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Effects of exogenous 5-aminolevulinic acid on photosynthesis, stomatal conductance, transpiration rate, and PIP gene expression of tomato seedlings subject to salinity stress

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ABSTRACT. The effects of exogenous 5-aminolevulinic acid (ALA) on photosynthesis, plant growth, and the expression of two aquaporin genes in tomato seedlings under control and salinity conditions were investigated. Exogenous ALA application significantly improved net photosynthetic rate (P_n), total chlorophyll content, and plant biomass accumulation of tomato seedlings under salinity stress. As revealed by real-time PCR analyses, after treatment with ALA alone, expression of both *LePIP1* and *LePIP2* in the two tomato cultivars was up-regulated at 2 h and subsequently decreased to normal levels. Under salinity stress, transcript levels of *LePIP1* in both leaves and roots of salt-sensitive cultivars (cv. Zhongza No.9) increased significantly and were considerably higher than in cultivars exposed to ALA alone. In contrast, the expression levels of *LePIP1* and *LePIP2* in cvs. Jinpeng No.1 cultivars were slightly lower under salinity stress than under ALA treatment. In addition, transcript levels of both *LePIP1* and *LePIP2* in the roots of Jinpeng No. 1 cultivars were considerably lower than those

in the roots of Zhongza No. 9 cultivars under salinity stress, regardless of ALA supplementation, implying that Jinpeng No. 1 cultivars had a better capacity to maintain membrane intrinsic protein stability. Further, ALA application distinctly counteracted the up- or down-regulation of *LePIP1* and *LePIP2* in both cultivars under salinity stress, in accordance with the improvements instomatal conductance, transpiration rate, and P_n of tomato leaves. The results presented here indicate that ALA controls aquaporin expression, thus, presumably ALA regulates water homeostasis and enhances salt tolerance of tomato seedlings.

Key words: Salinity stress; 5-aminolevulinic acid; Aquaporin; Tomato

INTRODUCTION

Salinity is one of the major threats to agricultural crop production worldwide. Soil salinity increases water potential in the apoplast of root cells and induces osmotic stress, thus, significantly reducing water uptake by roots. Plant growth and development is strongly dependent on the uptake and utilization of water. A number of reports have demonstrated that aquaporins play a vital role in regulating plant water movement and cellular osmotic potential under stress conditions (Srivastava et al., 2010; Liu et al., 2012). Different responses have been observed in the transcript levels of aquaporins under drought, salinity, and cold stress (Suga et al., 2002; Jang et al., 2004; Li et al., 2008), and these are thought to contribute to water transport and stress tolerance of plants. For example, over expression of a wheat aquaporin gene (*TaAQP8*) in transgenic tobacco enhanced plant salt tolerance (Hu et al., 2012). Meanwhile, the responses of aquaporin isoforms to phytohormones (gibberellic acid and abscisic acid) have been observed in many species, including radish (Suga et al., 2002), maize (Zhu et al., 2005), rice (Li et al., 2008), and so on. Thus, there may be a relationship between aquaporin expression and ALA treatment in improving tomato salt tolerance.

Adaption of plants to salinity stress is a genetically complex procedure. Numerous approaches have been used to alleviate salinity stress of various crops; one of which is the use of plant growth regulators (PGRs). In recent years, 5-aminolevulinic acid (ALA), a key precursor in the biosynthesis of all porphyrin compounds, including vitamin B12, chlorophyll, heme, and phytochrome, is considered to be a new PGR. ALA is reported to have the ability to promote plant growth and enhance stress tolerance, such as chilling (Korkmaz et al., 2010), drought (Liu et al., 2013), and salinity (Youssef and Awad, 2008; Naeem et al., 2012). Watanabe et al. (2000) evaluated12 different PGRs and found ALA to be the most effective in improving the salt tolerance of cotton. Thus far, research conducted on ALA has mainly focused on photosynthesis (Liu et al., 2013), chlorophyll accumulation (Memon et al., 2009), the antioxidant system (Nishihara et al., 2003; Sun et al., 2009), and ion uptake (Naeem et al., 2010). Although ALA has been reported to be capable of enhancing the salt tolerance of various plants, data on the molecular mechanism of exogenous ALA application are limited. Naeem et al. (2010) reported that spraying ALA on oilseed rape improved leaf water relations under salinity stress. Our previous research found that ALA could regulate the transcription of aquaporins in cucumber leaves and enhance the salt tolerance of the plants (Yan et al., 2014). Therefore, it is of interest to analyze the effects of ALA on the expression of aquaporins in tomato seedlings and to determine the interaction between aquaporin expression and water content of tomato leaves under different salinity conditions.

Tomato (*Solanum lycopersicum*) is one of the most popular vegetables in protected cultivation worldwide and is valued for its remarkable nutritional value. However, overcropping and fertilization exacerbate soil secondary salinization in facility cultivation, which dramatically reduces plant growth and fruit yield of tomato plants. Our previous research has found that foliar spray with a low concentration of ALA may enhance the salt tolerance of tomato seedlings. The objective of this research was to determine the effect of ALA on photosynthesis and plant growth of tomato seedlings under salinity stress, and to determine the effect of ALA on PIP aquaporin gene expression and its relationship with stomatal conductance (g_s) and transpiration rate (E) in tomato leaves. In gene expression studies, one can concentrate on only a few genes, which are seemingly of paramount relevance in salinity tolerance. Thus, the current research on salt tolerance focused on two aquaporins from the plasma membrane intrinsic proteins (PIPs) subgroup: *LePIP1* and *LePIP2*.

MATERIAL AND METHODS

Plant materials and stress treatments

Two tomato (*S. lycopersicum*) cultivars, cvs. Zhongza No. 9 (salt-sensitive) and Jinpeng No. 1 (relatively salt-tolerant), were used in this study. Seeds were surface-sterilized in 0.1% $HgCl_2$ for 5 min and washed with distilled water before sowing in washed commix medium (Xintiandi Co., Yangling, Shaanxi, China). When seedlings had four true leaves, seedlings of similar size were transplanted into plastic containers (12 plants per container) filled with 40 L of half-strength Hoagland nutrient solution (pH 6.5 ± 0.1, electrical conductivity: 1.4-1.8 dS/m, solution temperature: 20°-25°C) (Hoagland and Arnon, 1950) and aerated continuously with an air pump. Plants were grown in an environmentally-controlled greenhouse with day/night temperatures of 30° ± 2°/20° ± 2°C, a photoperiod of 14 h light (800 μmol photons·m^{-2}·s^{-1})/10 h dark, and relative humidity between 65 and 85%. The plastic containers were arranged in a randomized block design and the solution was renewed every four days.

After a two week acclimatization period, seedlings were separated into four groups: 1) control, sprayed with distilled water; 2) ALA, sprayed with 50 mg/L ALA (Sigma Chemical Co., St. Louis, MO, USA); 3) NaCl, treated in 100 mM NaCl and sprayed with distilled water; 4) NaCl+ALA, treated in 100 mM NaCl and sprayed with 50 mg/L ALA. The spraying treatment was applied between 7:00 and 8:00 a.m., both surfaces of all tomato leaves were sprayed with ALA solution or distilled water. The leaves and roots of tomato seedlings were collected in triplicate at 2, 4, 8, 12, and 24 h after treatment and frozen immediately into liquid nitrogen before storing at -80°C.

Analyses of chlorophyll content, net photosynthetic rate, and plant growth

Fully expanded mature leaves, used to measure chlorophyll content, were collected in triplicate on day 10 of the salt treatment. Leaf pieces (100 mg) were soaked in 10 mL of 80% aqueous acetone for 24 h in the dark. Chlorophyll content was calculated according to the method of Arnon (1949).

Net photosynthetic rate (P_n) was measured simultaneously with a LiCor-6400 portable photosynthesis system (LI-Cor Inc., Lincoln, NE, USA). All measurements were carried out with an internal light resource with PAR 800 μmol photons·m^{-2}·s^{-1}.

Dry and fresh weights of the tomato plants were measured 10 days after treatment. Six seedlings of each treatment were divided into shoots and roots and weighed immediately after harvesting and then dried in an oven at 75°C for 72 h and weighed again.

Analyses of stomatal conductance and transpiration rate

Stomatal conductance and transpiration rate were measured with a LiCor-6400 portable photosynthesis system (LI-Cor Inc., Lincoln, NE, USA) at 2, 4, 8, 12, and 24 h after salt treatment. All measurements were replicated six times, and the means ± SD were used for analysis.

RNA extraction

Total RNA from leaves and roots of tomato seedlings was extracted and purified using the RNA simple Total RNAKit (TIANGEN, China) following the manufacturer protocol. To remove any contaminating genomic DNA prior to cDNA synthesis, the RNA was treated with RNase-free DNAse I (Invitrogen, USA) following the manufacturer protocol, and the concentrations were accurately quantified spectrophotometrically by absorbance at 260 nm. The quality of RNA was assessed by calculating the ratio of A260/A280 nm. In order to monitor the integrity of RNA, total RNA samples were run on 1% agarose TAE gels and stained with ethidium bromide.

Real-time PCR

LePIP1 and *LePIP2* were acquired from the National Center for Biotechnology Information with the following Accession Nos. AY725511 and BT014251, respectively. The details of the primers used for real-time PCR are provided in Table 1 (He et al., 2011). The *actin-x* gene (X55749) was used as an internal control. Quantitative real-time PCR was performed as described previously (Yan et al., 2014). PCR amplification was performed using these primers and template cDNA under the following conditions: 1 cycle at 94°C for 3 min, followed by 40 cycles of 94°C for 30 s, 60°C for 30 s, and 72°C for 30 s (Table 1). The data were collected during the extension step (72°C for 30 s). Values for the cycle threshold (Ct) were determined using iQ5.0 software. Relative transcript levels were calculated as the ratio of AQP gene expression against *actin-x*. The data were analyzed using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001), the reactions were replicated three times, and the means ± SD were used for analysis.

Statistical analysis

All data presented are mean values for each treatment. Analysis of variance (ANOVA) was calculated using the SAS 8.1 software (SAS Institute, Cary, NC, USA). Significant differences among treatments were determined using the Duncan multiple comparison tests at the $P < 0.05$ level.

RESULTS

Effect of ALA on P_n, chlorophyll content, and biomass accumulation

P_n and chlorophyll content decreased rapidly for both tomato cultivars under salinity

conditions (Figures 1A and B). ALA treatment increased P_n by 27.72 and 31.37% for 'Zhongza No. 9' and 'Jinpeng No. 1', respectively, under salinity conditions. Meanwhile, the chlorophyll content of tomato seedlings was noticeably enhanced by exogenous ALA application, regardless of salinity.

The fresh and dry weights of shoots and roots (Figures 1C, D, E, and F) of tomato seedlings were significantly reduced after 10 days of salinity treatment compared with the control ($P < 0.05$). This effect was greater in 'Zhongza No. 9' (64.88, 59.07, 53.37, and 44.79%, respectively) than in 'Jinpeng No. 1'(36.21, 30.31, 28.25, and 27.59%, respectively). Exogenous ALA significantly alleviated the salt induced growth reduction in both cultivars. The dry weight of shoots and roots of 'Zhongza No. 9' were enhanced by 60.06 and 32.76%, respectively, and by 25.71 and 20.32% for 'Jinpeng No. 1', respectively. The results indicate that salinity stress remarkably inhibits the growth of both tomato cultivars, especially 'Zhongza No. 9', and treatment with ALA can significantly alleviate seedling damage.

Table 1. Primers used for RT-PCR analysis of gene expression in the roots and shoots of tomato seedlings.

Gene	Primer sequence (5' to 3')	Accession No.
LePIP1	F: ATGACGCACCCATACAAC R: TGCTAACAATGCTCCCAC	AY725511
LePIP2	F: AAGGATTACAAAGAGCCACC R: ACCAAAAGCCCAAGCAAC	BT014251
Actin-x	F: GATGGTGTCAGCCACAC R: ATTCCAGCAGCTTCCATTCC	X55749

F indicates forward and R indicates reverse.

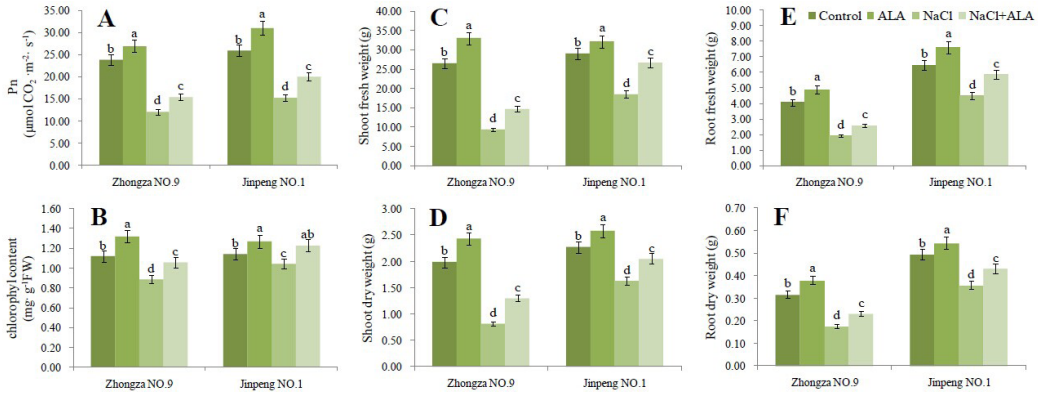

Figure 1. Effects of 5-aminolevulinic acid (ALA) on net photosynthetic rate (P_n) (**A**), chlorophyll content (**B**) and biomass (**C**, **D**, **E**, and **F**) of tomato seedlings subject to NaCl stress. Different letters denote statistically significant differences by the Duncan multiple comparison tests ($P < 0.05$).

Effect of ALA on stomatal conductance and transpiration rate

During salinity stress, g_s (Figures 2A and B) and E (Figures 2C and D) of tomato seedling leaves decreased drastically, reaching a minimum after 12 h of treatment. However, exogenous ALA noticeably increased g_s and E in both cultivars regardless of salinity, and more so in 'Jinpeng No. 1'.

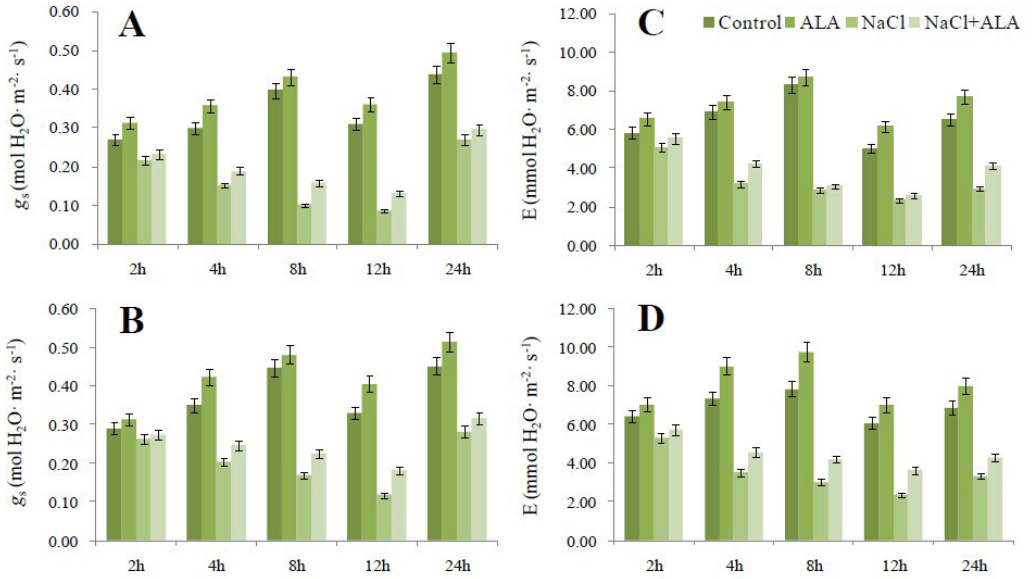

Figure 2. Effects of 5-aminolevulinic acid (ALA) on stomatal conductance (g_s) and transpiration rate (E) of tomato seedlings subject to NaCl stress. **A** and **C**, 'Zhongza No.9'; **B** and **D**, 'Jinpeng No.1'. Data are reported as means of six replicates ± SD.

Differential response of *LePIP1* and *LePIP2* genes to ALA treatment

The expression level of *LePIP1* in the leaves of both cultivars was up-regulated 2 h after ALA treatment and then returned to normal/decreased levels (Figure 3A). Similar patterns were observed in the expression of *LePIP2* in the leaves of both salt-sensitive and salt-tolerant tomato cultivars, both of which reached maximal levels at 2 h and decreased to normal levels after 4 h (Figure 3 B).

In the roots of tomato seedlings, the expression patterns were slightly different for both aquaporin genes. After the first 4 h of ALA treatment, *LePIP1* expression in 'Jinpeng No. 1' plants was induced by 2.1-fold of that in the control plants, and was subsequently down-regulated to normal levels at 8 h. *LePIP2* gene expression in 'Jinpeng No. 1' plants was almost the same as that of *LePIP1*. In the roots of the salt-sensitive cultivar (Zhongza No. 9) plants, both the *LePIP1* and *LePIP2* genes were down-regulated initially, followed by an increase after 8 or 12 h of ALA application, respectively, reaching maximum expression at 24h. Thus, the response and regulation of *LePIP1* and *LePIP2* genes to ALA treatment was slightly faster in the salt-tolerant tomato cultivar compared to the salt-sensitive cultivar (Figures 3C and D).

Differential response of *LePIP1* and *LePIP2* genes to salinity stress

Salinity stress resulted in increased levels of the *LePIP1* gene within the first 2 h (2.1-fold higher than that of the control), but remarkably expression decreased after 4 h of treatment in the leaves of 'Jinpeng No. 1' plants. In contrast, the transcription of *LePIP1* in the leaves of 'Zhongza No. 9' plants was initially depressed and then gradually increased by 2.9-

fold after 12 h of salinity stress. However, there were no significant changes in the transcript levels of the *LePIP2* gene in the leaves of both cultivars (Figures 4A and B).

During salinity stress, a remarkable 4.8-fold increase in the relative expression of the *LePIP1* gene was observed in the roots of 'Zhongza No. 9' plants at 12 h. Compared to the 'Zhongza No. 9' plants, the transcript level of *LePIP1* in the roots of 'Jinpeng No. 1' showed no significant changes (Figure 4 C). For *LePIP2* in the roots of 'Jinpeng No. 1', there was a slight increase in expression after treatment began and then expression was gradually down-regulated to normal levels. In the 'Zhongza No. 9' plants, expression decreased within 4 h of salt treatment, followed by an increase in expression, reaching maximum expression at 8 h (Figure 4D). The results indicate that under salinity stress, the transcript levels of both the *LePIP1* and *LePIP2* genes are higher in the salt-sensitive tomato cultivar than in the salt-tolerant cultivar.

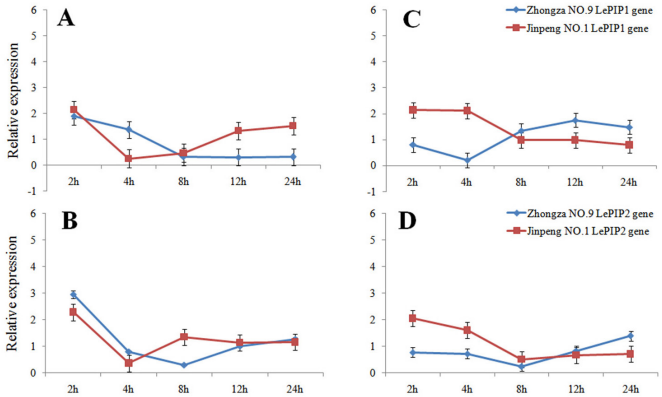

Figure 3. Relative expression of *LePIP1* (**A**, **C**) and *LePIP2* (**B**, **D**) genes in the leaves and roots of tomato seedlings exposed to 5-aminolevulinic acid (ALA) treatment. **A.** and **B.** Expression patterns of *LePIP1* and *LePIP2* genes in the leaves; **C.** and **D.** expression patterns of *LePIP1* and *LePIP2* genes in the roots. Amount of transcript was normalized to the tomato *actin-x* expression level. Data are reported as means of three replicates ± SD.

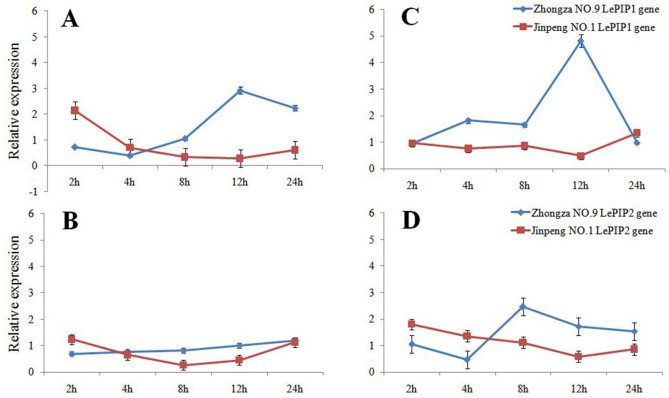

Figure 4. Relative expression of *LePIP1* (**A**, **C**) and *LePIP2* (**B**, **D**) genes in the leaves and roots of tomato seedlings subjected to NaCl stress. **A.** and **B.** Expression patterns of *LePIP1* and *LePIP2* genes in the leaves; **C.** and **D.** expression patterns of *LePIP1* and *LePIP2* genes in the roots. Amount of transcript was normalized to the tomato *actin-x* expression level. Data are reported as means of three replicates ± SD.

Differential response of *LePIP1* and *LePIP2* genes to ALA under salinity stress

As shown in Figure 5A, *LePIP1* gene expression in the leaves of 'Jinpeng No. 1' plants was up-regulated to 3.2-fold at 2 h following ALA application under salinity stress, after which there was a drastic decrease. In the 'Zhongza No. 9' plants, *LePIP1* gene expression was initially depressed and was then up-regulated to 1.7-fold following 4 h of ALA and salinity treatment. Subsequently, the expression level decreased rapidly. For *LePIP2* in the leaves of both cultivars, the transcript levels were almost the same as those under salinity treatment alone (Figure 5B).

Different trends in *LePIP1* expression levels were noted in the roots of salt-sensitive and salt-tolerant tomato cultivars. In the roots of salt-sensitive tomato cultivar plants, *LePIP1* expression increased rapidly by 3.4-fold of that in the control plants and peaked at 8 and 12 h. In the salt-tolerant plants, the transcript levels decreased after the treatment began, and then drastically increased by 4-fold (Figure 5 C). Similar to that of *LePIP1*, *LePIP2* also increased and peaked at 12 h in the roots of salt-sensitive tomato cultivar plants. By comparison, *LePIP2* was slightly down-regulated and showed little variation in the salt-tolerant tomato cultivar plants during the measurement time (Figure 5D).

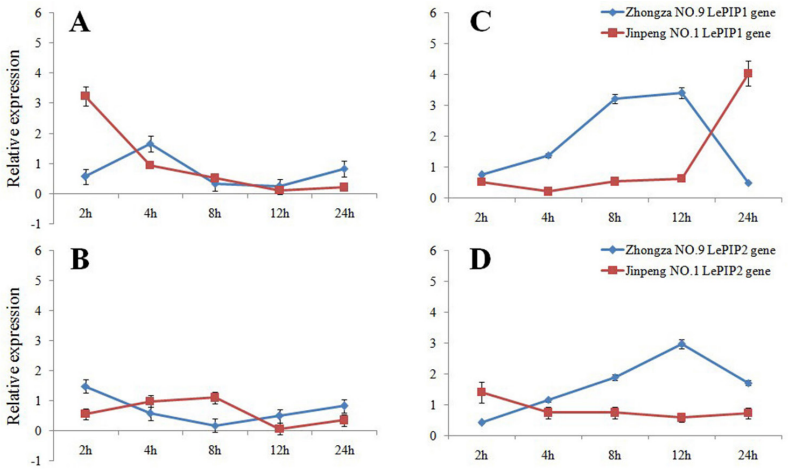

Figure 5. Relative expression of *LePIP1* (**A**, **C**) and *LePIP2* (**B**, **D**) genes in the leaves and roots of tomato seedlings subjected to NaCl and 5-aminolevulinic acid (ALA) stress. **A.** and **B.** Expression patterns of *LePIP1* and *LePIP2* genes in the leaves; **C.** and **D.** expression patterns of *LePIP1* and *LePIP2* genes in the roots. Amount of transcript was normalized to the tomato *actin-x* expression level. Data are reported as means of three replicates ± SD.

DISCUSSION

Soil salinity influences plant water up-take, photosynthesis and many other physiological metabolic activities, thus, strongly inhibiting plant growth. When salinity stress was induced in tomato seedlings, 'Jinpeng No. 1' plants demonstrated greater capacity for photosynthesis than 'Zhongza No. 9' plants. Compared with the control plants after 10 days of treatment, the declines in total chlorophyll content were less drastic in plants of the salt-tolerant 'Jinpeng No. 1'. In addition, further salinity tolerance was conferred to tomato seedlings following ALA treatment, as revealed by increasing P_n and chlorophyll content. Both the greater

P_n and the reduced chlorophyll degradation could be due to lower water loss from the leaves. Salt tolerance is usually assessed as the percent biomass production in saline versus control conditions (Munns, 2002). In this experiment, tomato plant growth was noticeably restrained by salinity stress; the fresh and dry weights of tomato seedlings were significantly reduced after 10 days of salinity treatment. Moreover, the decrease in weight was much more severe in 'Zhongza No. 9' plants compared to 'Jinpeng No. 1' plants. Exogenous ALA application remarkably reduced the damage caused by salinity stress and improved plant biomass accumulation in both tomato cultivars, which is consistent with previous research on cotton (Watanabe et al., 2000) and potato (Zhang et al., 2006).

Salinity stress decreases osmotic pressure dependent hydraulic conductance in melon roots (Carvajal et al., 2000). Likewise, root hydraulic conductance and E in citrus were remarkably reduced by long-term salt treatments (Rodriguez-Gamir et al., 2012). We also detected a marked reduction in g_s and E in tomato leaves when exposed to salinity stress. As expected, ALA application significantly increased the g_s and E of tomato leaves under control and stress conditions, especially in 'Jinpeng No. 1' plants. The same results have been observed in oilseed rape (Naeem et al., 2010). Previous research has also demonstrated the ability to improve g_s and E of watermelon and pakchoi plants under different conditions by exogenous ALA treatment (Sun et al., 2009; Memon et al., 2009). Furthermore, Naeem et al. (2011) reported that foliar spray with ALA improved relative growth rate, leaf osmotic potential, and relative water content in oilseed rape under salinity conditions.

The g_s and E are strongly related to leaf osmotic potential and relative water content. The results obtained for salinity treatment on pepper plants suggests that a decrease in g_s is in agreement with aquaporin functionality (Cabañero and Carvajal, 2007). Either, greater g_s and E or improved leaf osmotic potential and relative water content could be due to higher root water uptake. In our study, an obvious increase in g_s and E was observed following treatment with ALA, indicating one possible method of how tolerance was conferred to plants by ALA. Thus, we speculated on a reason for ALA improving g_s and E by analyzing the expression of related aquaporins in the leaves and roots of the tomato seedlings.

Higher g_s and E in leaves are beneficial for root water uptake and photosynthesis, and are, thus, beneficial for both plant growth and tolerance to abiotic stress. Aquaporins play an important role in plant water transport between cells and organelles and the medium under osmotic stress. A number of studies have established that aquaporins can be regulated significantly and differentially by various environmental stress conditions. For example, various aquaporin isoforms (of both the PIP1 and PIP2 subgroups) in roots of *Brassica juncea* demonstrated up-regulation upon salinity stress imposition, whereas they were down-regulated upon thiourea supplementation (Srivastava et al., 2010). Nevertheless, this down-regulation was found to contribute to a lower decline in the water retention ability of roots and was accompanied by transcriptional down-regulation under salinity conditions, hence, enabling plants to conserve water at an early stage of salinity stress. In contrast, transcription levels of both *LePIP1* and *LeTIP* genes in the roots were reduced under salt stress, and colonization of the tomato roots with arbuscular mycorrhizal fungi (AMF) distinctly enhanced this effect. However, colonization by AMF resulted in a drastic increase in the expression of *LePIP1*, *LePIP2*, and *LeTIP* genes in the leaves (Ouziad et al., 2006).

Water uptake and flow across cellular membranes is a fundamental requirement for plant growth and development. Aquaporins are known to significantly contribute to water movement in plants. Additionally, up- or down-regulation of aquaporin genes significantly

induces cellular membrane water permeability (Siefritz et al., 2001; Aharon et al., 2003), and either induced or suppressed regulation has been observed during osmotic stress. Katsuhara et al. (2003) observed that *HvPIP2*; *1* expression was decreased in the roots of barley under NaCl stress, while transcription levels of *HvPIP1*; *3* and *HvPIP1*; *5* remained unchanged. Recently, Li et al. (2008) reported that the expression of *OsTIP1*; *1*, *OsTIP1*; *2*, *OsTIP2*; *2,* and *OsTIP4*; *3* was considerably induced under 150 mM NaCl treatment, and the transcription levels of *OsTIP1*; *1* and *OsTIP1*; *2* increased by 3- to 12-fold. Research on radish seedlings suggests that NaCl stress results in up-regulation of *RsPIP2.1* expression, while polyethylene glycol treatment significantly down-regulates *RsPIP2.1* expression (Suga et al., 2002). Martre et al. (2002) utilized antisense RNA to depress transcription of *AtPIP1* and *AtPIP2* and found that transgenic plants showed a lower tolerance to water deficit. A previous study showed that *TaAQP* (a PIP1 subgroup gene) is up-regulated under salt stress and enhances salt tolerance in transgenic *Nicotiana tabacum* L. (Hu et al., 2012). However, over-expression of the *AtPIP1*; *2* gene in transgenic tobacco resulted in increased sensitivity to drought and salt stress (Aharon et al., 2003). Based on these results, it can be concluded that transcriptional regulation of aquaporins and their response to various environmental stimuli are very intricate procedures and depend on species, stress conditions, and evenplant organs. Differential expression levels of aquaporin isoforms may play important roles in water uptake and flow across cellular membranes under stress conditions. To date, research on the transcriptional regulation of aquaporins under salinity stress has mainly focused on PIPs and TIPs. Up-regulation of some of these isoforms could facilitate water permeability of membranes; meanwhile an increase in transcripts of aquaporins in plasma membranes may increase intracellular water loss, especially under stress conditions. Down-regulation of some aquaporin isoforms may play a role in limiting initial water loss during the early stages of salt stress and subsequently up-regulation by some aquaporins could enhance uptake of water and maintain better cellular water homeostasis in plants under high cellular salt conditions (Shao et al., 2008).

In our experiment, dynamic control of *LePIP1* and *LePIP2* transcript levels in leaves and roots of tomato seedlings were assayed after exposure to ALA, NaCl, and NaCl+ALA. The results indicate slight up-regulation in the expression of both *LePIP1* and *LePIP2* genes following spraying with ALA, which is in accordance with our previous research on cucumber (Yan et al., 2014). In addition, the response and regulation of *LePIP1* and *LePIP2* genes under ALA treatment was slightly faster and more stable in the salt-tolerant tomato cultivar compared to the salt-sensitive cultivar. When our tomato seedlings were subjected to salinity stress, numerous different responses were observed in both cultivars. *LePIP1* expression levels in the leaves of plants from 'Jinpeng No. 1' plants were up-regulated at 2 h, followed by a noticeable decrease, while the expression levels of *LePIP1* in roots and *LePIP2* in leaves and roots of 'Jinpeng No.1' plants did not show significant changes during the experimental period. By comparison, salinity stress remarkably induced the transcription of *LePIP1* in leaves and roots of 'Zhongza No. 9' plants by 2.9- and 4.8-fold, respectively, compared to control levels. The results demonstrate that transcripts of both the *LePIP1* and *LePIP2* genes in roots and the *LePIP1* gene in leaves of 'Zhongza No. 9' plants were higher than those in 'Jinpeng No. 1' plants, implying that the salt-sensitive species was more severely influenced by salinity. However, Liu et al. (2012) found that the salt-tolerant *Malus* species had relatively higher levels of aquaporin transcript. The differences in results may be due to the transcript differences between species. Our previous research has shown that low concentration ALA treatment can induce changes in *CsPIP1:1* and *CsNIP* transcriptional expression in cucum-

ber leaves subjected to NaCl stress, which is beneficial for maintaining water homeostasis in plants and is correlated with whole-plant resistance to salinity (Yan et al., 2014). In this study, the effect of ALA on salinity stressed tomato seedling aquaporin gene transcription was also investigated. Transcripts of *LePIP1* in leaves and roots of 'Jinpeng No. 1' plants were partially up-regulated by ALA application, while slight down-regulation was observed in the expression levels of *LePIP2*. For the salt-sensitive tomato cultivars, ALA may delay and counteract the up-regulated expression of *LePIP1* and *LePIP2* genes in both leaves and roots of tomato seedlings subjected to salinity stress. These results are partially inconsistent with the results observed for cucumber (Yan et al., 2014). The differences in *LePIP1* and *LePIP2* gene expression between 'Jinpeng No. 1' and 'Zhongza No. 9' plants under salinity conditions may play important roles in determining the salinity tolerance of the plants. Higher expression levels of PIPs and lower g_s and E was detected in salt-sensitive cultivars under salinity conditions, implying *LePIP1* and *LePIP2* may mediate stress sensitivity by enhancing water loss in the plant. Hence, the ability to maintain better water homeostasis by ALA application presumably enabled our tomato plants to tolerate those salinity conditions and accumulate more chlorophyll and biomass. However, many more studies need to be conducted to investigate the expression patterns of each aquaporin gene to determine the molecular mechanism by which ALA enhances plant salt tolerance and its relationship with water transport.

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