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Differential Responses of Two Tomato Cultivars (*Lycopersicon esculentum* L.) to NaCl Stress

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Abstract: A water culture experiment was conducted using two cultivars of tomato namely: Castle Rock (salt sensitive) and Super Strain –B (salt resistant), to study their response to NaCl stress. NaCl was supplied in three levels (0.0-10mmol-100mmol). Shoot, root dry weights, nutrients concentration, nutrient ratios, lipid peroxidation, POD and SOD activities were used as biochemical assays for assessing salt tolerance. The results of this study indicate that compared with Castle Rock the Na/K ratio as well as the lipid peroxidation in respect of MAD-content were much lower in Super Strain -B. The activities of POD and SOD were higher in Super Strain –B at 100 mmol NaCl. So the decreases in Na/K ratio and (MAD content), moreover, the increases in POD, SOD levels may be used for screening cultivar tolerance to NaCl stress. In addition, Super Strain –B was found to be more tolerant to NaCl salt stress compared to Castle Rock cultivar.

Keywords: Growth, nutritive status, lipid peroxidation, POD and SOD activities, salinity, salt tolerance, *Lycopersicon esculentum* L.)

Introduction

The disorders in plant nutrition produced by salinity either of soil or of irrigation water causes disturbances in plant growth and nutrient balance. It is currently assumed that the negative effect of the various environmental stresses is at least partially due to the generation of active oxygen species (AOS), or the inhibition of the system, which defense against them. AOS may lead to degradation of membrane lipids, proteins and DNA (Smirnoff, 1993), which have a great impact on growth parameters and makes a major contribution to nutrient imbalance. Plants have evolved a battery of antioxidant defense system including antioxidant enzymes (POD, SOD, Cat and Apx). The increase in AOS seems to occur as a response to all stresses, drought (Smirnoff, 1993), salt stress (Hernandez *et al.*, 1994 & Gossett *et al.*, 1996) and nutrient deficiency (Iturbe –Ormaetxe *et al.*, 1995). The results of most of these studies suggest that the tolerance of different plant species for environmental stresses is usually correlated with an efficient antioxidative system.

The objective of this study was to investigate the role of antioxidant in the mechanism of salt tolerance by comparing the extent of lipid peroxidation and the activities of antioxidant enzymes in relation to growth, nutrients concentration as well as, nutrient ratios in the young leaves of two cultivars differing in their sustain ability to salt stress.

Materials and Methods

Plant growth conditions: Seeds of two cultivars of tomato *Lycopersicon esculentum* L. (Castle Rock and Super Strain –B) were germinated in moist sand for 7 days. Then seedlings were transferred in plastic vessels containing full nutrient solution (FNS) of Hoagland and Arnon (1950) for 4 days. On 12th day seedlings were provided with three levels of NaCl (0.0-10 mmol-100 mmol). During the experimental period, seedlings grew at day/night temperature 25/20 °C, relative humidity of about 60% with 16h light and 8h dark periods at light intensity 300-350 μM. $m^{-2}sec^{-1}$. At 21-days, plants were harvested and prepared for nutrient analyses, enzymes activity and lipid peroxidation determinations.

Plant analyses: Three weeks later samples were taken for plant growth measurements in terms of shoot and root dry weight and the determination of nutrient concentrations according to Chapman and Pratt (1978).

Enzymatic extraction and determination: The activities of the POD and SOD in control and treated plants were measured. Leaf samples were ground with saccrose tris buffer (pH 7.8) in a cold mortar. The homogenate was filtered through 4 layers of nylon cloth and centrifuged at 15000 rpm for 15 min. The resulting crude supernatant was used for enzyme determinations.

Peroxidase (POD) was determined according to Chance and Maehly (1950). The activity (Unit /g^{-1} FW) was measured by determining the amount of enzyme causing increase in the absorbance at 470 nm.

Superoxide dismiutase (SOD) was assayed according to the photochemical method of Giannopolitis and Ries (1977). The activity (Unit/ g^{-1} FW) was measured by determining the amount of enzyme required to cause 50% inhibition of NBT reduction.

Lipid peroxidation: Lipid peroxidation was determined in 1 g leaf fresh weight by measuring the amount of malondialdehyde (MAD; E= 156.0 mM cm^{-1}), a product of lipid peroxidation, by the thiobarbituric acid reaction (described by Joseph *et al.*, 1988). MAD content expressed as (μmol MAD /g^{-1} FW).

Results and Discussion

Growth: Data in Table (1) show considerable variation in two cultivars. NaCl reduced shoot and root growth of both cultivars, the effects were more pronounced in Castle Rock. There was loss in shoot and root dry weights of Castle Rock from 58 to 25% at 100 mmol NaCl. On the contrary, Super Strain -B was almost not affected by increasing NaCl level in the growth root media. Considerable decrease was observed in root /shoot ratio in Castle Rock cultivar.

The reduction in dry weight in Castle Rock may be attributed to lack of energy (Helal & Mengel, 1979;1981) and depleted storage carbohydrates to a great extent rendering low plant biomass content (Gao *et al.*, 1998; Schmidhalter *et al.*, 1999).

Nutrients uptake: The results in Table (2) reveal that Ca uptake was reduced by both cultivars. Castle rock was much more affected (46%) than Super strain-B (52%) at 100-mmol NaCl.

Table 1: Shoot and root dry weight (g/plant) in tomato cultivars as affected by NaCl stress.

Treatments	Shoot D.W.	%	Root D.W.	%	Total plant	%	Root/shoot
Castle Rock							
Control	0.12	(100)	0.04	(100)	0.16	(100)	0.33
10 mmol	0.09	(75)	0.02	(50)	0.11	(69)	0.22
100 mmol	0.07	(58)	0.01	(25)	0.08	(50)	0.14
Super Strain-B							
Control	0.19	(100)	0.07	(100)	0.26	(100)	0.37
10 mmol	0.18	(95)	0.07	(100)	0.25	(96)	0.39
100 mmol	0.17	(90)	0.07	(100)	0.24	(92)	0.41

Table 2: Ca, K and Na uptake (mg/plant) in the leaves of tomato cultivars as affected by NaCl salt stress

Treatments	Ca	%	K	%	Na	%
Castle Rock						
Control	3.10	(100)	3.98	(100)	0.68	(100)
10 mmol	2.07	(67)	2.65	(67)	2.90	(426)
100 mmol	1.42	(46)	1.75	(44)	3.60	(529)
Super Strain -B						
Control	6.42	(100)	8.30	(100)	3.09	(100)
10 mmol	4.10	(64)	9.22	(111)	2.60	(84)
100 mmol	3.35	(52)	7.75	(93)	2.95	(95)

Table 3: Nutrients concentration ratios in leaves of tomato cultivars as affected by NaCl stress

Treatments	Na/Ca	Na/K	K/Ca
Castle Rock			
Control	0.22	0.17	1.29
10 mmol	1.40	1.09	1.27
100 mmol	2.53	2.06	1.23
Super Strain-B			
Control	0.48	0.37	1.29
10 mmol	0.63	0.28	2.25
100 mmol	0.88	0.38	2.32

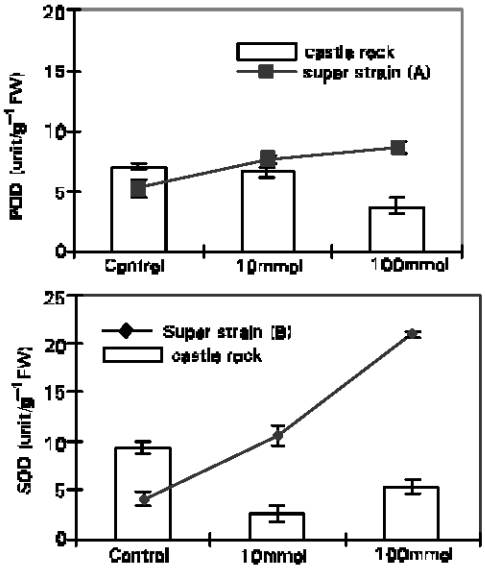

Fig. 1: Peroxidase and superoxide dismutase in the leaves of Castle rock and Super strain -B under different levels of NaCl. Values indicate mean ± SD with n = 3.

Castle Rock showed the same trend in K uptake, while Super Strain-B showed less decrease (93%) at high level of NaCl in the root growth medium. On the other hand Na uptake increased with the increase in NaCl level, compared with

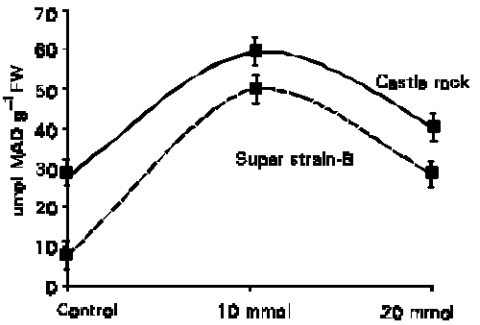

Fig. 2: Lipid peroxidation in the leaves of Castle rock and Super strain -B under different levels of NaCl. Values indicate mean ± SD with n = 3.

Super Strain-B. The higher reduction in Ca and K concentration in Castle-Rock suggest that high sodium uptake induced the low K and Ca uptake that has been implicated in growth and yield reduction of tomato crops (Crvajal *et al.*, 1999). Besides salinity effects, it is worth to note that NaCl causes toxic effects, (Ayers and Westcot, 1985) and nutrient imbalances (Marschner, 1995). The depression in nutrient uptake in Castle-Rock might be a pH effect, (Roemheld and Marschner, 1986) or depressive effects of water potential which resist the passive uptake of these nutrients (Crvajal *et al.*, 1999)

Nutrients concentration ratios: Concerning the nutrient concentration ratios, Table 3 reveals that the ratios were affected by increasing NaCl level. The increase in NaCl level induced the increases in Na/Ca ratios in both cultivars. The increase was more pronounced in Super Strain-B. While K/Ca ratio increased only for Castle Rock. This may be attributed to the increase in Na ions, which diminished the concentrations of K and Ca giving large values for Na/Ca. Consequently, the Na/K ratio was also different and showed less value for Super Strain –B cultivar. In this respect it was reported that Na/K ratio might be considered as an indicator of crop tolerance to NaCl stress because the roots of the most tolerate cultivars are characterized by their high selectivity of

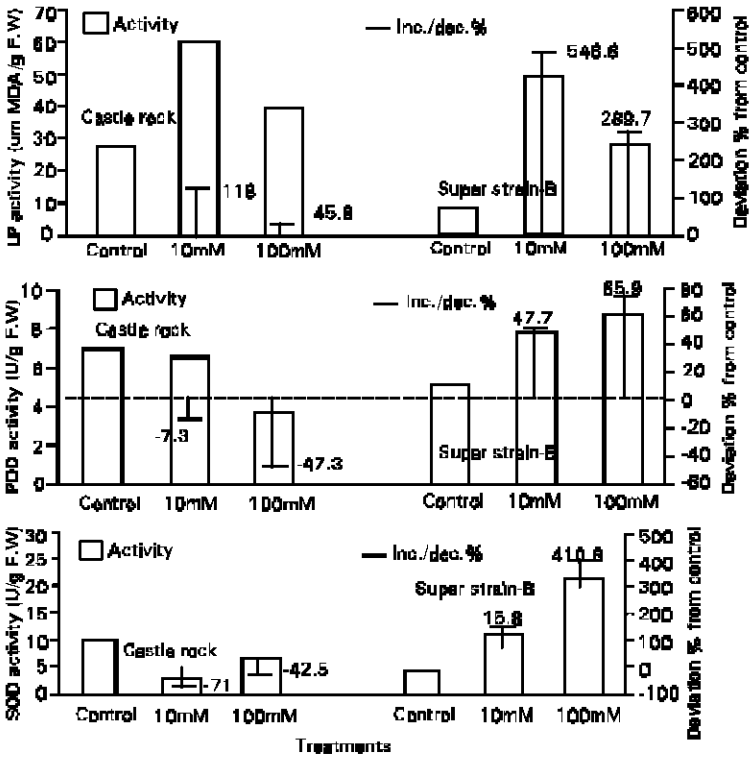

Fig. 3: Changes decrease-increase % of lipid peroxidation, POD and SOD activities in the leaves of Castle Rock and Super Strain –B under different levels of NaCl.

K even at high concentration of NaCl, which allows the maintenance of the transport of K and the limitation of Na in the shoots (Tattini *et al.*, 1992 & Carvajal *et al.*, 1999).

POD and SOD activities: POD level was not altered by both cultivars at 10 mmol NaCl. However Super Strain –B showed a pronounced increase at 100 mmol NaCl (8.6 units/g^{-1}FW) compared with Castle Rock cultivar (3.7 units/g^{-1}FW).

Total SOD level in relation to NaCl was increased much more in Super Strain- B than Castle Rock cultivar. It was approximately 4-fold than in Castle Rock at 10 and 100 mmol NaCl (Fig. 1). Increasing levels of antioxidant such as (POD and SOD) involved in super oxide detoxification have been also found by Del Rio *et al.* (1992).

Increases in SOD and POD in Super Strain- B may be attributed to the better protection of Super Strain -B, which seems to have a high capacity to protect cells against the toxic effects of O^{-}_{2}. radicals as a result of tissue injury (Reuveni *et al.*, 1993 & Hernandez *et al.*, 1994). The increase in POD and SOD levels in Super Strain- B cultivar under NaCl stress conditions result ed from the inherited capability (Gosset *et al.*, 1994a;1996) and reflect the changes in the mechanical properties of the cell wall which in turn, could be related to the salt adaptation process (Hernandez *et al.*, 1994).

Lipid peroxidation: Lipid peroxidation (MAD content) was lower in Super Strain -B than in Castle Rock cultivar under control conditions. Compared with control, NaCl stress 10-100mmol caused an increase in the levels of MAD content in both cultivars. As compared with Castle Rock cultivar, plants of Super Strain –B had much lower level of lipid peroxidation (49.12 and 28.75 μmol MAD/g FW) than Castle Rock (59.6 and 40.11 μmol MAD/g^{-1}FW) under both levels of NaCl (Fig. 2).

MAD in Super Strain -B cultivar seems to add more to its tolerance compared with Castle Rock cultivar, as it was more protected from oxidative damage under salt stress. In this regard, Scandalios (1993) found that active oxygen species bring about peroxidation of membrane lipids that lead to membrane damage (Zhang and Kirkham, 1996). As was observed in (Fig. 3) Super Strain -B cultivar expressed much higher increase in MAD accumulation (546.6% and 289.7%) than Castle Rock (118% and 45.8%) at different levels of NaCl compared to control treatment. Higher increases in POD activity (47.7%-65.9%) and (151.8%-410.8%) in SOD activity were observed in Super Strain-B. On contrary, the activities of POD and SOD in Castle Rock, were decreased by 7.3-47.3% and 71-42.5% respectively, under two levels of NaCl. This may be attributed to that salinity stress could modify the membrane structure and stimulate O^{-}_{2} radicals production, which facilitate the lipid peroxidation (MAD), one of the decomposition products of polly unsaturated fatty acids showing greater increase in total POD and low MAD that indicate the involvement of POD in all membrane integrity (Zhang and Kirkham, 1996).

Based on the results of this study, it can be concluded that exposure of Castle Rock and Super Strain -B cultivars to different levels of NaCl salinity stress resulted in different responses in growth, nutrients content and nutrient ratios, as well as the induction of peroxidase, superoxide dismutase activities, and the MAD content. Such alteration in the induction varied between cultivars. So, the results of the

present study proved that Super Strain-B cultivar was genetically more tolerant than Castle Rock. This was a result of inefficient uptake of Na leading to low values of Na/K ratio and low content of MAD and the induction of POD and SOD levels.

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