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BRIEF COMMUNICATION

Effect of salt stress on lipid peroxidation and superoxide dismutase and peroxidase activities of *Lycopersicon esculentum* and *L. pennellii*

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Abstract

In this study, a relationship between lipid peroxidation, the antioxidant defense system and salt stress in salt-sensitive cultivated tomato (*Lycopersicon esculentum*) and its salt-tolerant wild relative (*L. pennellii*) was established. Superoxide dismutase (SOD) activities were significantly higher in the leaves of *L. pennellii* than those of *L. esculentum* after 12 and 84 d. POX activity showed a gradual increase in both cultivars under 70 mM NaCl. POX activity in *L. pennellii* significantly increased after 6 and 84 d whereas showed no remarkable change in leaves of *L. esculentum* under 140 mM NaCl. A higher salinity tolerance of *L. pennellii* was also correlated with a lower lipid peroxidation, which might be due to a higher content of antioxidant enzymes studied.

Additional key words: antioxidant enzymes, ascorbate peroxidase, glutathione reductase, malondialdehyde, NaCl, tomato.

Salinity in soil and irrigation water is one of the major factors that limit crop productivity. The cultivated tomato *Lycopersicon esculentum* is considered to be salt-sensitive while its wild relative species *L. pennellii* has been observed to be more salt tolerant (Bolarin *et al.* 1991, Cuartero *et al.* 1992). The salt tolerance of these two species was based mainly on growth and survival under salt stress (Rush and Epstein 1981). Attempts have also been made to evaluate physiological and biochemical changes such as ion exchange, the role of solute contribution to the osmotic adjustment and polyamine levels with respect to differential salinity tolerance in *L. esculentum* and *L. pennellii*. However, involvement of antioxidant enzymes such as superoxide dismutase (SOD; EC 1.15.1.1) and peroxidase (POX; EC 1.11.1.7) in salinity tolerance of tomato plants is poorly discussed. Recently, Shalata and Tal (1998) and Mittova *et al.* (2002) studied the response of the antioxidant system in the leaves of the cultivated tomato and its wild salt-tolerant relative *L. pennellii*. However, in their works they employed relatively short duration of exposure (only 22 d). Therefore, how antioxidant system in the leaves of two species is included in the response to osmotic effect (within days after commence of NaCl) and salt-specific effect (within weeks after commence of NaCl) of salt stress comparatively needs to be addressed. Hence, in the present work, the changes in lipid peroxidation and antioxidant enzyme activities such as SOD and POX in salt-sensitive *L. esculentum* and salt-tolerant *L. pennellii* were studied.

Two species of tomato, salt-sensitive (*Lycopersicon esculentum* Mill. cv. Riogrande) and salt-tolerant (*L. pennellii* (Correll) D'Arcy, PE 47) were used. Seeds were germinated in Perlite and grown in a growth chamber under controlled environmental conditions under the 16-h photoperiod, temperature of 30/18 °C, relative humidity of 60 - 70 %. The photosynthetic photon flux density (PPFD) was 350 $\mu mol^{-1} m^{-2} s^{-1}$ at plant height.

Seedlings were watered with full strength Hoagland solution regularly in two days intervals until 40th day of plant age. On the 41st day, the two sets of plants were watered with Hoagland nutrient solution containing either 70 mM or 140 mM NaCl while control plants were watered as before. Tomato plants were harvested and sampled after 0, 6, 12 and 84 d of salt treatment initiation. For enzyme analysis, the first fully expanded leaves

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Abbreviations: APOX - ascorbate peroxidase; BSA - bovine serum albumine; DAB - diaminobenzidine-tetrahydrochloride dehydrate; EDTA - ethylenediamine tetraacetic acid; GR - glutathione reductase; MDA - malondialdehyde; NBT - nitroblue tetrazolium; POX - peroxidase; PVPP - polyvinylpolypyrrolidone; ROS - reactive oxygen species; SOD - superoxide dismutase.

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were sampled and frozen in liquid nitrogen immediately and then stored at -40 °C until analysis.

Samples for SOD, POX and total protein analyses were prepared by homogenizing 0.5 g of frozen leaf material in 3 cm^3 of cold 50 mM Na phosphate buffer (pH 7.8) containing 1 mM EDTA and 2 % (m/v) PVPP. The homogenate was centrifuged at 13 000 *g* for 40 min at 0 °C. All spectrophotometric analyses were conducted on a *Shimadzu* (Kyoto, Japan) *UV-1600* spectrophotometer.

The SOD activity assay was based on the method of Beauchamp and Fridovich (1971). One unit of enzyme activity was defined as the quantity of SOD required to produce a 50 % inhibition in the photochemical reduction of nitro blue tetrazolium (NBT) at 560 nm. The reaction mixture (3 cm^3) contained 50 mM Na phosphate buffer (pH 7.8), 0.033 mM NBT, 10 mM L-methionine, 0.66 mM EDTA and 0.0033 mM riboflavin. Reactions were carried out for 10 min at 25 °C, under PPFD of about 300 $\mu mol\ m^{-2}s^{-1}$.

The POX activity was based upon the method described by Herzog and Fahimi (1973). The reaction mixture (3 cm^3) contained 2.75 cm^3 of DAB solution [dissolved gelatine solution and 150 mM Na phosphate-citrate buffer (pH 4.4)], 0.2 cm^3 of plant extract, and 0.05 cm^3 of 0.6 % (m/v) H_2O_2. The increase in A_{465} was followed for 3 min.

Total soluble protein content in the enzyme extracts of tomato leaves was determined according to Bradford

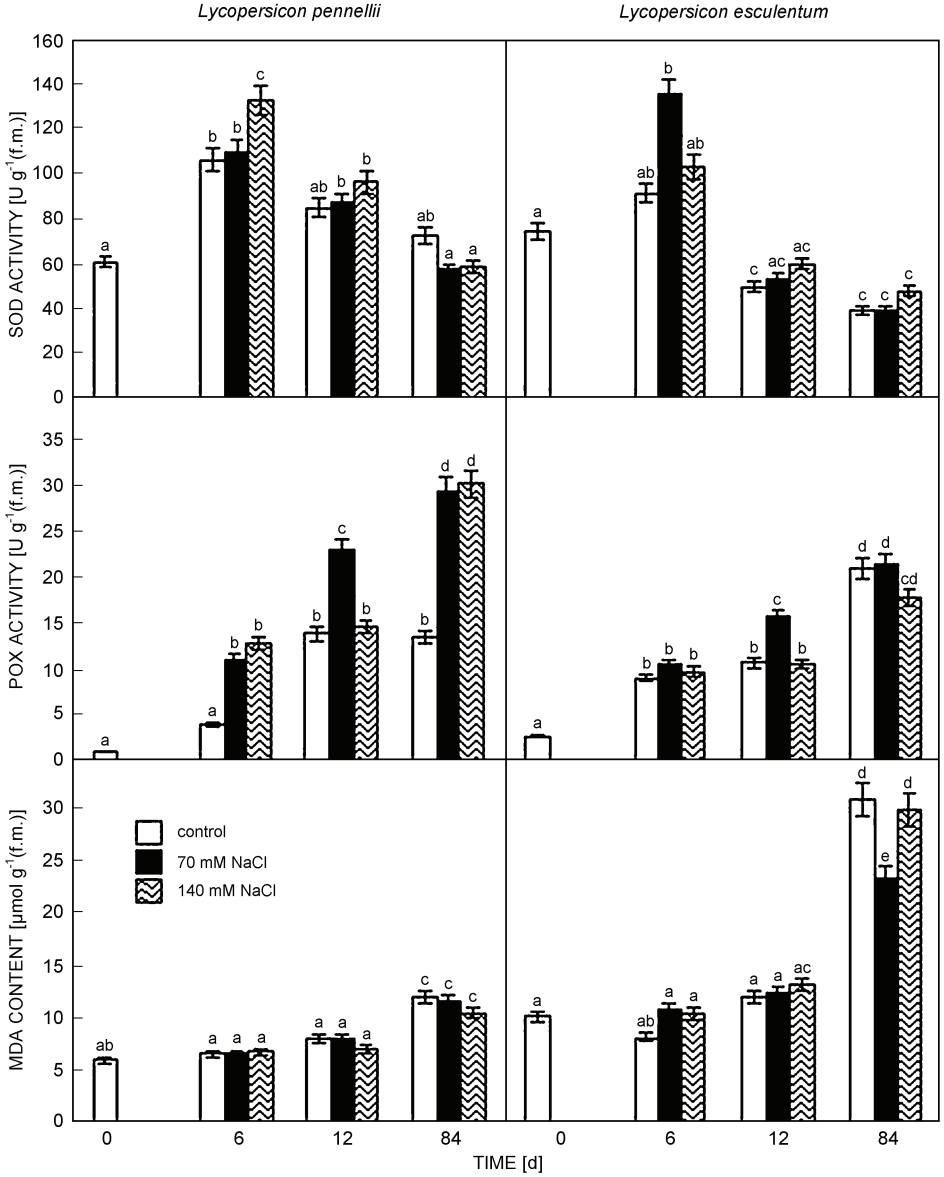

Fig. 1. Superoxide dismutase (SOD) and peroxidase (POX) activities and lipid peroxidation (MDA content) in leaves of *Lycopersicon pennellii* and *L. esculentum* in response to different concentrations of NaCl. Data represents the average of two experiments with three replicates (n = 6). *Vertical bars* indicate means ± SE. *Bars* with different letters are significantly different at $P < 0.05$ within each cultivar.

(1976) using BSA as a standard.

Lipid peroxidation was determined by estimating the malondialdehyde (MDA) content by the thiobarbituric acid reaction (Madhavara Rao and Sresty 2000). The concentration of MDA was calculated from the absorbance at 532 nm (correction was done by sub-tracting the absorbance at 600 nm for unspecific turbidity) by using coefficient of absorbance 155 mM^{-1} cm^{-1}.

All data were subjected to a one-way analysis of variance (*ANOVA*) and general linear model for repeated measures (GLM). The mean differences were compared by LSD test and $P < 0.05$ was considered significantly different. Each data point was mean of three replication from two independent experiments ($n = 6$).

SOD activities in leaves of two species were monitored on days 6, 12 and 84 d under 70 and 140 mM NaCl treatments (Fig. 1). Salt stress-induced enhancements in SOD activity were observed in leaves of *L. pennellii* (25 %) under 140 mM NaCl and of *L. esculentum* (48 %) under 70 mM NaCl on day 6. We observed a higher activity of SOD in all groups of *L. pennellii* on days 12 and 84. Our results are in agreement with the findings of Shalata and Tal (1998) who also observed higher SOD activities in leaves of *L. pennellii*. A higher constitutive and/or induced activity of SOD has also been reported in wild beet species (Bor *et al.* 2003) confirming a correlation between increased SOD activity and relative tolerance to salt in different genotypes.

Under control conditions, an age-dependent increase was found in POX activities in leaves of *L. pennellii* after 6 d. However, there was no attractive change in POX activity on day 84. In *L. esculentum*, a remarkable age-dependent increase was found in POX activity on day 84. Except for the plants treated with 140 mM NaCl on day 12, POX activity gradually increased in leaves of *L. pennellii* subjected to two NaCl concentrations throughout the experimental period (Fig. 1). Under 70 mM NaCl stress, these increases were 175 %, 67 % and 119 % over the controls in leaves of *L. pennellii* on days 6, 12 and 84, respectively. Increases in POX activity in leaves of *L. pennellii* under 140 mM NaCl stress were 217 and 126 % on days 6 and 84, respectively. However, POX activity did not change in leaves of *L. esculentum* except for the plants grown under 70 mM NaCl on day 12 and the plants grown under 140 mM NaCl on day 84. POX activity in leaves of *L. esculentum* increased by 47 % under 70 mM NaCl on day 12 and decreased by 15 % under 140 mM NaCl on day 84. When we compared two species, POX activities were usually higher in leaves of *L. pennellii* than in *L. esculentum* throughout the experiment. Increase in POX activity has also been reported in salt stressed cotton species (Meloni *et al.* 2003). Furthermore, improved tolerance of a salt-tolerant rice cultivar Pokkali has been due to the increased activities of SOD, POX and APOX enzymes under salt stress (Demiral and Türkan 2004). Greater SOD, POX and APOX activities and thus improved growth has also been reported in shoots of soybean plants under salt stress (Ghorbanli *et al.* 2004). On the other hand, shelf life of tomato plants has been positively correlated with enhanced activities of APOX, GR, CAT and POX in tomato fruits (Mondal *et al.* 2004).

Lipid peroxidation in leaves of *L. pennellii* was almost the same throughout the experimental period (Fig. 1). However, MDA content in leaves of *L. esculentum* showed a significant age-dependent increase on day 84 under control conditions. MDA contents in this species were always higher than in *L. pennellii* under both salt treatments. A lower level of lipid peroxidation, hence a lower degree of membrane damage in *L. pennellii* than in *L. esculentum* leaves might be resulted from the higher SOD and POX activities in *L. pennellii*. These results are in a good agreement with the results of Shalata and Tal (1998) who found a lower level of lipid peroxidation and higher constitutive antioxidant enzyme activities in leaves of *L. pennellii* under salt stress. Similarly, Meloni *et al.* (2003) and Agarwal and Pandey (2004) found that lesser degree of membrane damage and higher activity of SOD and POX in NaCl-treated cotton and in senna (*Cassia angustifolia* Vahl.) plants, respectively, were correlated with higher salinity tolerance.

In conclusion, salinity tolerance of *L. pennellii* is associated with higher SOD and POX activities and lower level of lipid peroxidation than in *L. esculentum*. *L. pennellii* exhibited better protection from oxidative damage maintaining cellular membranes under not only short-term but also long-term salt stresses.

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