponically were transferred either to a new nutrient solution with 150 mM NaCl for salt stress treatment or without salt as the control. The third leaf from the top from different individuals was taken at the three time points: 0 h (9:30 am), 2 h (11:30 am), 24 h (9:30 am). Small pieces of leaf tissue, which were the abaxial of leaf and were sampled and fixed with 2.5% solution of glutaraldehyde in 0.1 M phosphate buffer (pH 7.2), post-fixed for about 30 min in 1% osmium tetroxide. After washing with distilled water several times, the specimens were dehydrated with ethanol series (30%, 50%, 70% and 100%) and then transferred to propylene oxide for 30 min, followed by washing with isoamyl acetate for 40 min. All specimens were critical point-dried using liquid CO_2 and then coated with a thin layer of gold. The stomatal morphology was observed with a Hitachi S-450 scanning electron microscope (Hitachi, Tokyo, Japan). Stomata were counted on ten fields per sample and statistically analyzed.

2.4. Determination of hormones content in leaves and roots

The culture conditions and treatments of tomato plants were the same as described above. The samplings were carried out at different time points: 0 h (9:30 am), 1 h (10:30 am), 2 h (11:30 am), 6 h (15:30 pm), 12 h (21:30 pm), 24 h (9:30 am). Root tissues and the third leaf from the top were taken at each time point, quickly preserved in liquid nitrogen and kept in an ultra-low temperature freezer (−80 °C). Approximately 0.5 g of tissue was weighed and homogenized in small volumes of pre-cooled 80% methanol with