Effect of Salicylic Acid on Oxidative Stress Caused by NaCl Salinity in Lycopersicum Esculentum Mill

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ABSTRACT
Of the effects of salinity stress is the production of Reactive Oxygen Species (ROS) and creation of oxidative stress, where ROS rapidly attack all kinds of biomolecules such as nucleic acids, proteins, lipids and amino acids and lead to abnormal metabolic function. Salicylic acid reduces the effects of oxidative stress. In this research, first, seeds were cultured in the pots containing perlite and then they were put in a growth room under controlled conditions at 27±2 and 23±2°C in light (16 h) and dark (8 h) conditions, respectively with luminous intensity of 15 klx and moisture of 75%. Five concentrations of NaCl including 0, 25, 50, 75 and 100 nM and 4 concentrations of salicylic acid including 0, 1.5 and 1.5 mM were used. Then, peroxidation rates of lipids, ascorbic acid and dehydroascorbic acids were measured to study the effects of oxidative stress. High salinity significantly increased malondialdehyde rate. At different salinity levels, a decrease in other aldehydes was found and salicylic acid decreased its effect in most treatments. Content of ascorbic and dehydroascorbic acids increased by salinity and its salicylic acid was reduced.

Key words: Lycopersicum esculentum mill, oxidative stress, NaCl salinity, salicylic acid

INTRODUCTION
If Electric Conduction (EC) of soil water solution is above 4 ds m⁻¹ (almost equivalent to 40 mM NaCl), the soil is called saline. Salinity of the resulting soil occurs due to either natural stages or irrigation of products by saline water in most of arid and semiarid areas of the world which prevents the plant from growing and yielding; in the worst case, it causes death of plants (Meloni et al., 2004). Salinity stress leads to oxidative stress and production of Reactive Oxygen Species (ROS); accumulation of free radicals of H₂O₂ and OH damages biomolecules and membranes. But, plants have enzymatic and non-enzymatic antioxidant defense system in organelles of each cell. Catalase, peroxidase, superoxide dismutase are enzymatic antioxidants and metabolites with low molecular weight (ascorbate, glutathione, phenol compounds, tocopherols and carotenoids) are non-enzymatic ones. These antioxidants play the detoxification role for ROS in ascorbate-glutathione cycle (Jithesh et al., 2006).

Salicylic acid is a plant phenol and today it is studied as an internal regulating hormone. Its role has been well proved in defense mechanisms against living and non-living stresses and it participates in the adjustment of physiological stages of the plant. Furthermore it significantly affects different aspects of a plant life such as its growth and development, photosynthesis, evaporation, absorption and ion transfer (Sakhabutdinova et al., 2005). For instance, pretreatment
with salicylic acid increases hydrogen peroxide in banana. Although, \( \text{H}_2\text{O}_2 \) is poisonous in high concentrations its low concentrations can play the role of messages in message transmission processes and activate genes associated with resistance in the plant. The activity of antioxidant enzymes is directly or indirectly increased by salicylic acid (Hung et al., 2005).

Since the most important agent for soil salinity is sodium chloride its study seems necessary. In this research, the effect of salicylic acid on tomato (\textit{Lycopersicon esculentum} Mill. cv. Rio grand) was investigated under NaCl salinity stress.

**MATERIALS AND METHODS**

**Pot culturing:** First, the seeds were disinfected by sodium hypochlorite and then 5 seeds were cultured in each pot containing perlite in a growth room under controlled light (16 h) and dark (8 h) conditions at 27±2 and 23±2°C with luminous intensity of 15 klx and moisture of 75%, respectively. Then, the pots were irrigated for one month (until the plant reached growth stage of three or four leaves) with food solution at the pH of 6.5 every other day and with distilled water (50 mL from each). Then, the treatment stage started.

**Treatment method in the pot:** In this test, 5 concentrations of NaCl including 0, 25, 50, 75 and 100 mM and 4 concentrations of salicylic acid including 0, 1.5 and 1.5 mM were used. In a design, factorial tests including 20 types of treatments, each with 3 iterations (totally 60 pots) were performed.

In the treatment stage, first, salicylic acid was sprayed on leaves every other day for 2 weeks and then saline treatments were used with food solution in the volume of 50 mL every other day for 15 days.

**Measuring peroxidation of lipids:** Measuring concentration of malon deialdehyde (MDA) (Heath and Packer, 1968): According to this method, 0.2 g fresh tissue of a leaf was weighed and ground in a porcelain mortar containing 5 mL of Trichloro acetic acid (TCA) 0.1%. The extract was centrifuged using the centrifuge (Model: Napeo2028K) for 5 min at 10000 g. Four milliliter of 20% solution of TCA containing 0.5% of Thiobarbituric Acid (TBA) was added to 1 mL of supernatant obtained by centrifugation. The mixture was heated in the hot water bath at 95°C for 30 min. Then it was immediately cooled in ice and the mixture was centrifuged at 10000 g for 10 min. Absorption intensity of this solution was read using a spectrophotometer at wavelength of 532 nm. The considered matter for absorption in this wavelength (MDA-TBA) was a red complex. Absorption of other non-specific pigments was determined at 600 nm and was deducted from this value. In order to calculate the concentration of MDA, extinction coefficient equivalent to 155 cm mM\(^{-1}\) was used. The results obtained from measurement were calculated and presented in terms of fresh weight.

**Measuring other aldehydes (propanol butanal, hexanan, heptanal and propanol demethyl acetate):** The work procedure and test stages were similar to the measurement of aldehydes, except for the fact that its absorption intensity was read at the wavelength of 455 nm. Absorption of other non-specific pigments was read at 600 nm and was deducted from this value. In order to calculate the concentration of these aldehydes, extinction coefficient equivalent to 0.458×105 cm mM\(^{-1}\) was used. This extinction coefficient was the mean extinction coefficient for the five considered aldehydes. The obtained results were calculated and presented in terms of fresh weight (Meirs et al., 1992).
Measuring ascorbic acid and dehydroascorbic acids: In order to measure the rate of ascorbic acid and dehydroascorbic acids, 0.5 g fresh leaf tissue was weighed, ground in 10 mL metaphosphoric acid and centrifuged at 10000 g for 15 min (De Pinto et al., 1999).

Measuring method of ascorbic acid: In order to measure ascorbic acid, 0.3 mL of centrifuged extract was poured into the test tube and the required solutions were added in the following manner: first, 750 µL potassium phosphate buffer was added to it. Then, 300 µL of distilled water was added. The mixture was completely blended using vortex and placed at room temperature for 10 min. Then, 600 µL of TCA was added and then 600 µL of orthophosphoric acid was added (this stage was performed under a hood). Then, 600 µL of alpha-alpha depyridyl and then 10 µL of FeCl₃ were also added. The mixture was well blended using vortex and was put in hot water bath at 40°C for 20 min. Test tube was taken out of the hot water bath its content was mixed again with vortex and placed in hot water bath at 40°C for 20 min. Absorption intensity was read at wavelength of 525 nm. In order to set the spectrophotometer, the solution which contained 300 µL of 5% metaphosphoric acid and other solutions, was used instead of the plant extract as the control (De Pinto et al., 1999).

Measuring method of dehydroascorbic acid: In order to measure this matter, 300 µL of the centrifuged extract was taken according to the previous method and 750 µL of the potassium phosphate buffer was added to it. Other stages were performed as follows: 150 µL of the Dithiotheritol DTT was added and the mixture was put at room temperature for 10 min. Then, 150 µL of N-ethyl malamid was added to it and the mixture was well blended using vortex and placed at room temperature for 10 min. Other stages (from adding TCA on) were performed like the ones for dehydroascorbic acid. At the end, absorption intensity was determined at wavelength of 525 nm. In order to set the spectrophotometer, the solution which contained 300 µL of 5% metaphosphoric acid and other solutions, was used instead of the plant extract as the control. In order to calculate the concentration of ascorbic acid and dehydroascorbic acid, the standard curve relating to each one of these matters was used. Measurement results were calculated and presented in terms of mg g⁻¹ fw (De Pinto et al., 1999).

Statistical analysis: Statistical analysis was performed randomly in this research and 3 iterations were considered for each treatment. The means were compared using SPSS software and Duncan test with the confidence of 95%. In order to evaluate the effect of two treatments on the measured attribute, all the obtained data were analyzed using ANOVA in SPSS software.

RESULTS
Peroxidation of leaf membrane lipids: Only salinity had a significant effect on the content of malon deialdehyde so that salinities of 50, 75 and 100 mM caused a significant increase of deialdehyde malon (Fig. 1). Interaction of salinity and salicylic acid on the content of other aldehydes was also significant. Different salinity levels of other aldehydes were lower than those of the control. SA increased other aldehydes rates at zero salinity level and decreased other aldehydes at other levels, except for concentration of 1.5 SA at the level of 50 mM and concentrations of 1 and 1.5 mM at 100 mM (Fig. 2).

Ascorbic acid: Salinity caused a significant increase of ascorbic acid in the leaf. In studying the simultaneous effect of salinity and salicylic acid at all salinity levels, salicylic acid decreased ascorbic
Fig. 1: Effect of NaCl salinity on concentration of malon dealdehyde

Fig. 2: Effect of the interaction of NaCl salinity with salicylic acid (SA) on concentration of other aldehydes

Fig. 3: Effect of the interaction between NaCl salinity and salicylic acid (SA) on content of ascorbic acid of leaf

acid. This decrease was found in concentrations of 0.5 and 1 mM in the salicylic acid (Fig. 3). Simple effects of SA and salinity on the content of root ascorbic acid were significant so that there was a

Fig. 4(a-b): (a) Effect of NaCl salinity on content of ascorbic acid of root and (b) Effect of salicylic acid (SA) on content of ascorbic acid of root

Fig. 5(a-b): (a) Effect of NaCl salinity on content of dehydroascorbic acid of leaf and (b) Effect of salicylic acid (SA) on content of dehydroascorbic acid of leaf

significant increase in ascorbic acid of the root at concentrations of 75 and 100 mM. Twenty-five and 50 mM of salinity did not cause a significant difference in ascorbic acid of root compared with the control (Fig. 4a). In studying the SA effect on ascorbic acid of root (Fig. 4b), different concentrations of SA caused a significant decrease in ascorbic acid of root compared with the control.

**Dehydroascorbic acid:** Only simple effects of salinity and SA on the rate of ascorbic acid of leaf were significant so that studying salinity effect (Fig. 5a) showed that all salinity concentrations caused a significant increase in dehydroascorbic acid. Considering Fig. 5b, SA at its different concentrations caused a decrease in dehydroascorbic acid of leaf. Interaction of salinity and SA on dehydroascorbic acid of root was also significant. Figure 6 shows that salinity at concentrations of 75 and 100 mM caused a significant increase in dehydroascorbic acid of root and SA decreased all salinity levels at all levels, except in zero mM of salinity and there was increase in 1 mM of SA. The highest decrease of dehydroascorbic acid was found at concentration of 1.5 mM of salicylic acid.

**DISCUSSION**

**Peroxidation of membrane lipids:** Superoxide radicals produced by environmental stresses cause peroxidation of lipids (Sairam and Tyagi, 2004). The result of peroxidation of membrane lipids includes compounds such as malon dialdehyde, propanol butanal, hexanen, heptanal and
propyl dimethyl acetal. These matters are used as an indicator for measuring lipid peroxidation. An increase in lipid peroxidation is regarded an indicator of oxidative stress Heath and Packer (1968) and Meirs et al. (1992). Oxidative loss can be measured by changes in lipid peroxidation Borsani et al. (2001). Antioxidant defense system (ascorbate, glutathione, carotenoids and other isoprenoids, flavonoids and enzymatic antioxidants) helps plants in environmental stresses. SA-pretreated tomato causes more accumulation of putrescine, spermine and spermidine which reduces oxidative losses and protects the membranes under salinity stress (Szepesi, 2006). There are pieces of evidence which show that SA increases antioxidant power by affecting H₂O₂ and protects the plant against oxidative stress (Johnston et al., 2002). Some tests have shown that SA increases NaCl-induced lipid peroxidation. Salinity and SA both increase gene expression of peroxidase glutathione enzyme (Borsani et al., 2001).

In the present research on tomato, salinity increased content of malon dialdehyde and decreased content of other aldehydes at different salinity levels and in SA treatment. α-tocopherol is known to interact with oxygen and oxidized lipids to stabilize them against other oxidative interactions. It has been proved that SA treatment decreases lipid peroxidation (Khodary, 2004). A decrease in lipid peroxidation is a photosynthetic protection which leads to the improvement of cellular diffusion. Studies have shown that SA prevents damage of unsaturated fatty acids, decreases membrane permeation and protects thylakoid membrane at the time of salinity in Arabidopsis thaliana. In addition, SA decreases malon dialdehyde produced in salinity stress in the leaves and roots of barley (El-Tayeb, 2005).

**Ascorbic acid and dehydroascorbic acid:** Ascorbic acid is a strong antioxidant which is found in many plant cells, organelles and apoplastic and acts as the reducer of many free radicals while performing this action directly through the reduction of radicals or indirectly through reduction of alpha tocopherol. About 90% of this acid exists in its reduced form in leaves and chloroplasts under physiological conditions and its intracellular concentration is at the mM level (Yordanova et al., 2003). Use of ascorbic acid causes reduction of Reactive Oxygen Species (ROS) resulting from salinity stress in Arabidopsis thaliana (Borsani et al., 2001). In a research on Lemna minor it was found that salinity caused an increase in non-enzymatic antioxidants of ascorbate and glutathione in roots (Panda and Upadhyay, 2003). Tomato pretreatment under salinity stress with SA increases
antioxidative mechanisms and enhances activity of root peroxidase ascorbate (Szepesi, 2006). Other studies have shown that salicylic acid activates many enzymes of catalase, peroxidase, superoxide dismutase and peroxidase ascorbate (Hung et al., 2005).

Moreover, studies have shown that SA treatment in short term increases ascorbate level in Sinapis arvensis and dehydroascorbate level while long term treatment of SA increases rate of ascorbate and dehydroascorbate compared with the control (Dat et al., 1998). In the present research, salinity caused an increase in ascorbic acid of leaf which was decreased by SA. In root, high concentration of salinity caused an increase in ascorbic acid. In studying the effect of SA, higher concentrations of this hormone caused more reduction. Salinity and SA increased and decreased dehydroascorbate acid of leaf, respectively and while studying the effect of treatments on dehydroascorbate acid of root, high concentrations of salinity (75 and 100 mM) increased it which was reduced by salicylic acid. An increase in dehydroascorbate acid can indicate an increase in activity of the enzymes involved in oxidation-reduction cycle of ascorbic acid and an increase in ascorbic acid is probably related to the increase of total ascorbic acid of the plant.

CONCLUSION

This study showed that Salicylic acid reduces the effects of oxidative stress Caused by NaCl Salinity in Lycopersicum Esculentum Mill.

REFERENCES


