

THE ALLEVIATION OF THE ADVERSE EFFECTS OF SALT STRESS IN THE TOMATO PLANT BY SALICYLIC ACID SHOWS A TIME- AND ORGAN-SPECIFIC ANTIOXIDANT RESPONSE

IRMA TARI^{*}, JOLÁN CSISZÁR, EDIT HORVÁTH, PÉTER POÓR, ZOLTÁN TAKÁCS,
AND ÁGNES SZEPESI

*Department of Plant Biology, University of Szeged,
Közép fasor 52, H-6726, Szeged, Hungary*

Received January 8, 2015; revision accepted March 14, 2015

In the last decade contradictory results have been published as to whether exogenous salicylic acid (SA) can increase salt stress tolerance in cultivated plants by inducing an antioxidant response. Salt stress injury in tomato was mitigated only in cases when the plant was hardened with a high concentration of SA ($\sim 10^{-4}$ M), low concentrations were ineffective. An efficient accumulation of Na^+ in older leaves is a well-known response to salt stress in tomato plants (*Solanum lycopersicum* cv. Rio fuego) but it remains largely unexplored whether young and old leaves or root tissues have a distinct antioxidant status during salt stress after hardening with 10^{-7} M or 10^{-4} M SA. The determination of superoxide dismutase (SOD) and catalase (CAT) activity revealed that the SA-induced transient increases in these enzyme activities in young leaf and/or root tissues did not correlate with the salt tolerance of plants. Salt stress resulted in a tenfold increase in ascorbate peroxidase (APX) activities of young leaves and significant increases in APX and glutathione reductase (GR) activities of the roots hardened with 10^{-4} M SA. Both total ascorbate (AsA) and glutathione pools reached their highest levels in leaves after 10^{-7} M SA pre-treatment. However, in contrast to the leaves, the total pool of AsA decreased in the roots under salt stress and thus, due to low APX activity, active oxygen species were scavenged by ascorbate non-enzymatically in these tissues. The increased GR activities in the roots after treatment with 10^{-4} M SA enabled plants to enhance the reduced glutathione (GSH) pool and maintain the redox status of AsA under high salinity, which led to increased salt tolerance.

Key words: Antioxidant enzymes, ascorbate, glutathione, salicylic acid, salt stress, tomato.

INTRODUCTION

Salt stress is one of the most important environmental factors responsible for the reduced yield of cultivated plants. The exposure of plants to high salinity induces osmotic and ionic stress as well as the formation of reactive oxygen species (ROS), which accelerate lipid peroxidation, protein carbonylation and DNA damage (Munns, 2008). Hence, the excess of ROS must be eliminated and their removal from tissues is accomplished by several antioxidant enzymes and non-enzymatic antioxidants. Sensitivity to salinity stress varies among plant species and it is specific for plant organs and the developmental stage (Shalata et al., 2001).

Several studies have supported the major role of salicylic acid (SA) in mediating the response of plants to abiotic and biotic stress by the induction of antioxidant defense. The cellular antioxidant sys-

tems consist of non-enzymatic components including ascorbic acid (AsA), glutathione (GSH) and tocopherol and antioxidant enzymes generating or scavenging H_2O_2 such as superoxide dismutase (SOD) or catalase (CAT), ascorbate peroxidase (APX), guaiacol peroxidase (POD) and glutathione reductase (GR). AsA is regenerated from its oxidized forms, monodehydroascorbate (MDHA) and dehydroascorbate (DHA) either non-enzymatically at the expense of reduced glutathione (GSH) or via coordinated enzymatic reactions of ascorbate-glutathione cycle, which includes MDHA-, DHA- and glutathione disulfide (GSSG) reductase (MDHAR, DHR and GR) (Asada, 1999; Foyer and Noctor, 2011). The antiox-

Abbreviations: APX – ascorbate peroxidase; AsA – ascorbic acid; CAT – catalase; DHA – dehydroascorbate; DHAR – dehydroascorbate reductase; GR – glutathione reductase; GSH – reduced glutathione; GSSG – oxidized glutathione; MDA – malondialdehyde; O_2^- – superoxide anion radical; ROS – reactive oxygen species; SA – salicylic acid; SOD – superoxide dismutase.

^{*}e-mail: tari@bio.u-szeged.hu

idant mechanisms have been found in almost all cellular compartments that proceed via the activity of various isoenzymes (Kocsy et al., 2013).

Treatment with exogenous SA has been shown to decrease the harmful effect of abiotic stresses, such as high salinity (Tari et al., 2002a). The effect of SA depends not only on the concentration but on plant species, developmental stage or mode of application (reviewed by Horváth et al., 2007).

SA alleviates abiotic stress-induced damage by eliciting oxidative stress, which enhances the expression and activity of redox-controlled antioxidant enzymes (Ananieva et al. 2004; Li et al., 2013; Csiszár et al., 2014). SA may interact with ROS and regulate cellular redox homeostasis, which leads to changes in NPR1 transcription factor activity and defense gene expression (Strauss et al., 2010). The putative mechanism of SA action was the inhibition of CAT, one of the most important enzymes participating in H_2O_2 elimination (Dat et al., 1998). This led to H_2O_2 accumulation in tissues, though the effect proved to be isoenzyme-specific in maize (Horváth et al., 2002) or transient in cereals (Janda et al., 2003). It was also found that both APX (Durner and Klessig, 1995) and CAT inhibition could be involved in SA action (Horváth et al., 2002), however the effect of SA depended on the mode of application. In cucumber plants, foliar spray with 1 mM SA increased the photosynthetic efficiency, SOD and CAT activity under heat stress and as a result H_2O_2 was efficiently removed from leaf tissues. When SA was applied to the nutrient solution CAT activity was inhibited, which resulted in serious oxidative damage to plants subjected to high temperature (Shi et al., 2006).

The improvement of salt tolerance by exogenous SA is inconsistent. Borsani et al. (2001) found that greater oxidative damage occurred under salt stress in the wild type than in the SA-deficient transgenic *Arabidopsis* line expressing a salicylate hydroxylase (NahG) gene. This result suggests that SA may enhance the stress injury in plants exposed to high salinity. In wheat, exogenous SA counteracted the salt stress-induced growth inhibition in a salt tolerant genotype, but no improvement occurred in a salt sensitive wheat cultivar (Arfan et al., 2007).

In most of the cases, hardening with SA to a subsequent abiotic stress was investigated in short-term experiments (Wang et al., 2005; Shi et al., 2006). However, our knowledge about oxidative stress and antioxidant response during salt stress after a long-term SA pre-treatment is incomplete. Moreover, most of the earlier studies focused on the antioxidant response of leaves irrespective of their position on the stem although the Na^+ accumulation and the antioxidant status can be different in young and old leaves. Since the major effect of salinity stress is the reduction of root growth, the first level

of defence therefore has to be induced in the root system and the antioxidant status of roots plays a pivotal role in acclimation.

The objective of this study was to elucidate the time- and organ-specific induction of oxidative defense responses to high salinity in tomato hardened with various concentrations of SA. In our earlier work it was found that pre-treatment with 10^{-7} or 10^{-4} M SA for a long-term period resulted in an osmotic adjustment (Szepesi et al., 2005; 2009). This osmotic adaptation in concert with the time- and tissue-specific accumulation of sugars (Poór et al., 2011), sorbitol (Tari et al., 2010), abscisic acid and polyamines (Szepesi et al., 2009) contributed to the increased salt tolerance of plants after a pre-treatment with 10^{-4} M SA, but not with 10^{-7} M SA. SA stimulated pre-adaptation status was beneficial in the acclimation to subsequent salt stress via reducing malondialdehyde (MDA) content (Szepesi et al., 2009) and decreasing the accumulation of H_2O_2 in the leaves or ROS and nitric oxide generation in root apices after salt exposure (Gémes et al., 2011).

However, the role and the mechanism of antioxidant defense at the whole plant level have not yet been completely clarified. The objective of this work was to study the changes in enzymatic and non-enzymatic antioxidant systems during salt stress induced by 100 mM NaCl in young as well as in adult leaves and roots of the tomato plant after long-term pre-treatment with low (10^{-7} M) and high (10^{-4} M) SA concentrations. The question is whether the acclimation to high salinity can be characterized by changes in certain antioxidant enzymes or non-enzymatic antioxidants after SA pre-treatments or if it is the total antioxidant response that has to be measured and compared in various organs and tissues as a function of time and SA concentration. The information obtained from this study could improve our knowledge about the oxidative stress response of plants after successful chemical hardening.

MATERIALS AND METHODS

PLANT MATERIAL AND GROWTH CONDITIONS

Seeds of tomato plants (*Solanum lycopersicum* cv. Rio Fuego) were germinated at 26°C for 3d in the dark, and the seedlings were subsequently transferred to perlite for 2 weeks and then to aerated modified Hoagland nutrient solution as described earlier (Poór et al., 2011). The nutrient solution was renewed every second day. Plants were grown in a controlled environment under 300 $\mu\text{mol m}^{-2} \text{s}^{-1}$ light intensity and 12/12-h light/dark period, with a 24/22°C day/night temperature and 55-60% relative humidity. Prior to being subjected to salinity stress,

the plants were pre-treated for three weeks with 10^{-7} and 10^{-4} M SA. Salt stress was imposed by transferring the plants into the hydroponic culture containing 100 mM NaCl. The samples were prepared from the second, fully expanded young leaves (7th leaf), from the basal, old leaves (3rd leaf) and roots in three replicates 7 d after the salt treatment. The experiments were repeated 3–5 times and the samples were taken between 10–12 a.m.

ENZYME ASSAYS

The plant tissue was homogenized on ice at a ratio of 1:3 (w:v) of fresh weight and 50 mM phosphate buffer (pH 7.0), containing 1 mM EDTA, 1 mM phenylmethylsulfonyl fluoride (PMSF) and 1% (w:v) polyvinyl-pyrrolidone. For the APX assay, 1 mM ascorbate was added to the extraction buffer. The homogenate was filtered through two layers of cheese-cloth and centrifuged for 20 min at 12,000 g, at 4°C and the supernatant was used for enzyme activity assays. SOD (EC 1.15.1.1) activity was determined by measuring the ability of the enzyme to inhibit the photochemical reduction of nitroblue-tetrazolium (NBT) in the presence of riboflavin in the light (Beauchamp and Fridovich, 1971). CAT (EC 1.11.1.6) activity was determined by the decomposition of H_2O_2 and was measured spectrophotometrically by following the decrease in absorbance at 240 nm (Aebi, 1984). GR (EC 1.6.4.2) activity was determined by measuring the absorbance increment at 412 nm when 5,5'-dithio-bis(2-nitrobenzoic acid) (DTNB) was reduced by GSH, generated from glutathione disulfide (GSSG) (Csiszár et al., 2004). APX (EC 1.11.1.11) activity was assayed according to the method of Nakano and Asada (1987). The hydrogen peroxide-dependent oxidation of ascorbate was followed by a decrease in the absorbance at 290 nm ($\epsilon=2.8 \text{ mM}^{-1} \text{ cm}^{-1}$). APX activity was expressed as $\mu\text{mol ascorbate oxidized min}^{-1}\text{g}^{-1}$ fresh mass (FM). All assays were done at 4°C and results were expressed on fresh mass basis.

ASCORBATE EXTRACTION AND DETERMINATION

Ascorbate was extracted and assayed according to Law et al. (1983). One gram of fresh leaf or root material was ground with a mortar and pestle with 3 mL of 5% trichloroacetic acid (TCA). The homogenate was centrifuged at 6000 rpm for 20 min at 4°C. The 100 μL aliquot of the supernatant was transferred to an Eppendorf tube. To assay total ascorbate, 100 μL of 10 mM dithiothreitol (DTT) was added; after 10 min of incubation at room temperature 100 μL of 0.5% (w:v) N-ethylmaleimid (NEM) was added to remove the excess of DTT. The reduced AsA sample was diluted with 200 μL of water instead. Five-hundred microliters of 10% (w:v)

TCA was added to both samples. To determine ascorbate, this extract was mixed with 400 μL of 43% (v:v) H_3PO_4 , 400 μL of 4% (w:v) 2-dipyridyl 200 μL 3% (w:v) $FeCl_3$ and incubated for 60 min. Ascorbate concentrations were determined spectrophotometrically at 525 nm. DHA content was calculated as the difference between the concentration of total and reduced ascorbate.

GLUTATHIONE EXTRACTION AND DETERMINATION

Leaf or root samples (1g FM) were ground with a mortar and pestle in liquid nitrogen and extracted with 5 mL of cold 5% TCA. The homogenate was centrifuged at 6000 rpm for 20 min at 4°C and the supernatant was used for the assay. One hundred microliters of extract were transferred to an Eppendorf-type tube and 100 μL of H_2O (total glutathione assay) or 2-vinylpyridine to mask GSH (GSSG assay) was added. Samples were mixed and incubated for 1h at 25°C. Total and oxidized glutathione concentrations were determined spectrophotometrically at 405 nm using an enzymatic assay (modified method of Griffith, 1980). The reaction mixture contained 0.1 M phosphate buffer pH 7.5, 1 mM 5,5'-dithiobis(2-nitrobenzoic acid) DTNB, 1 mM NADPH, 1 U of glutathione reductase (GR baker's yeast, Sigma) and 100 μL of the tissue extract obtained as described above in 1 mL volume. GSH content was calculated from the difference between the concentration of total and oxidized glutathione. Standard curves were obtained for total glutathione and GSSG within the 0–2 μM range.

STATISTICAL ANALYSIS

The experiment was repeated at least three times. Statistical analysis was carried out with SigmaPlot 11.0 statistical software (SigmaPlot, Milano, Italy). The data were subjected to an analysis of variance (ANOVA) and the differences between the means were compared by Fisher's least-significant differences test (LSD). All data presented are means \pm SE.

RESULTS

10^{-7} M SA pre-treatment induced about 150–200% increases in the SOD activity of young leaves and roots after a two-day incubation, while 10^{-4} M SA concentration caused only 50–70% enhancement in young leaves (Fig. 1). Salt stress activated SOD to a very high extent in young leaves and roots of control plants, which was further enhanced in roots pre-treated with 10^{-7} M SA, but a smaller induction could be measured in these organs after 10^{-4} M SA pre-treatment (Fig. 1).

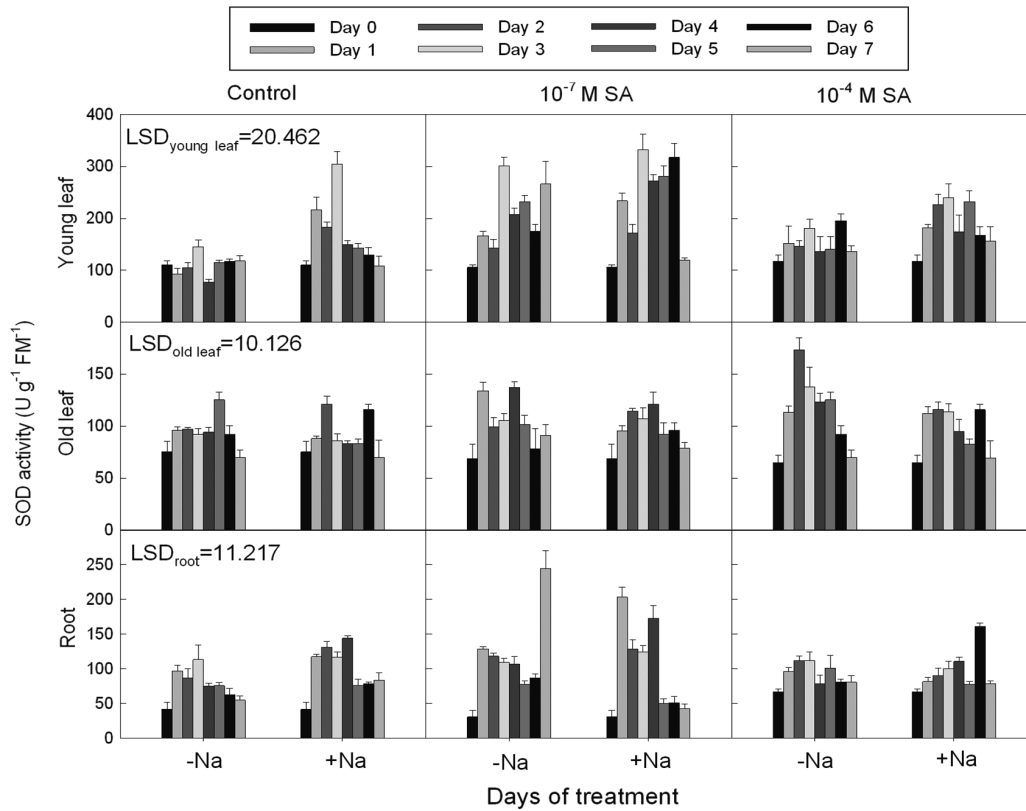


Fig. 1. Effect of salt stress induced by 100 mM NaCl on SOD activity in the young and basal leaves and roots of tomato plants subjected to 10^{-7} M or 10^{-4} M salicylic acid pre-treatments. Bars represent means \pm SE (n=9), and the differences between means were compared using Fisher's least significant difference (LSD) (P=0.05).

In accordance with most of the data in the literature, CAT activity fluctuated even in control samples as a function of time and exhibited a slight decrease to the end of the experiment in young leaves after SA treatments compared to the untreated control. However, the enzyme activities were stimulated both in the absence and in the presence of 100 mM NaCl in old leaves (Fig. 2). Time-dependent changes in CAT activity were much more pronounced in the roots. After 10^{-7} M SA pre-treatment a further increase in CAT activity was observed in the roots when plants were exposed to salt stress but this change was diminished at the end of the stress period. In contrast, plants pre-treated with 10^{-4} M SA could maintain similar CAT activity in the roots as salt-treated control up to the end of the experiment (Fig 2).

Compared to the control, leaves pre-treated with 10^{-7} M SA did not show significant changes in APX activity in the absence or in the presence of 100 mM NaCl but 10^{-4} M SA pre-treatment induced obvious stimulation in young and old leaves (Fig. 3). Significant increases in APX activity were found in young leaves of salt-stressed plants, which cannot be observed in old leaves. Moreover, much higher enzyme activities could be detected in the roots than in the leaf

tissues even in control plants, which were further enhanced by salt stress. In contrast to plants hardened with low SA concentration a gradual increase in APX activity could be observed upon exposure to 100 mM NaCl in plants hardened with 10^{-4} M SA (Fig. 3).

Higher GR activities were found in young leaves than in old ones (Fig. 4) and in the latter, GR activity was induced by SA pre-treatments only slightly. However, in young leaves a considerable induction in enzyme activity was detected after SA pre-treatments after the 7th day of salt exposure. While a time-dependent decline in GR activity was observed in control roots during salt stress, the roots pre-treated with 10^{-4} M SA could maintain high enzyme activities to the end of the experiments. Thus, GR activity displayed an increasing trend during salt acclimation in 10^{-4} M SA pre-treated young leaves and roots (Fig. 4).

As shown in Fig. 5 10^{-7} M SA pre-treatment induced more significant changes in total AsA content in the leaves than 10^{-4} M SA, although in the latter case AsA levels were also significantly increased compared to controls. The enhanced AsA concentrations in the leaves hardened with 10^{-7} M SA were reduced under salt stress. However, plants pre-treated with 10^{-4} M SA could maintain higher AsA

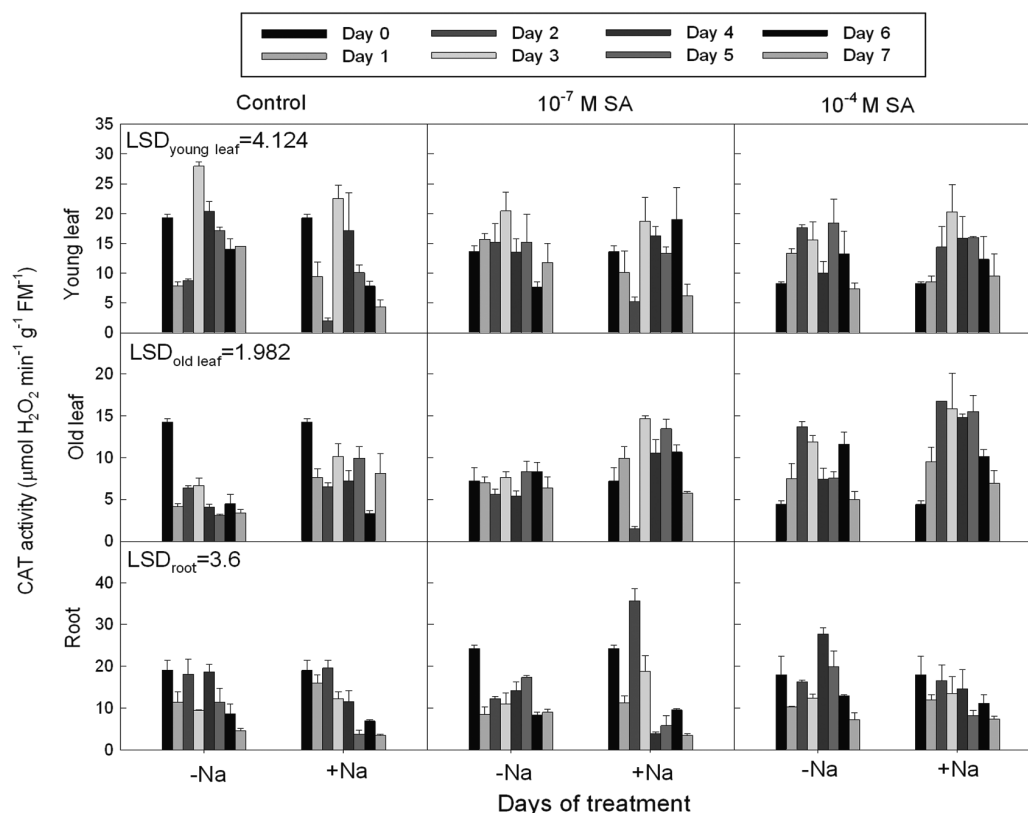


Fig. 2. Effect of salt stress induced by 100 mM NaCl on CAT activity in the young and basal leaves and roots of tomato plants subjected to 10^{-7} M or 10^{-4} M SA pre-treatments. Bars represent means \pm SE (n=9).

pool in the roots under salt stress than those treated with 10^{-7} M SA concentration at the end of the experiment. The proportion of reduced AsA was 31% in the roots of control plants, which decreased to 27% after pre-treatment with 10^{-7} M SA and was 49% after pre-treatment with 10^{-4} M SA. After seven days, in plants under salt stress this ratio was adjusted to 45% in all of the treatments, but the latter represented a significantly higher pool of the reduced AsA in the roots hardened with 10^{-4} M SA.

Contrary to the trends seen for GSH contents in the controls, a significant increase was observed in the GSH level of leaves after 10^{-7} and 10^{-4} M SA pre-treatments. Our results demonstrated that 10^{-7} M SA pre-treatment induced more pronounced changes in young leaves than in old ones and enhanced the glutathione pool to a higher extent than 10^{-4} M SA both in the absence or in the presence of NaCl. Contrasting results were found in the roots. The root tissues of plants hardened with 10^{-4} M SA, maintained a higher GSH content under salt stress than controls and plants hardened with a low SA, while the GSH contents of leaves were lower than observed in plants hardened with 10^{-7} M SA concentration (Fig. 6). In the roots exposed to high salinity

the proportion of the reduced glutathione decreased from 83% to 78 and 81% in control and 10^{-7} M SA pre-treated samples, respectively and in the case of 10^{-4} M SA-treated roots it was reduced from 93% to 83%. Since the total glutathione pool increased by more than 200% to the end of the experiments in the roots treated with high SA, these tissues could maintain the redox status of the increased ascorbate pool after one week of salt stress.

DISCUSSION

In recent decades a considerable improvement in salinity tolerance has been achieved in crop species and in the most important vegetables, both by conventional selection techniques and by the tools of molecular biology. The typical agronomic selection parameters for salinity tolerance are yield, survival of stress, maintenance of photosynthetic activity and biomass production, as well as growth, especially root elongation and biochemical markers such as the accumulation of soluble sugars, sugar alcohols, quaternary ammonium compounds, polyamines and amino acids (Ashraf and Harris, 2004). Juan et

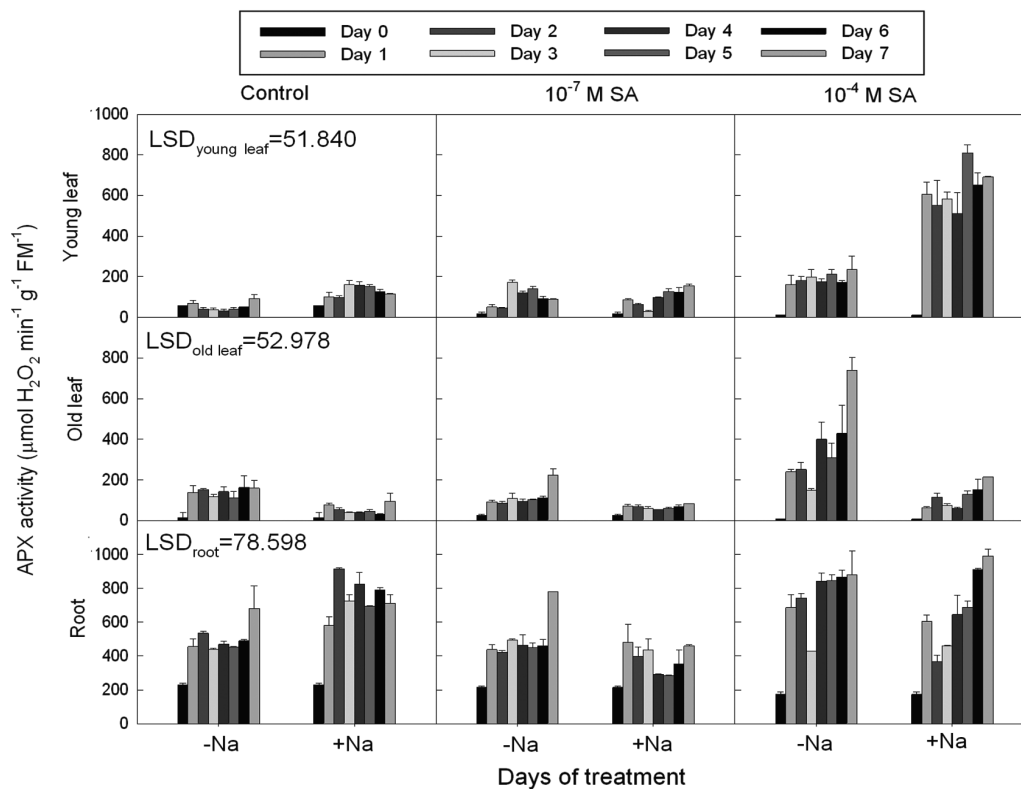


Fig. 3. Effect of salt stress induced by 100 mM NaCl on APX activity in the young and basal leaves and roots of tomato plants subjected to 10⁻⁷ M or 10⁻⁴ M SA pre-treatments. Bars represent means \pm SE (n=9).

al. (2005) found that the most tolerant genotypes of *S. lycopersicum* could be characterized by increased K^+/Na^+ ratios, carotenoid and sucrose contents and a reduced level of lipid peroxidation in the leaves during salt stress, suggesting a central role of the photosynthetic performance in the selection and breeding of cultivars for salt tolerance, though many genes and QTLs have also been described (Foolad et al., 2001; Cuartero et al., 2002).

A genotype tolerance should be evaluated on the basis of salt tolerance strategies of plants. It was found that *Solanum pimpinellifolium* 'PE-2', a wild relative of the cultivated tomato behaved like a halophyte and accumulated Na^+ in the upper leaves which was accompanied by a high Na^+/K^+ ratio (Cuartero et al., 1992). Salt tolerance of the cultivated tomato can be significantly enhanced by over-expressing an NHX-type Na^+/H^+ antiporter localized to the vacuolar membrane which enables plants to compartmentalize Na^+ in the vacuole (Yarra et al., 2012). Comparative transcriptomic profiling of the salt-tolerant *S. pimpinellifolium* 'PI365967' and the salt sensitive tomato cultivar Moneymaker revealed that a gene encoding SA binding protein 2 (SABP2), which functions in converting methylsalicylate into SA, was induced by salinity only in the wild species (Sun et al., 2010). Genes coding for

plasma membrane ATPase1, cell wall peroxidase and cytosolic APX as well as lactoylglutathione lyase involved in methylglyoxal detoxification were up-regulated only in 'PI365967', but other peroxidases and glutathione S-transferases (GSTs) were induced in Moneymaker under salt stress.

High activities of SOD and CAT in wild tomato, *Solanum pennellii* correlated also with higher salt tolerance and better protection against oxidative stress as compared with a salt sensitive, cultivated genotype (Shalata et al., 2001). Determining the role of various antioxidant enzymes in the salt stress response of *S. pennellii* as compared to cultivated species *S. lycopersicum*, the stress tolerance of plants was correlated with increased activities of SOD and various peroxidases (Alscher et al., 2002; Li et al., 2014).

Whilst investigating the root proteome in various tomato genotypes it was found that several proteins of antioxidant defence e.g. cytosolic APX1, various peroxidases, GSTs and glutathione peroxidases exhibited significant changes under salt stress (Manaa et al., 2011).

Based on biochemical and physiological indicators summarized in the Introduction we found that, in spite of significant increases in SOD activity after

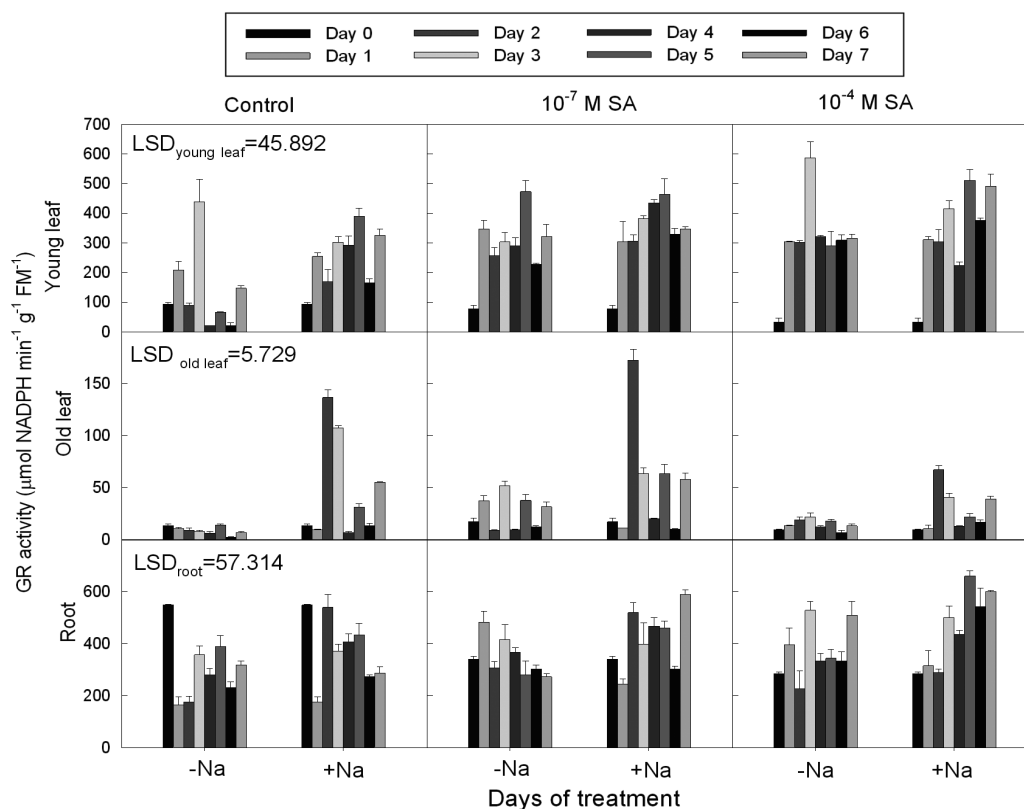


Fig. 4. Effect of salt stress induced by 100 mM NaCl on GR activity in the young and basal leaves and roots of tomato plants subjected to 10^{-7} M or 10^{-4} M SA pre-treatments. Bars represent means \pm SE (n=9).

salt stress in young leaves and roots and much higher CAT activities in the roots of plants hardened with low SA concentration, the salt stress resulted in more severe damage to these plants (Szepesi et al., 2009; Poór et al., 2011).

ROS scavenging by AsA is carried out by direct chemical reactions or by means of APX activity. The effect of SA on APX activity depended on the position and age of the leaves. It was an unexpected result that APX activity was only moderately enhanced on the effect of SA in young leaves. Since the induction of SOD was not accompanied by a similar induction of APX at low SA, the inactivation of H_2O_2 required more effective non-enzymatic antioxidant system in these tissues. However, in the roots exposed to high SA concentration, APX activity clearly increased compared to untreated control plants and to plants hardened with 10^{-7} M SA, and exhibited an enhanced activity to the end of the experiment in the presence of 100 mM NaCl. Thus, APX activity in young leaves and in the roots but not in old leaves is a good parameter for the estimation of successful antioxidant defense under salt stress in our system.

The accumulation of non-enzymatic antioxidants ascorbate and glutathione plays an important role in scavenging ROS under abiotic stresses. The

total AsA and glutathione levels increased in young leaves on the effect of SA compared to untreated control plants and it was more pronounced in leaves hardened with 10^{-7} M SA. In leaf tissues, after a transient decline, the total AsA content exhibited two maxima at day 2 and 7 after SA treatment and this tendency remained under salt stress.

Besides AsA, GSH is one of the most important non-enzymatic antioxidants and its accumulation was due to the enhanced expression of glutathione synthase (GS) after salt exposure (Li et al., 2013).

In the roots the concentration of these important antioxidants were lower than in the leaves but we found significant increases in GSH levels in the salt-stressed roots hardened with high SA. The GSH pool was in more reduced status in these root tissues by the activation of GR than that of roots exposed to 100 mM NaCl. This higher redox charge favours acclimation to salt stress and growth of root apices under high salinity.

The inability of the roots pre-treated with 10^{-7} M SA to increase the concentrations of ascorbate and glutathione during salt stress correlated well with their higher sensitivity to excess of NaCl (Figs 3, 4). In earlier experiments we found six members among tomato GST genes with DHAR activity and two of

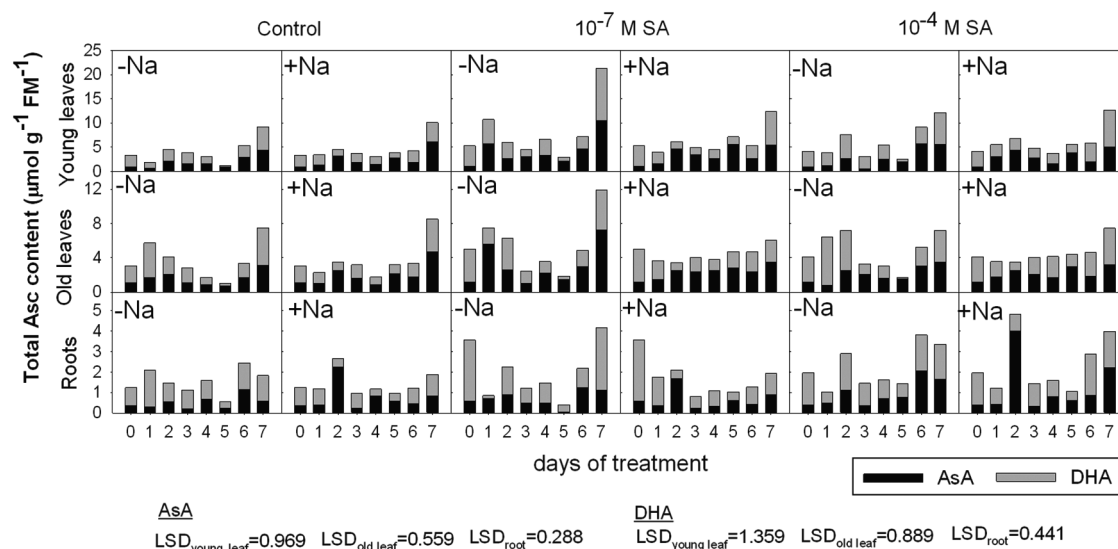


Fig. 5. Effect of salt stress induced by 100 mM NaCl on reduced AsA and DHA content in the young and basal leaves and roots of tomato plants subjected to 10⁻⁷ M or 10⁻⁴ M SA pre-treatments. Bars represent means±SE (n=9).

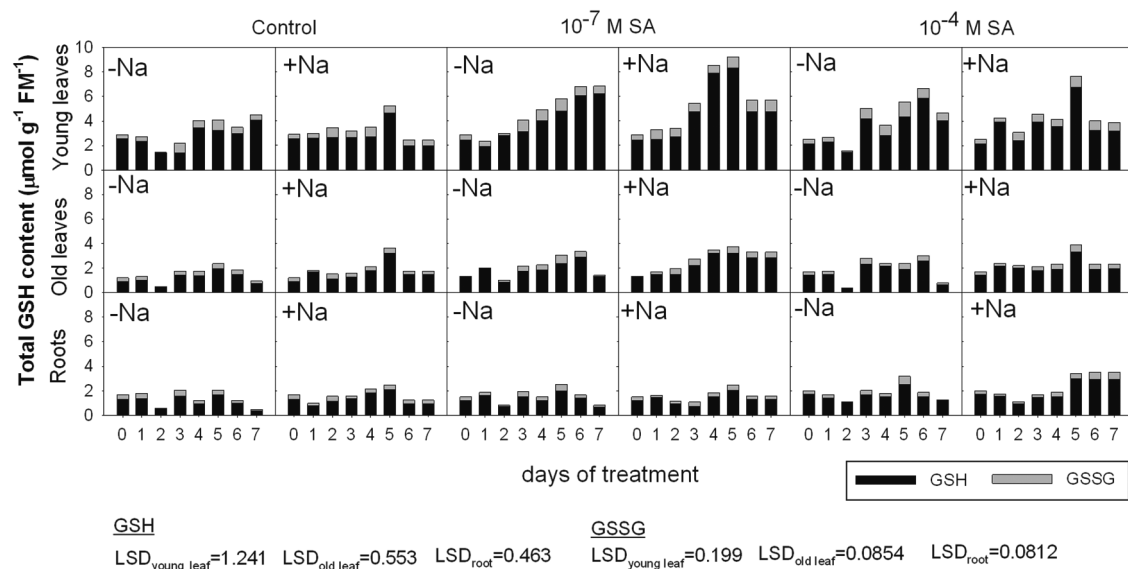


Fig. 6. Effect of salt stress induced by 100 mM NaCl on reduced and oxidized glutathione content in the young and basal leaves and roots of tomato plants subjected to 10⁻⁷ M or 10⁻⁴ M SA pre-treatments. Bars represent means±SE (n=9).

them, *SIDHAR2* and *SIDHAR5* were significantly down-regulated under salt stress in the roots of plants hardened with low SA concentration, but their expression and activity were maintained after a treatment with 10⁻⁴ M SA compared to salt-treated control (Csiszár et al., 2014). This coincided with a significant reduction in the extractable DHAR activity of the same tissue, which cannot be observed in roots hardened with high SA concentration. Similar results were obtained by Ananieva et al. (2004), who found that SA antagonized the oxidative stress

caused by paraquat via elicitation of an antioxidative response by increasing DHAR and peroxidase activity in barley. The regeneration of dehydroascorbate may be more effective in the presence of high GSH pool. Thus, the tolerance to salinity stress is associated with increased glutathione pool, APX and GR activities in the root tissues of the tomato hardened with high SA concentration.

The maintenance of root growth correlated well with the concentration and redox status of non-enzymatic antioxidants. The growth inhibition induced by

Cd in the root apices of barley (Bocová et al., 2012) and onion (Córdoba-Pedregosa et al., 2003) exhibited a close correlation with the decrease in ratio of the apoplastic ascorbate/dehydroascorbate. Thus, the redox status of the apoplastic ascorbate in the root elongation zone was the most important factor in the maintenance of root growth under abiotic stress.

The central role of glutathione in growth and development is also well documented (Tari et al., 2002b; Kocsy et al., 2013). The GSH pool may also determine cell division frequency and cell elongation in the root tissues. Exogenous SA affected the biomass production and the growth rate of plants according to an optimum curve, 10^{-7} M SA increased the growth rate while 10^{-4} M SA was inhibitory (Szepesi et al., 2009). Although both low and high concentration of SA improved the photosynthetic activity of the tomato plant under salt stress, soluble sugars were transported to the roots much more efficiently and contributed to osmotic adjustment in those plants which were hardened with high SA concentrations (Poór et al., 2011). We also found that in contrast to low SA, tomato plants pre-treated with high SA concentration could maintain root elongation under salt stress (Szepesi et al., 2009), which is one of the most important physiological traits of salt tolerance.

In conclusion, the successful hardening with SA to salt stress is a complex physiological response and better protection originates from a more efficient antioxidant response of roots. Since the mass of the plant body determines the energy requirements of salt acclimation the smaller biomass of plants pre-treated with 10^{-4} M SA before exposure to high salinity proved to be an advantageous feature.

AUTHORS' CONTRIBUTIONS

JCs, EH and PP carried out the measurement of antioxidant enzyme activities, ZT carried out the repetition of the experiments, ÁSz and IT were responsible for the analysis of the non-enzymatic antioxidants, analysed the data and wrote the manuscript. All Authors declare that they have no conflicts of interest.

ACKNOWLEDGEMENTS

The authors thank Kispálné Szabó Ibolya for her excellent technical assistance. This work was financed by grants from the Hungarian National Scientific Research Foundation (OTKA K101243 and OTKA K76854). This research for EH was supported by the European Union and the State of Hungary, co-financed by the European Social Fund in the framework of TÁMOP 4.2.4.A/2-11-1-2012-0001 'National Excellence Program'.

REFERENCES

- AEBI H. 1984. Catalase *in vitro*. *Methods in Enzymology* 105: 121–126.
- ALSCHER RG, ERTURK N, and HEATH LS. 2002. Role of superoxide dismutases (SODs) in controlling oxidative stress in plants. *Journal of Experimental Botany* 53: 1331–1341.
- ANANEVA EA, CHRISTOV KN, and POPOVA LP. 2004. Exogenous treatment with salicylic acid leads to increased antioxidant capacity in leaves of barley plants exposed to paraquat. *Journal of Plant Physiology* 161: 319–328.
- ARFAN M, ATHAR HR, and ASHRAF M. 2007. Does exogenous application of salicylic acid through the rooting medium modulate growth and photosynthetic capacity in two differently adapted spring wheat cultivars under salt stress? *Journal of Plant Physiology* 164: 685–694.
- ASHRAF MPJC, and HARRIS PJC. 2004. Potential biochemical indicators of salinity tolerance in plants. *Plant Science* 166: 3–16.
- ASADA K. 1999. The water-water cycle in chloroplasts: scavenging of active oxygens and dissipation of excess photons. *Annual Review of Plant Biology* 50: 601–639.
- BORSANI O, VALPUESTA V, and BOTELLA MA. 2001. Evidence for a role of salicylic acid in the oxidative damage generated by NaCl and osmotic stress in *Arabidopsis* seedlings. *Plant Physiology* 126: 1024–1030.
- BEAUCHAM PC, and FRIDOVICH I. 1971. Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. *Analytical Biochemistry* 44: 276–287.
- BOCOVÁ B, MISTRÍK I, PAVLOVKIN J, and TAMÁS L. 2012. Cadmium disrupts apoplastic ascorbate redox status in barley root tips. *Acta Physiologiae Plantarum* 34: 2297–2302.
- CÓRDOBA-PEDREGOSA MC, CÓRDOBA F, VILLALBA JM, and GONZÁLES-REYES JA. 2003. Zonal changes in ascorbate and hydrogen peroxide contents, peroxidase and ascorbate-related enzyme activities in onion roots. *Plant Physiology* 131: 697–707.
- CSISZÁR J, SZABÓ M, ERDEI L, MÁRTON L, HORVÁTH F, and TARI I. 2004. Auxin autotrophic tobacco callus tissues resist oxidative stress: the importance of glutathione S-transferase and glutathione peroxidase activities in auxin heterotrophic and autotrophic calli. *Journal of Plant Physiology* 161: 691–699.
- CSISZÁR J, HORVÁTH E, VÁRY Z, GALLÉ Á, BELA K, BRUNNER S, and TARI I. 2014. Glutathione transferase supergene family in tomato: Salt stress-regulated expression of representative genes from distinct GST classes in plants primed with salicylic acid. *Plant Physiology and Biochemistry* 78: 15–26.
- CUARTERO J, YEO AR, and FLOWERS TJ. 1992. Selection of donors for salt tolerance in tomato using physiological traits. *New Phytologist* 121: 63–69.
- CUARTERO J, ROMERO-ARANDA R, YEO AR, and FLOWERS TJ. 2002. Variability for some physiological characters affecting salt tolerance in tomato. *Acta Horticulturae* 573: 435–441.
- DAT JF, LOPEZ-DELGADO H, FOYER CH, and SCOTT IM. 1998. Parallel changes in H₂O₂ and catalase during thermotolerance induced by salicylic acid or heat acclimation in mustard seedlings. *Plant Physiology* 116: 1351–1357.

- DURNER J, and KLESSIG DF. (1995) Inhibition of ascorbate peroxidase by salicylic acid and 2,6-dichloroisonicotinic acid, two inducers of plant defense responses. *Proceedings of National Academy of Sciences U.S.A.* 92: 11312–11316.
- FOOLAD MR, ZHANG LP, and LIN GY. 2001. Identification and validation of QTLs for salt tolerance during vegetative growth in tomato by selective genotyping. *Genome* 44: 444–454.
- FOYER CH, and NOCTOR G. 2011. Ascorbate and glutathione: the heart of the redox hub. *Plant Physiology* 155: 2–18.
- GÉMES K, POÓR P, HORVÁTH E, KOLBERT ZS, SZOPKÓ D, SZEPESI Á, and TARI I 2011. Cross-talk between salicylic acid and NaCl-generated reactive oxygen species and nitric oxide in tomato during acclimation to high salinity. *Physiologia Plantarum* 142: 179–192.
- GRIFFITH OW. 1980. Determination of glutathione and glutathione disulfide using glutathione reductase and 2-vinylpyridine. *Analytical Biochemistry* 106: 207–212.
- HAO JH, DONG CJ, ZHANG ZG, WANG XL, and SHANG QM 2012. Insights into salicylic acid responses in cucumber (*Cucumis sativus* L.) cotyledons based on a comparative proteomic analysis. *Plant Science* 18: 69–82.
- HORVÁTH E, JANDA T, SZALAI G, and PÁLDI E. 2002. *In vitro* salicylic acid inhibition of catalase activity in maize: differences between the isozymes and a possible role in the induction of chilling tolerance. *Plant Science* 163: 1129–1135.
- HORVÁTH E, SZALAI G, and JANDA T. 2007. Induction of abiotic stress tolerance by salicylic acid signaling. *Journal of Plant Growth Regulation* 26: 290–300.
- JANDA T, SZALAI G, RIOS-GONZALEZ K, and VEISZ O, and PÁLDI E. 2003. Comparative study of frost tolerance and antioxidant activity in cereals. *Plant Science* 164: 301–306.
- JUAN M, RIVERO RM, ROMERO L, and RUIZ JM. 2005. Evaluation of some nutritional and biochemical indicators in selecting salt-resistant tomato cultivars. *Environmental and Experimental Botany* 54:193–201.
- KOCSY G, TARI I, VANKOVÁ R, ZECHMANN B, GULYÁS Z, POÓR P, and GALIBA G. 2013. Redox control of plant growth and development. *Plant Science* 211: 77–91.
- LAW MY, CHARLES SA, and HALLIWELL B. 1983. Glutathione and ascorbic acid in spinach (*Spinacia oleracea*) chloroplasts. The effect of hydrogen peroxide and of paraquat. *Biochemical Journal* 210: 899–903.
- LI G, PENG X, WEI L, and KANG G. 2013. Salicylic acid increases the contents of glutathione and ascorbate and temporally regulates the related gene expression in salt-stressed wheat seedlings. *Gene* 529: 321–325.
- LI T, HU Y, DU X, TANG H, SHEN C, and WU J. 2014. Salicylic acid alleviates the adverse effects of salt stress in *Torrey grandis* cv. Merrillii seedlings by activating photosynthesis and enhancing antioxidant systems. *PLoS One*, 9(10), e109492.
- MANAA A, AHMED HB, VALOT B, BOUCHET JP, ASCHI-SMITI S, CAUSSE M, and FAUROBERT M. 2011. Salt and genotype impact on plant physiology and root proteome variations in tomato. *Journal of Experimental Botany* 62: 2797–2813.
- MUNNS R, and TESTER M. 2008. Mechanisms of salinity tolerance. *Annual Review of Plant Biology* 59: 651–681.
- NAKANO Y, and ASADA K. 1987. Purification of ascorbate peroxidase in spinach chloroplasts; its inactivation in ascorbate-depleted medium and reactivation by monodehydroascorbate radical. *Plant Cell Physiology* 28: 131–140.
- POÓR P, GÉMES K, HORVÁTH F, SZEPESI Á, SIMON ML, and TARI I. 2011. Salicylic acid treatment via the rooting medium interferes with stomatal response, CO₂ fixation rate and carbohydrate metabolism in tomato, and decreases harmful effects of subsequent salt stress. *Plant Biology* 13: 105–114.
- SHALATA A, MITTOVA V, VOLOKITA M, GUY M, and TAL M. 2001. Response of cultivated tomato and its wild salt-tolerant relative *Lycopersicon pennellii* to salt-dependent oxidative stress. The root antioxidative system. *Physiologia Plantarum* 112: 487–494.
- SHI Q, BAO Z, ZHU Z, YING Q, and QIAN Q. 2006. Effects of different treatments of salicylic acid on heat tolerance, chlorophyll fluorescence, and antioxidant enzyme activity in seedlings of *Cucumis sativus* L. *Plant Growth Regulation* 48: 127–135.
- STRAUS MR, RIETZ S, VER LOREN VAN THEMAAT E, BARTSCH M, and PARKER JE. 2010. Salicylic acid antagonism of EDS1-driven cell death is important for immune and oxidative stress responses in *Arabidopsis*. *Plant Journal* 62: 628–640.
- SZEPESI A, CSISZÁR J, BAJKÁN S, GÉMES K, HORVÁTH F, ERDEI L, DEÉR A, SIMON LM, and TARI I. 2005. Role of salicylic acid pre-treatment on the acclimation of tomato plants to salt- and osmotic stress. *Acta Biologica Szegediensis* 49: 123–125.
- SZEPESI Á, CSISZÁR J, GÉMES K, HORVÁTH E, HORVÁTH F, SIMON ML, and TARI I. 2009. Salicylic acid improves acclimation to salt stress by stimulating abscisic aldehyde oxidase activity and abscisic acid accumulation, and increases Na⁺ content in leaves without toxicity symptoms in *Solanum lycopersicum* L. *Journal of Plant Physiology* 166: 914–925.
- SUN W, XU X, ZHU H, LIU A, LIU L, LI J, and HUA X. 2010. Comparative transcriptomic profiling of a salt-tolerant wild tomato species and a salt-sensitive tomato cultivar. *Plant Cell Physiology* 51: 997–1006.
- TARI I, CSISZÁR J, SZALAI G, HORVÁTH F, PÉCSVÁRADI A, KISS G, SZEPESI Á, SZABÓ M, and ERDEI, L. 2002a. Acclimation of tomato plants to salinity stress after a salicylic acid pre-treatment. *Acta Biologica Szegediensis* 46: 55–56.
- TARI I, SZALAI G, LÓRINCZ ZS, and BÁLINT A. 2002b. Changes in thiol content in roots of wheat cultivars exposed to copper stress. *Biologia Plantarum* 45: 255–260.
- TARI I, KISS G, DEÉR AK, CSISZÁR J, ERDEI L, GALLÉ Á, GÉMES K, HORVÁTH F, POÓR P, SZEPESI Á, and SIMON LM. 2010. Salicylic acid increased aldose reductase activity and sorbitol accumulation in tomato plants under salt stress. *Biologia Plantarum* 54: 677–683.
- WANG LJ, and LI SH. 2006. Salicylic acid-induced heat or cold tolerance in relation to Ca²⁺ homeostasis and antioxidant systems in young grape plants. *Plant Science* 170: 685–694.