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BRIEF COMMUNICATION

Exogenous salicylic acid alleviates NaCl toxicity and increases antioxidative enzyme activity in *Lycopersicon esculentum*

Y. HE* and Z.J. ZHU*,**[1]

*Department of Horticulture, Zhejiang University, Hangzhou 310029, P.R. China**
*Department of Horticulture, Zhejiang Forestry University, Lin'an, Hangzhou 311300, P.R. China***

Abstract

Effects of exogenous salicylic acid (SA) on plant growth, contents of Na, K, Ca and Mg, activities of superoxide dismutase (SOD), guaiacol peroxidase (GPX), ascorbate peroxidase (APX), dehydroascorbate reductase (DHAR), glutathione reductase (GR) and catalase (CAT), and contents of ascorbate and glutathione were investigated in tomato (*Lycopersicon esculentum* L.) plants treated with 100 mM NaCl. NaCl treatment significantly increased H_2O_2 content and lipid peroxidation indicated by accumulation of thiobarbituric acid reactive substances (TBARS). A foliar spray of 1 mM SA significantly decreased lipid peroxidation caused by NaCl and improved the plant growth. This alleviation of NaCl toxicity by SA was related to decreases in Na contents, increases in K and Mg contents in shoots and roots, and increases in the activities of SOD, CAT, GPX and DHAR and the contents of ascorbate and glutathione.

Additional key words: ascorbate, ascorbate peroxidase, glutathione, lipid peroxidation, salt stress, superoxide dismutase.

Salinity causes water stress, ion imbalance, stomatal closure and reduced CO_2 assimilation. There is accumulating evidence that high salinity induces the overproduction of reactive oxygen species (ROS; Hernández *et al*. 1999, Zhu 2001). ROS cause lipid peroxidation, protein denaturation and DNA damage (Feranda 2004). ROS content is controlled by an antioxidant system including low molecular antioxidants (ascorbate, glutathione, *etc*.) and antioxidative enzymes such as superoxide dismutase (SOD, EC 1.15.1.1), guaiacol peroxidase (GPX, EC 1.11.1.7), catalase (CAT, EC 1.11.1.6), ascorbate peroxidase (APX, EC 1.11.1.11), dehydroascorbate reductase (DHAR, EC 1.8.5.1) and glutathione reductase (GR, EC 1.6.4.2). SOD is crucial for the removal of $O_2^{\bullet-}$ (Takahashi and Asada 1983). Hydrogen peroxide is scavenged by APX, CAT, GPX, GR and DHAR.

Salicylic acid (SA) has various physiological functions including induction of plant resistance to biotic and abiotic stresses (Mauch-Mani and Métraux 1998, Metwally *et al*. 2003, He *et al*. 2005, Mahdavian *et al*. 2008). It has been reported that SA ameliorates the damaging effects of salinity by sustaining photosynthetic and transpiration rate and content of K^+ (El-Tayeb 2005, Arfan *et al*. 2007, Stevens *et al*. 2006). However, the influence of SA on antioxidative system of plants has not been well understood. In view of all before-mentioned reports, we suppose that SA may alleviate toxic effects of salinity on tomato in different ways. Therefore the influence of SA on NaCl-induced changes of growth and antioxidant system, as well as on element distribution in tomato seedlings have been investigated in this study.

The experiment was carried out in a greenhouse of Zhejiang University. Tomato (*Lycopersicon esculentum* L. cv. Hezuo 903) seeds were germinated on moisture filter paper in dark at 28 °C for 3 d, and then they were grown in vermiculite for 20 d. The seedlings were transplanted into 10 dm^3 plastic containers (four seedlings per container) containing aerated full nutrient solution: 5 mM $Ca(NO_3)_2$, 5 mM KNO_3, 2.5 mM KH_2PO_4, 2 mM $MgSO_4$, 29.6 μM H_3BO_3, 10 μM $MnSO_4$, 50 μM Fe-EDTA, 1.0 μM $ZnSO_4$, 0.05 μM H_2MoO_4, 0.95 μM $CuSO_4$. The pH of the nutrient solution was maintained close to 6.5 adding H_2SO_4 or

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Abbreviations: APX - ascorbate peroxidase; ASC - reduced ascorbate; CAT - catalase; DHA - dehydroascorbate; DHAR - dehydroascorbate reductase; EDTA - ethylenediaminetetra-acetic acid; GPX - guaiacol peroxidase; GR - glutathione reductase; GSH - reduced glutathione; GSSG - oxidized glutathione; NBT - nitroblue tetrazolium; PVP - polyvinylpyrrolidone; RH - relative humidity; ROS - reactive oxygen species; SA - salicylic acid; SOD - superoxide dismutase; TBARS - thiobarbituric acid reactive substances.
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[1] Corresponding author; fax: (+86) 571 86971354, e-mail: zhjzhu@zju.edu.cn

KOH. The seedlings were cultivated under in a growth chamber (relative humidity 70 %, day/night temperature 28/20 °C, irradiance 800 μmol m^{-2} s^{-1} and 12-h photoperiod) for 10 d, and then pretreated with a foliar spray of 1 mM SA. Salt treatment started by adding NaCl to the nutrient solution. The experimental design consisted of a control (no SA or NaCl) and three treatments (SA, NaCl, SA + NaCl) was arranged in a randomized, complete block design with four replicates, giving a total of 16 pots. The youngest fully developed leaves were taken for assays of antioxidant enzymes after 7 d and 14 d of NaCl treatment.

After 14 d of treatment, the plants were harvested. The shoots and roots were dried at 70 °C to constant mass. The dried samples were digested in a mixture of 38.1 % H_2SO_4 (m/v) and 3 % H_2O_2 (v/v), and the concentrations of K, Na, Ca and Mg were determined with atomic absorption spectrometry (*Shimadzu AA-6300*, Tokyo, Japan). H_2O_2 content was determined according to the method of Patterson *et al*. (1984). Lipid peroxidation was determined in terms of thiobarbituric acid reactive substances (TBARS) concentration according to the method of Cakmak and Marschner (1992).

For the enzyme assays, 0.3 g leaves were ground with 2 cm^3 ice-cold 25 mM HEPES buffer (pH 7.8) containing 0.2 mM EDTA, 2 mM ascorbate and 2 % PVP. The homogenates were centrifuged at 4 °C for 20 min at 12 000 *g* and the resulting supernatants were used for the determination of enzymatic activity and protein content assays (Zhu *et al*. 2000). All spectrophotometric analyses were conducted on a *Shimadzu UV-2410PC* spectrophotometer. Protein content was determined using a Coomassie brilliant blue with bovine serum albumin as the standard (Bradford 1976). The activity of SOD was assayed by measuring its ability to inhibit the photochemical reduction of nitroblue tetrazolium (Rao and Sresty, 2000). CAT activity was measured as the decline in absorbance at 240 nm due to the decomposition of H_2O_2 (Cakmak and Marschner 1992). GPX activity was measured as the increase of absorbance due to polymerization of guaiacol to tetraguaiacol at 470 nm (Egley 1983). APX activity was measured according to Nakano and Asada (1981) as the decrease in absorbance at 290 nm due to ascorbate oxidation. The assay of DHAR activity was carried out by measuring the increase in absorbance at 265 nm due to ASC formation (Nakano and Asada 1981). The GR activity was measured by following the decrease in absorbance at 340 nm due to NADPH oxidation (Foyer and Halliwell 1976). Reduced ascorbate (ASC), dehydroascorbate (DHA) and total ascorbate (ASC + DHA) were determined by the method of Hodges *et al*. (1996). Reduced glutathione (GSH) and oxidized glutathione (GSSG) were determined according to the method from Vlachaki and Meyn (1998).

Data presented are the mean values of four replicates. Statistical assays were carried out by analysis of variance (*ANOVA*) using SAS software (SAS Institute, Cary, NC, USA) and means were compared by the least significant difference (LSD) test and those at $P < 0.05$ were considered significantly different.

After 14 d of 100 mM NaCl treatment, the shoot and root dry mass reduced to 23.7 and 27.3 %, respectively, as compared to control (Table 1). However, this inhibition was alleviated by a foliar spray of 1 mM SA, the shoot and root dry mass of tomato plants in combination treatment of SA and NaCl decreased to 45.8 and 63.7 %, respectively. Similarly to previous studies (*e.g*. Silva *et al*. 2008), NaCl

Table 1. Effects of foliar spray of 1 mM SA on shoot and root dry mass, leaf and root Na, K, Ca and Mg content and K/Na ratio, and leaf ASC, DHA, ASC/DHA ratio, GSH, GSSG and GSH/GSSG ratio in tomato plants grown with or without 100 mM NaCl in nutrient solutions for 14 d. Values with the same letter are not significantly different at $P < 0.05$.

		Control	SA	NaCl	SA + NaCl
Dry mass [g $plant^{-1}$]	shoot	6.31 ± 0.09 a	6.44 ± 0.20 a	1.50 ± 0.05 c	2.95 ± 0.26 b
	root	1.32 ± 0.07 a	1.35 ± 0.10 a	0.36 ± 0.08 c	0.86 ± 0.04 b
Na content [mg g^{-1}(d.m.)]	leaf	3.93 ± 0.22 c	4.02 ± 0.36 c	40.97 ± 1.55 a	25.16 ± 1.34 b
	root	6.03 ± 0.45 c	7.16 ± 0.63 c	53.98 ± 2.67 a	45.36 ± 1.39 b
K content [mg g^{-1}(d.m.)]	leaf	42.42 ± 0.09 a	41.18 ± 1.51 a	30.40 ± 0.91 c	34.60 ± 0.18 b
	root	54.72 ± 2.11 a	53.31 ± 3.28 a	35.62 ± 0.38 c	39.32 ± 1.15 b
K/Na	leaf	10.80 ± 0.32 a	10.24 ± 0.51 a	0.74 ± 0.02 c	1.38 ± 0.01 b
	root	9.08 ± 0.05 a	8.82 ± 0.43 a	0.66 ± 0.03 c	0.87 ± 0.01 b
Ca content [mg g^{-1}(d.m.)]	leaf	42.43 ± 2.93 a	38.39 ± 2.00 a	37.85 ± 4.14 a	38.26 ± 3.16 a
	root	12.84 ± 1.78 b	25.05 ± 1.15 a	6.03 ± 0.50 c	13.65 ± 0.41 b
Mg content [mg g^{-1}(d.m.)]	leaf	52.55 ± 3.47 a	48.04 ± 3.69 a	15.04 ± 2.83 c	28.40 ± 3.52 b
	root	46.02 ± 6.51 b	63.50 ± 1.56 a	27.68 ± 0.48 c	40.28 ± 5.68 b
ASC content [μg g^{-1}(f.m.)]	leaf	1.12 ± 0.14 a	1.08 ± 0.08 a	0.40 ± 0.04 c	0.87 ± 0.03 b
DHA content [μg g^{-1}(f.m.)]	leaf	0.41 ± 0.06 a	0.42 ± 0.05 a	0.38 ± 0.03 a	0.41 ± 0.05 a
ASC/DHA	leaf	2.81 ± 0.51 a	2.27 ± 0.46 a	1.13 ± 0.05 c	2.17 ± 0.23 b
GSH content [μg g^{-1}(f.m.)]	leaf	11.62 ± 0.37 a	11.07 ± 0.08 a	4.24 ± 0.15 c	6.79 ± 0.19 b
GSSG content [μg g^{-1}(f.m.)]	leaf	9.22 ± 0.40 a	9.72 ± 0.29 a	8.89 ± 0.09 a	9.79 ± 0.28 a
GSH/GSSG	leaf	1.26 ± 0.09 a	1.14 ± 0.03 a	0.48 ± 0.02 c	0.69 ± 0.04 b

Table 2 Effects of foliar spray of 1 mM SA on concentrations of H_2O_2 and TBARS, activities of SOD, CAT, GPX, APX, DHAR and GR in the leaves of tomato plants grown with or without 100 mM NaCl in nutrient solutions. Values with the same letter are not significantly different at $P < 0.05$.

	Treatment	Control	SA	NaCl	SA + NaCl
H_2O_2 content [nmol g^{-1}(f.m.)]	14 d	12.54 ± 0.77 c	12.99 ± 1.40 c	28.25 ± 1.14 a	19.19 ± 0.10 b
TBARS content [nmol g^{-1}(f.m.)]	14 d	29.70 ± 1.79 c	29.80 ± 2.68 c	60.65 ± 5.86 a	39.40 ± 2.16 b
SOD activity	7 d	224.89 ± 9.76 c	197.28 ± 13.42 c	305.32 ± 13.48 b	374.79 ± 15.86 a
[U mg^{-1}(protein) min^{-1}]	14 d	196.69 ± 11.00 c	198.99 ± 4.61 c	299.76 ± 19.02 b	394.37 ± 19.42 a
CAT activity	7 d	3.30 ± 0.47 c	5.58 ± 0.71 a	4.34 ± 0.16 b	5.00 ± 0.39 a
[μmol(H_2O_2) mg^{-1}(protein) min^{-1}]	14 d	6.63 ± 0.23 a	7.01 ± 0.18 a	2.41 ± 0.27 c	3.41 ± 0.17 b
APX activity	7 d	6.98 ± 1.00 c	6.23 ± 0.39 c	9.22 ± 1.17 b	15.01 ± 1.38 a
[μmol(ASC) mg^{-1}(protein) min^{-1}]	14 d	10.09 ± 1.44 c	15.43 ± 1.67 a	13.95 ± 0.74 b	18.08 ± 1.13 a
GPX activity	7 d	81.66 ± 6.39 c	84.25 ± 9.70 c	271.27 ± 6.55 a	212.35 ± 17.04 b
[μmol(guaiacol) mg^{-1}(protein) min^{-1}]	14 d	83.94 ± 5.72 c	62.19 ± 2.48 c	199.39 ± 12.19 a	149.42 ± 15.61 b
DHAR activity	7 d	91.45 ± 4.67 a	99.25 ± 5.59 a	32.97 ± 0.96 c	81.38 ± 2.77 b
[μmol(ASA) mg^{-1}(protein) min^{-1}]	14 d	80.28 ± 1.54 a	85.44 ± 2.00 a	48.83 ± 4.11 b	85.95 ± 16.77 a
GR activity	7 d	9.61 ± 0.29 b	8.68 ± 1.16 b	18.22 ± 1.56 a	16.22 ± 1.10 a
[μmol(NADPH) mg^{-1}(protein) min^{-1}]	14 d	9.05 ± 0.15 b	8.83 ± 0.31 b	13.33 ± 0.87 a	13.24 ± 1.21 a

treatment significantly increased Na content and reduced K content in both leaves and roots, and SA considerably decreased Na content and inhibited the K content reduction in salt-stressed plants (Table 1) and therefore increased their K/Na ratio. SA did not influence Ca content in leaves, but increased Ca content in roots under salt stress. Salt stress decreased the Mg content in the leaves and roots. SA significantly increased the Mg content in the salt-stressed leaves, non-stressed and salt-stressed roots. The results were in agreement with those in barley and maize (El-Tayeb 2005, Gunes *et al*. 2007).

The content of TBARS, produced during peroxidation of membrane lipids, is often used as an indicator of oxidative damage. In the present study, increase in the contents of H_2O_2 and TBARS was observed in salt-stressed tomato plants (Table 2) and SA alleviated oxidative damage caused by NaCl. However, no influence was observed when SA was supplied to non-stressed plants.

SOD forms the first line in removing ROS and enhanced SOD activity suggests its important role in removing $O_2^{\bullet-}$ induced by salt stress. SA increased SOD activity under NaCl stress (Table 2). In H_2O_2 scavenging, CAT, GPX and ascorbate-glutathione cycle play a vital role. In the present experiment, APX activity was increased by salt stress, but it was significantly decreased by SA (Table 2). The similar result has been observed in barley (Metwally *et al*. 2003). The inhibition of APX increased cellular H_2O_2 content during the early time of SA treatment was observed by Ding *et al*. (2002) and Shi *et al*. (2006). CAT activity was declined after 14 d of salt stress, and GPX activity was increased after NaCl treatment (Table 2), which might be considered as a common response to various stresses (Janda *et al*. 2003). SA increased both CAT and GPX activities under salt-stress, which might decrease H_2O_2 content and thus alleviate oxidative stress.

Activity of DHAR decreased severely by salt stress, and the decrease was mitigated by SA. ASC content was greatly decreased in tomato leaves treated with NaCl (Table 1). DHA is reduced to ASC by DHAR using GSH as a reductant. As DHA content was not changed (Table 1), the decrease of DHAR activity may contribute to the decline of ASC content. Since SA spray increased the activity of DHAR, the content of ASC and the ratio of ASC to DHA under salt stress (Table 1), this might be one way how SA alleviated salt toxicity. Although NaCl treatment enhanced GR activity, the GSH content was reduced and the GSSG content was not changed, probably due to the limitation of NADPH as substrate during the reaction. SA had no influence in GR activity, however, increased GSH and total glutathione content under salt stress, and thus promoted GSH/GSSG ratio (Table 1), suggesting that SA increased the scavenging capacity of ROS.

In summary, the results in the present study indicated that SA foliar spray maintained ion balance, increased capacity of antioxidant system and decreased lipid peroxidation in tomato leaves under salt stress.

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