

# Salt resistance of tomato species grown in sand culture

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## ABSTRACT

In this study, Na<sup>+</sup>, Cl<sup>-</sup>, K<sup>+</sup>, Ca<sup>2+</sup>, chlorophyll and proline levels and the rate of lipid peroxidation level in terms of malondialdehyde (MDA), were investigated in tissues of 15 different tomato cultivars in salt tolerance. As a material, 15 different tomato genotypes were used during a 28-day period and 150 mmol NaCl was applied in sand culture. While one of tomato genotypes was a wild type belonging to *Lycopersicum peruvianum*, the others were local genotypes belonging to *Lycopersicum esculentum* L. Better NaCl-stress tolerance in salt-tolerant cultivars as compared to salt-sensitive cultivars observed during the present investigation might be due to restriction of Na<sup>+</sup> accumulation and ability to maintain high K<sup>+</sup>/Na<sup>+</sup> ratio in tissue. The chlorophyll level decreased more in salt-sensitive than in salt-resistant cultivars, whereas proline level increased more in salt-sensitive than in salt-resistant cultivars. The exposure to NaCl induced a significant increase in MDA level in both salt-resistant and salt-sensitive cultivars; yet, MDA level was higher in salt-sensitive cultivars. As a result, exclusion or inclusion of Na<sup>+</sup>, Cl<sup>-</sup>, K<sup>+</sup> and Ca<sup>2+</sup> MDA levels, chlorophyll and proline levels may play a key protective role against stress and these features can be used as identifiers for tolerance to salt.

**Keywords:** chlorophyll; ion accumulation; *Lycopersicum esculentum*; MDA; proline; salinity tolerance

Salt stress is recognized as a major factor that limits plant growth, physiology and productivity, mainly by inducing osmotic effects, ion-specific effects and oxidative stress (Okhovatian-Ardakani et al. 2010). Moreover, membrane stability, chlorophyll biosynthesis, carbon and nitrogen metabolism and rates of photosynthesis and respiration are also seen to be significantly hampered due to high salinity (Ashraf 2004). High-salt stress disrupts homeostasis in water potential and ion distribution at both the cellular and the whole plant levels (Errabii et al. 2007). Excess of Na<sup>+</sup> and Cl<sup>-</sup> ions may lead to conformational changes in the protein structure, while osmotic stress leads to turgor loss and cell volume change (Errabii et al. 2007). However, the precise mechanisms underlying these effects are not fully understood because the resistance to salt stress is a multigenic trait (Errabii et al. 2007). To achieve salt tolerance, plant cells evolve several biochemical and physiological pathways. These processes are thought to operate

additively to ensure plants and cells survival, and they include the exclusion of Na<sup>+</sup> ions and their compartmentation into vacuoles as well as accumulation of compatible solutes such as proline, glycinebetaine and polyols (Errabii et al. 2007).

Mechanisms of salt tolerance, not yet clear, can be, to some extent, explained by stress adaptation effectors that mediate ion homeostasis, osmolytic biosynthesis, toxic radical scavenging, water transport and long-distance response coordination (Jaleel et al. 2007).

Undoubtedly, plant breeders have made a significant achievement in the past few years, improving the salinity tolerance in a number of potential crops using artificial selection and conventional breeding approaches. However, most of the selection procedures were based on differences in agronomic characters (Ashraf and Harris 2004). Agronomic characters represent the combined genetic and environmental effects on plant growth and include the integration of the physiological mechanisms

conferring salinity tolerance. Typical agronomic selection parameters for salinity tolerance are yield, survival, plant height, leaf area, leaf injury, relative growth rate, and relative growth reduction (Ashraf and Harris 2004, Okhovatian-Ardakani et al. 2010). Many scientists suggested that selection is more convenient and practicable if the plant species possess distinctive indicators of salt tolerance at the whole plant, tissue or cellular level (Ashraf and Harris 2004). Physiological criteria are able to supply more objective information than agronomic parameters or visual assessment when screening for component traits of complex characters (Ashraf and Harris 2004). Some researches suggested that plant physiologists could improve the salt tolerance of plants only by defining genes or characters for geneticists or breeders to exploit (Ashraf and Harris 2004). However, knowledge of heritability and the genetic mode of salinity tolerance is lacking because few studies have yet been conducted in these areas. Indeed, genetic information is lagging behind the physiological information (Ashraf and Harris 2004). Despite a great deal of research into salinity tolerance of plants, mainly on water relations, photosynthesis and accumulation of various inorganic ions and organic metabolites (Ashraf and Harris 2004), the metabolic sites at which salt stress damages plants and, conversely, the adaptive mechanisms utilized by plants to survive saline stress are still not well understood. The main enigma is that there are no well-defined plant indicators for salinity tolerance that could practically be used by plant breeders for improvement of salinity tolerance in a number of important agricultural crops. This is partly due to the fact that the mechanisms of salt tolerance are so complex that variation occurs not only among species but, in many cases, also among cultivars within a single species (Ashraf and Harris 2004).

During the recent years, tissue culture has gained importance in the development of plants against various abiotic stresses as well as in elucidating mechanisms operating at the cellular level by which plants survive under various abiotic stresses including salinity (Jain et al. 2001). Plant tissue culture allows to control the stress homogeneity and to characterize the cell behaviour under stress conditions, independently of the regulatory systems that take place at the whole plant level (Lutts et al. 2004).

The objective of the present investigation was to study the effect of salinity stress on  $\text{Na}^+$ ,  $\text{Cl}^-$ ,  $\text{K}^+$ ,  $\text{Ca}^{2+}$  and proline contents, the rate of lipid peroxidation level in terms of malondialdehyde (MDA) and chlorophyll content in salt-sensitive

and -tolerant tomato cultivars in order to evaluate the relative significance of these parameters in imparting tolerance to NaCl oxidative stress.

## MATERIAL AND METHODS

In the experiment, the seeds of 15 tomato genotypes, 14 of which belonged to local *Lycopersicum esculentum* species (Table 1) and the other one belonged to *Lycopersicum peruvianum* wild species (PI-899-01), were used. The seeds were cleaned from bacteria and fungi by applying the superficial sterilization method of Ellis et al. (1988). Plants were placed in a climate chamber at  $25 \pm 2^\circ\text{C}$  and  $65 \pm 5\%$  humidity under 16/8 light/dark, 13 500 lux (90% fluorescent, 10% incandescent) in sand culture (with Arnon and Hoagland solution). When the root length of the seedlings reached 1 cm, magentas were placed in a 16 h light/8 h dark photoperiod. They were left to grow for 28 days in a media containing 150 mmol NaCl. At the end of the 28<sup>th</sup> day, the tissues harvested were kept in a deep freezer at  $-80^\circ\text{C}$  and homogenized into liquid nitrogen for the analyses.

A randomized parcels experimental design was carried out with three replications in the factorial order. Time, one of these three factors, has a replicated measurement quality. The factors were compared by the repeated measurement variance analysis from the view point of the properties concerned. The differences between the levels of the factors were examined by the least important difference (LID) multiple comparison method. The calculations were made by using MINITAB 13.0 statistical packet program. Statistica V.6.0 packet program was used for the multiple comparisons of the properties.

For ion measurements, tissues were first rinsed for 5 min with cool distilled water in order to remove free ions from the apoplasm without substantial elimination of cytosolic solutes. Tissues were oven-dried at  $80^\circ\text{C}$  for 48 h and then were ground. The dry matter obtained was used for mineral analysis. The major cations were extracted after digestion of dry matter with  $\text{HNO}_3$ . The extract was filtered prior to analysis.  $\text{Na}^+$  and  $\text{K}^+$  concentrations were determined using a flame spectrophotometer (Eppendorf flame photometer, PHF 90D, LeMans, France). The  $\text{Ca}^{2+}$  concentration was quantified by an atomic absorption spectrophotometer (Shimadzu AA-6200, Kyoto, Japan) (Guerrier and Patolia 1989). For  $\text{Cl}^-$  content estimation, ions were extracted with hot distilled

Table 1. Salt tolerant and sensitive species used in the experiment, respectively

Genotypes	Species	Location
PI-899-01	<i>Lycopersicum peruvianum</i>	Wild
TR-40361	<i>Lycopersicum esculentum</i>	Mardin/Kızıltepe
TR-40395	<i>Lycopersicum esculentum</i>	Diyarbakır/Seyhkent
TR-48938	<i>Lycopersicum esculentum</i>	Kastamonu/Tosya
TR-VF-12	<i>Lycopersicum esculentum</i>	
TR-49646	<i>Lycopersicum esculentum</i>	Kars/Kotek
TR-52414	<i>Lycopersicum esculentum</i>	
TR-H-2274	<i>Lycopersicum esculentum</i>	
TR-40397	<i>Lycopersicum esculentum</i>	Diyarbakır/Merkez
TR-47865	<i>Lycopersicum esculentum</i>	Sanlıurfa/Bozova
TR-52377	<i>Lycopersicum esculentum</i>	Erzurum/Tortum
TR-63233	<i>Lycopersicum esculentum</i>	İzmir
TR-52428	<i>Lycopersicum esculentum</i>	Erzurum/Tortum
TR-61697	<i>Lycopersicum esculentum</i>	Mugla/Milas
TR-52361	<i>Lycopersicum esculentum</i>	Kars/Kotek

water (80°C) during 2 h. Chloride was determined (Buchler-Cotlove chloridometer) as described by Taleisnik et al. (1997).

One plant per replicate was used for chlorophyll determination. Prior to extraction, fresh leaf samples were cleaned with deionized water to remove any surface contamination. Chlorophyll extraction was carried out on fresh fully expanded leaf material; 1 g leaf sample was ground in 90% acetone using a pestle and mortar. The absorbance was measured with a UV/Visible spectrophotometer (Pye Unicam SP6-550, UK) and chlorophyll concentrations were calculated using the equation proposed by (Luna et al. 2000).

Lipid peroxidation in leaf tissue was colorimetrically determined by measuring malondialdehyde (MDA), a major thiobarbituric acid reactive species (TBARS) and product of lipid peroxidation according to Lutts et al. (1996). Levaes (0.2 g) were ground in 5 ml of trichloroacetic acid (TCA, 0.1%, w/v). The homogenate was centrifuged at 12 000 g for 20 min and 3 ml of the supernatant was added to 3 ml 0.5% thiobarbituric acid (TBA) in 20% TCA. The mixture was heated at 95°C for 30 min, and the reaction was stopped by putting the reaction tubes in an ice bath. The samples were then centrifuged at 12 000 g for 20 min. Absorbance of the supernatant was measured at 532 nm and the amount of nonspecific absorption at 600 nm was subtracted from this value using a spectrophotometer (Analytic Jena 40 Model). The amount of MDA was calculated from the extinction coefficient of 155 mmol/cm.

Proline was also determined spectrophotometrically following the ninhydrin method described by Bates et al. (1973) using L-proline as a standard. 0.2 g of leaf sample was homogenized in 10 ml of 3% (w/v) aqueous sulphosalicylic acid and filtered. In 2 ml of the filtrate, 2 ml of acid ninhydrin was added, followed by the addition of 2 ml of glacial acetic acid and boiled for 60 min. The mixture was extracted with toluene, and the free proline was quantified spectrophotometrically at 520 nm from the organic phase using a Shimadzu spectrophotometer (Duisburg, Germany).

Tables indicate mean values  $\pm$  SE. Differences between the values for control and treated leaves were analyzed by one-way ANOVA, taking  $P < 0.001$  as significance level, according to LSD multiple range tests.

## RESULTS

In the absence of stress, in all tissues, the  $\text{Na}^+$  level differed significantly ( $P < 0.001$ ) among the cultivars and was lower in salt-resistant than in salt-sensitive cultivars. The exposure to NaCl induced a significant increase in  $\text{Na}^+$  (Table 2) levels in both salt-resistant and salt-sensitive cultivars; yet,  $\text{Na}^+$  levels were higher in salt-sensitive cultivars. In the absence and present of stress, in all tissues,  $\text{Cl}^-$  level differed significantly ( $P < 0.001$ ) among the cultivars. The exposure to NaCl induced a significant increase in  $\text{Cl}^-$  (Table 3) levels in both salt-resistant and salt-sensitive cultivars.

Table 2. Na<sup>+</sup> (mg/g dry weight) contents in root, stems and leaves of the *Lycopersicum esculentum* species in the presence of 150 mmol NaCl. Results are expressed as mean  $\pm$  SE ( $n = 3$ )

Genotype	Root		Stem		Leaf	
	control	salt	control	salt	control	salt
PI-899-01	3.8 $\pm$ 0.4	5.0 $\pm$ 0.6	3.6 $\pm$ 0.3	4.5 $\pm$ 0.6	3.3 $\pm$ 3	4.2 $\pm$ 0.4
TR-40361	4.5 $\pm$ 0.8	7.2 $\pm$ 1.3	4.2 $\pm$ 0.7	6.8 $\pm$ 1.2	4.0 $\pm$ 7	6.4 $\pm$ 1.0
TR-40395	8.8 $\pm$ 0.3	12.0 $\pm$ 0.6	8.3 $\pm$ 0.3	11.3 $\pm$ 0.5	7.8 $\pm$ 3	11.5 $\pm$ 0.5
TR-48938	10 $\pm$ 0.3	16.0 $\pm$ 0.4	9.4 $\pm$ 0.3	15.0 $\pm$ 0.4	8.8 $\pm$ 2	14.0 $\pm$ 0.4
TR-VF-12	9.9 $\pm$ 0.2	15.9 $\pm$ 0.3	9.3 $\pm$ 0.2	14.8 $\pm$ 0.2	8.8 $\pm$ 1	14.0 $\pm$ 0.2
TR-49646	8.0 $\pm$ 0.5	12.8 $\pm$ 0.8	7.5 $\pm$ 0.4	12.0 $\pm$ 0.7	7.1 $\pm$ 4	11.3 $\pm$ 0.7
TR-52414	5.5 $\pm$ 0.2	8.8 $\pm$ 0.4	5.2 $\pm$ 0.2	8.3 $\pm$ 0.4	4.9 $\pm$ 2	7.8 $\pm$ 0.3
TR-H 2274	11 $\pm$ 0.7	17.8 $\pm$ 1.0	11.0 $\pm$ 0.5	16.0 $\pm$ 1.0	8.9 $\pm$ 4	12.9 $\pm$ 0.9
TR-40397	11 $\pm$ 0.3	18.0 $\pm$ 0.5	11.0 $\pm$ 0.3	17.0 $\pm$ 0.5	9.9 $\pm$ 3	16.0 $\pm$ 0.4
TR-47865	7.9 $\pm$ 0.4	12.6 $\pm$ 0.6	7.4 $\pm$ 0.3	11.8 $\pm$ 0.5	6.9 $\pm$ 3	11.0 $\pm$ 0.5
TR-52377	6.8 $\pm$ 0.5	10.9 $\pm$ 0.8	6.4 $\pm$ 0.5	10.3 $\pm$ 0.8	6.0 $\pm$ 5	9.7 $\pm$ 0.7
TR-63233	11 $\pm$ 0.4	15.8 $\pm$ 0.6	9.9 $\pm$ 0.4	14.8 $\pm$ 0.6	8.3 $\pm$ 4	11.8 $\pm$ 0.6
TR-52428	10 $\pm$ 0.4	16.4 $\pm$ 0.6	10.0 $\pm$ 0.1	15.5 $\pm$ 0.6	9.4 $\pm$ 1	14.5 $\pm$ 0.5
TR-61697	6.8 $\pm$ 0.6	10.9 $\pm$ 1.0	6.4 $\pm$ 0.6	10.0 $\pm$ 0.9	6.0 $\pm$ 5	9.6 $\pm$ 0.9
TR-52361	8.5 $\pm$ 0.3	13.6 $\pm$ 0.4	8.0 $\pm$ 0.2	12.8 $\pm$ 0.4	7.5 $\pm$ 2	12.0 $\pm$ 0.4
LSD (genotype $\times$ NaCl treatment) ( $P < 0.001$ )	2.2		2.3		2.64	

In the absence of stress, in all tissues, the K<sup>+</sup> level differed significantly ( $P < 0.001$ ) among the cultivars and was lower in salt-sensitive than in

salt-resistant cultivars. The exposure to NaCl induced a significant increase in K<sup>+</sup> (Table 4) levels in both salt-resistant and salt-sensitive cultivars,

Table 3. Cl<sup>-</sup> (mg/g dry weight) contents in root, stems and leaves of the *Lycopersicum esculentum* species in the presence of 150 mmol NaCl. Results are expressed as mean  $\pm$  SE ( $n = 3$ )

Genotype	Root		Stem		Leaf	
	control	salt	control	salt	control	salt
PI-899-01	2.0 $\pm$ 0.1	3.0 $\pm$ 0.1	20.0 $\pm$ 0.1	29.0 $\pm$ 0.1	12.0 $\pm$ 0.1	18.0 $\pm$ 0.1
TR-40361	5.0 $\pm$ 0.1	11.0 $\pm$ 0.1	5.0 $\pm$ 0.1	10.0 $\pm$ 0.1	4.0 $\pm$ 0.1	10.0 $\pm$ 0.1
TR-40395	9.0 $\pm$ 0.1	15.0 $\pm$ 0.1	8.0 $\pm$ 0.1	15.0 $\pm$ 0.1	7.0 $\pm$ 0.1	14.0 $\pm$ 0.1
TR-48938	11.0 $\pm$ 0.1	15.0 $\pm$ 0.1	10.0 $\pm$ 0.1	14.0 $\pm$ 0.1	10.0 $\pm$ 0.1	20.0 $\pm$ 0.1
TR-VF-12	7.0 $\pm$ 0.1	14.0 $\pm$ 0.1	6.0 $\pm$ 0.1	13.0 $\pm$ 0.1	6.0 $\pm$ 0.1	7.0 $\pm$ 0.1
TR-49646	3.0 $\pm$ 0.1	7.0 $\pm$ 0.1	3.0 $\pm$ 0.1	7.0 $\pm$ 0.1	3.0 $\pm$ 0.1	6.0 $\pm$ 0.1
TR-52414	5.0 $\pm$ 0.1	11.0 $\pm$ 0.1	5.0 $\pm$ 0.1	11.0 $\pm$ 0.1	5.0 $\pm$ 0.1	10.0 $\pm$ 0.1
TR-H-2274	2.0 $\pm$ 0.1	5.0 $\pm$ 0.1	2.0 $\pm$ 0.1	7.0 $\pm$ 0.1	2.0 $\pm$ 0.1	5.0 $\pm$ 0.1
TR-40397	10.0 $\pm$ 0.1	23.0 $\pm$ 0.1	9.0 $\pm$ 0.1	22.0 $\pm$ 0.1	8.0 $\pm$ 0.1	20.0 $\pm$ 0.1
TR-47865	5.0 $\pm$ 0.1	10.0 $\pm$ 0.1	4.0 $\pm$ 0.1	10.0 $\pm$ 0.1	4.0 $\pm$ 0.1	9.0 $\pm$ 0.1
TR-52377	4.0 $\pm$ 0.1	10.0 $\pm$ 0.1	8.0 $\pm$ 0.1	14.0 $\pm$ 0.1	4.0 $\pm$ 0.1	10.0 $\pm$ 0.1
TR-63233	3.0 $\pm$ 0.1	6.0 $\pm$ 0.1	3.0 $\pm$ 0.1	8.0 $\pm$ 0.1	2.0 $\pm$ 0.1	5.0 $\pm$ 0.1
TR-52428	10.0 $\pm$ 0.1	22.0 $\pm$ 0.1	10.0 $\pm$ 0.1	21.0 $\pm$ 0.1	9.0 $\pm$ 0.1	20.0 $\pm$ 0.1
TR-61697	5.0 $\pm$ 0.1	11.0 $\pm$ 0.1	5.0 $\pm$ 0.1	11.0 $\pm$ 0.1	5.0 $\pm$ 0.1	10.0 $\pm$ 0.1
TR-52361	2.0 $\pm$ 0.1	6.0 $\pm$ 0.1	2.0 $\pm$ 0.1	5.0 $\pm$ 0.1	2.0 $\pm$ 0.1	5.0 $\pm$ 0.1
LSD (genotype $\times$ NaCl treatment) ( $P < 0.001$ )	2.5		2.3		1.8	

Table 4. K<sup>+</sup> (mg/g dry weight) contents in root, stems and leaves of the *Lycopersicum esculentum* species in the presence of 150 mmol NaCl. Results are expressed as mean ± SE (*n* = 3)

Genotype	Root		Stem		Leaf	
	control	salt	control	salt	control	salt
PI-899-01	4.2 ± 0.1	6.7 ± 0.1	4.0 ± 0.1	6.2 ± 0.1	3.8 ± 0.1	5.8 ± 0.1
TR-40361	2.3 ± 0.1	3.6 ± 0.1	2.1 ± 0.1	3.4 ± 0.1	2.2 ± 0.1	3.2 ± 0.1
TR-40395	2.1 ± 0.1	3.4 ± 0.1	1.9 ± 0.1	3.3 ± 0.1	1.8 ± 0.1	3.1 ± 0.1
TR-48938	1.8 ± 0.1	2.9 ± 0.1	1.7 ± 0.1	2.7 ± 0.1	1.6 ± 0.1	2.5 ± 0.1
TR-VF-12	2.7 ± 0.1	4.2 ± 0.1	2.6 ± 0.1	4.0 ± 0.1	2.4 ± 0.1	3.7 ± 0.1
TR-49646	2.1 ± 0.1	3.3 ± 0.1	1.9 ± 0.1	3.1 ± 0.1	1.8 ± 0.1	2.9 ± 0.1
TR-52414	2.8 ± 0.1	4.5 ± 0.1	2.6 ± 0.1	4.2 ± 0.1	2.5 ± 0.1	3.9 ± 0.1
TR-H-2274	1.8 ± 0.1	3.0 ± 0.1	1.7 ± 0.1	2.7 ± 0.1	1.6 ± 0.1	2.5 ± 0.1
TR-40397	1.8 ± 0.1	2.9 ± 0.1	1.7 ± 0.1	2.7 ± 0.1	1.7 ± 0.1	2.5 ± 0.1
TR-47865	2.0 ± 0.1	3.3 ± 0.1	1.9 ± 0.1	3.0 ± 0.1	1.8 ± 0.1	2.8 ± 0.1
TR-52377	2.9 ± 0.1	4.6 ± 0.1	2.7 ± 0.1	4.3 ± 0.1	2.5 ± 0.1	4.1 ± 0.1
TR-63233	1.9 ± 0.1	3.1 ± 0.1	1.9 ± 0.1	2.9 ± 0.1	1.7 ± 0.1	2.8 ± 0.1
TR-52428	3.0 ± 0.1	4.7 ± 0.1	2.8 ± 0.1	4.5 ± 0.1	2.6 ± 0.1	4.2 ± 0.1
TR-61697	2.1 ± 0.1	3.5 ± 0.1	1.9 ± 0.1	3.2 ± 0.1	1.8 ± 0.1	3.4 ± 0.1
TR-52361	2.1 ± 0.1	3.3 ± 0.1	1.9 ± 0.1	3.1 ± 0.1	1.7 ± 0.1	2.9 ± 0.1
<i>LSD</i> (genotype × NaCl treatment) ( <i>P</i> < 0.001)	0.6		0.5		0.5	

yet K<sup>+</sup> levels were higher in salt-resistant cultivars and K<sup>+</sup> variation was lower than that of Na<sup>+</sup>, Cl<sup>-</sup> and Ca<sup>2+</sup>.

In the absence of stress, in all tissues, the Ca<sup>2+</sup> level differed significantly (*P* < 0.001) among the cultivars and was lower in salt-sensitive than in

Table 5. Ca<sup>2+</sup> (mg/g dry weight) contents in root, stems and leaves of the *Lycopersicum esculentum* species in the presence of 150 mmol NaCl. Results are expressed as mean ± SE (*n* = 3)

Genotype	Root		Stem		Leaf	
	control	salt	control	salt	control	salt
PI-899-01	37 ± 1.6	65 ± 0.7	35 ± 0.6	65 ± 0.6	23 ± 0.9	38 ± 1.2
TR-40361	57 ± 1.5	45 ± 1.2	51 ± 1.1	59 ± 1.2	45 ± 1.1	56 ± 1.2
TR-40395	45 ± 1.4	58 ± 1.2	39 ± 0.1	51 ± 0.4	25 ± 0.2	33 ± 0.4
TR-48938	41 ± 1.2	63 ± 1.4	35 ± 1.2	45 ± 0.5	26 ± 0.5	34 ± 1.2
TR-VF-12	45 ± 1.3	56 ± 1.1	39 ± 1.2	51 ± 1.1	31 ± 1.1	39 ± 0.6
TR-49646	53 ± 1.9	58 ± 1.6	45 ± 1.7	55 ± 0.6	38 ± 0.8	48 ± 0.5
TR-52414	52 ± 2.3	60 ± 1.8	44 ± 1.2	52 ± 0.6	37 ± 0.8	46 ± 0.8
TR-H-2274	58 ± 2.1	69 ± 1.6	54 ± 0.3	64 ± 0.5	46 ± 0.4	58 ± 0.5
TR-40397	55 ± 1.7	63 ± 1.5	51 ± 0.4	58 ± 0.5	35 ± 0.3	45 ± 0.6
TR-47865	54 ± 2.4	66 ± 1.1	47 ± 1.3	58 ± 0.4	39 ± 0.5	49 ± 1.3
TR-52377	32 ± 1.4	42 ± 1.5	26 ± 1.8	35 ± 0.8	18 ± 1.2	28 ± 0.9
TR-63233	36 ± 2.2	53 ± 1.8	31 ± 0.8	45 ± 0.9	27 ± 1.2	35 ± 0.6
TR-52428	36 ± 1.8	62 ± 1.3	28 ± 0.6	51 ± 1.2	21 ± 0.8	34 ± 0.5
TR-61697	57 ± 0.9	68 ± 1.2	48 ± 0.5	58 ± 0.8	41 ± 1.1	51 ± 0.5
TR-52361	39 ± 1.8	64 ± 1.2	31 ± 0.8	57 ± 0.9	24 ± 0.4	31 ± 0.3
<i>LSD</i> (genotype × NaCl treatment)	1.7 ( <i>P</i> < 1.2)		0.95 ( <i>P</i> < 0.6)		0.8 ( <i>P</i> < 0.7)	



Table 6. Chlorophyll (mg/g fresh weight) and malondialdehyde (MDA) ( $\mu\text{mol/g}$  fresh weight) levels in leaves of the *Lycopersicum esculentum* species in the presence of 150 mmol NaCl. Results are expressed as mean  $\pm$  SE ( $n = 3$ )

Genotype	Chlorophyll		MDA	
	control	salt	control	salt
PI-899-01	331 $\pm$ 0.2	312 $\pm$ 0.1	32 $\pm$ 0.2	33 $\pm$ 0.2
TR-40361	243 $\pm$ 0.1	146 $\pm$ 0.2	24 $\pm$ 0.1	43 $\pm$ 0.1
TR-40395	244 $\pm$ 0.2	167 $\pm$ 0.1	25 $\pm$ 0.1	43 $\pm$ 0.2
TR-48938	263 $\pm$ 0.1	158 $\pm$ 0.3	25 $\pm$ 0.3	42 $\pm$ 0.1
TR-VF-12	281 $\pm$ 0.3	156 $\pm$ 0.1	25 $\pm$ 0.3	44 $\pm$ 0.2
TR-49646	228 $\pm$ 0.2	137 $\pm$ 0.1	25 $\pm$ 0.3	43 $\pm$ 0.4
TR-52414	217 $\pm$ 0.2	134 $\pm$ 0.1	24 $\pm$ 0.4	43 $\pm$ 0.5
TR-H-2274	168 $\pm$ 0.1	86.2 $\pm$ 0.3	28 $\pm$ 0.1	48 $\pm$ 0.1
TR-40397	265 $\pm$ 0.1	159 $\pm$ 0.2	25 $\pm$ 0.2	43 $\pm$ 0.1
TR-47865	288 $\pm$ 0.4	173 $\pm$ 0.1	25 $\pm$ 0.2	42 $\pm$ 0.3
TR-52377	212 $\pm$ 0.1	127 $\pm$ 0.3	27 $\pm$ 0.1	44 $\pm$ 0.1
TR-63233	167 $\pm$ 0.3	92.1 $\pm$ 0.3	35 $\pm$ 0.1	66 $\pm$ 0.2
TR-52428	248 $\pm$ 0.1	147 $\pm$ 0.4	26 $\pm$ 0.1	43 $\pm$ 0.1
TR-61697	211 $\pm$ 0.2	126 $\pm$ 0.1	25 $\pm$ 0.4	45 $\pm$ 0.2
TR-52361	250 $\pm$ 0.4	152 $\pm$ 0.1	25 $\pm$ 0.2	44 $\pm$ 0.4
LSD (genotype $\times$ NaCl treatment) ( $P < 0.01$ )	1.33		1.55	

salt-resistant cultivars. The exposure to NaCl induced a significant increase in  $\text{Ca}^{2+}$  level (Table 5) in both salt-resistant and salt-sensitive cultivars (especially in the wild type).

In the absence of stress, the chlorophyll level in leaf tissue differed significantly ( $P < 0.01$ ) among the cultivars and was higher in salt-resistant than in salt-sensitive cultivars. The exposure to NaCl induced a significant decrease in chlorophyll level (Table 6) in both salt-resistant and salt-sensitive cultivars; yet, the chlorophyll level was more decreased in salt-sensitive than in salt-resistant cultivars.

The lipid peroxidation levels in leaves of 15 tomato cultivars, measured as the content of MDA, are given in Table 6. In the absence of stress, the MDA level differed significantly ( $P < 0.01$ ) among the cultivars and was higher in salt-resistant than in salt-sensitive cultivars. The exposure to NaCl induced a significant increase in the MDA level in salt-sensitive cultivars compared to a lower increase in salt-resistant wild type.

In the absence of stress, the proline level differed significantly ( $P < 0.01$ ) among the cultivars and was higher in salt-resistant than in salt-sensitive cultivars. The exposure to NaCl induced a signifi-

Table 7. Proline levels ( $\mu\text{mol/g}$  fresh weight) in leaves of the *Lycopersicum esculentum* species in the presence of 150 mmol NaCl. Results are expressed as mean  $\pm$  SE ( $n = 3$ )

Genotype	Proline	
	control	salt
PI-899-01	31 $\pm$ 0.2	34 $\pm$ 0.2
TR-40361	25 $\pm$ 0.1	36 $\pm$ 0.1
TR-40395	24 $\pm$ 0.1	35 $\pm$ 0.2
TR-48938	24 $\pm$ 0.3	37 $\pm$ 0.1
TR-VF-12	24 $\pm$ 0.3	35 $\pm$ 0.2
TR-49646	25 $\pm$ 0.3	38 $\pm$ 0.4
TR-52414	23 $\pm$ 0.4	35 $\pm$ 0.5
TR-H-2274	27 $\pm$ 0.1	42 $\pm$ 0.1
TR-40397	23 $\pm$ 0.2	34 $\pm$ 0.1
TR-47865	24 $\pm$ 0.2	35 $\pm$ 0.3
TR-52377	27 $\pm$ 0.1	41 $\pm$ 0.1
TR-63233	32 $\pm$ 0.1	48 $\pm$ 0.2
TR-52428	25 $\pm$ 0.1	37 $\pm$ 0.1
TR-61697	24 $\pm$ 0.4	44 $\pm$ 0.2
TR-52361	26 $\pm$ 0.2	39 $\pm$ 0.4
LSD (genotype $\times$ NaCl treatment) ( $P < 0.01$ )	1.55	

cant increase in the proline level (Table 7) in both salt-resistant and salt-sensitive cultivars. But the proline level was more increased in salt-sensitive than in salt-resistant cultivars.

## DISCUSSION

NaCl is the dominant salt, and the high concentration of ions, particularly  $\text{Na}^+$  in the growing medium can, if taken up by the plant, result in cytoplasmic toxicity (Parvaiz and Satyawati 2008). An excess of  $\text{Na}^+$  disturbs cellular ion homeostasis, and leads to other effects such as interference in enzyme activities and oxidative stress (Afzal et al. 2008, Okhovatian-Ardakani et al. 2010).  $\text{Cl}^-$  is the most prevalent anion under saline conditions. To prevent these problems, plants suppress the influx and accumulation of  $\text{Na}^+$  in the cytoplasm, especially in leaves (Esmaili et al. 2008, Patel and Pandey 2008). We found that, in all tissues under salt stress, the  $\text{Na}^+$  and  $\text{Cl}^-$  contents increased dramatically in salt-sensitive cultivars. Even under the control conditions, in all tissues  $\text{Na}^+$  contents in the salt-sensitive plants were twice higher than those in the salt-resistant plants (Tables 2 and 3). Some researchers found high accumulation of  $\text{Cl}^-$  in the leaves of salt-sensitive lines of barley. In most crop species salt tolerance was found to be associated with the accumulation of only low amounts of both  $\text{Na}^+$  and  $\text{Cl}^-$  in the shoots (Ashraf 2004). Salt tolerance in most plants is associated with low uptake and accumulation of  $\text{Na}^+$ , which is mediated through the control of influx and/or by active efflux from the cytoplasm to the vacuoles and also back to the growth medium (Ashraf 2004). The fact that NaCl affected the relative growth rate value at the least extent is mostly due to the selective accumulation of  $\text{Na}^+$  and  $\text{Cl}^-$  ions. A moderate increase of  $\text{Na}^+$  and  $\text{Cl}^-$  within callus tissue might avoid water loss and ensure an economic way to adjust osmotically (Errabii et al. 2007). However, when the ability of the cells to compartmentalize the ions into the vacuole is exceeded, ions build up in the cytoplasm and lead to severe ion imbalances and to conformational changes in the plasma membrane electrical potential (Errabii et al. 2007). The salt-resistant plants accumulated less  $\text{Na}^+$  and  $\text{Cl}^-$  compared to the salt-sensitive ones.

Under saline conditions, due to excessive amounts of exchangeable  $\text{Na}^+$ , high  $\text{Na}^+/\text{K}^+$  and  $\text{Na}^+/\text{Ca}^{2+}$  ratios occur in the soil. Plants subjected to such environments, take up high amounts of

$\text{Na}^+$  (Esmaili 2008), whereas the uptake of  $\text{K}^+$  and  $\text{Ca}^{2+}$  is considerably reduced (Patel and Pandey 2008). Reasonable amounts of both  $\text{K}^+$  and  $\text{Ca}^{2+}$  are required to maintain the integrity and functioning of cell membranes (Ashraf 2004). In *Agropyron* spp., the high salt tolerance of *A. elongatum* relative to *A. intermedium*, is associated with its higher uptake of  $\text{K}^+$  under saline conditions (Ashraf 2004, Afzal et al. 2008, Patel and Pandey 2008). Researchers concluded that roots of the salt-tolerant *Beta vulgaris* had a greater affinity for  $\text{K}^+$  relative to  $\text{Na}^+$  than did the salt-sensitive *Phaseolus vulgaris*. Similarly, in soybean, researchers found that the salt-tolerant cultivar Lee accumulated more  $\text{K}^+$  in its leaves than did the salt-sensitive cultivar Jackson (Ashraf 2004). In this study, we showed that only the salt-tolerant cultivars maintained higher  $\text{K}^+$  contents and lower  $\text{Na}^+$  contents in the all tissues of the plant (Table 4). Furthermore, salt-tolerant cultivars excluded approximately three times more  $\text{Na}^+$  than did the salt-sensitive plants. These results suggest that salt-tolerant cultivars have a mechanism to select potassium in the presence of sodium, and that this ability is related to maintain high potassium contents under salt stress (Esmaili et al. 2008).

Calcium is known to play a crucial role in maintaining the structural and functional integrity of plant membranes in addition to its considerable roles in cell wall stabilization, regulation of ion transport and selectivity and activation of cell wall enzymes (Mengel and Kirkby 1987, Rengel 1992). The maintenance of calcium acquisition and transport under salt stress is an important determinant of salinity tolerance (Afzal et al. 2008). In most cases salt tolerance of a crop cultivar can be increased by an increase in the  $\text{Ca}^{2+}$  concentration in the saline growth medium. For example, supplemental calcium alleviated the adverse effect of salt stress on the germination and vegetative growth of bean (Awada et al. 1995) and pigeonpea (Subbarao et al. 1990). Some researchers reported that supplemental  $\text{Ca}^{2+}$  in the presence of salinity improved the growth of *Brassica juncea* and *B. napus* but not that of *B. carinata* and *B. rapa*, showing genotypical difference in the plant growth responses to the addition of  $\text{Ca}^{2+}$ .  $\text{Ca}^{2+}$  maintenance and  $\text{Na}^+$  exclusion, which is related to salinity tolerance in *Lycopersicon esculentum*, were genetically controlled with additive major genetic components (Afzal et al. 2008, Esmaili et al. 2008). In this study, we showed that only the salt-tolerant cultivars maintained higher  $\text{Ca}^{2+}$  contents in all tissues of the plant (Table 5).

Salinity reduces net photosynthetic rate, transpiration rate, and stomatal conductance in plant species and degrades chlorophyll at high salinity. Salinity tolerance is related to the maintenance of net photosynthetic rate and stomatal conductance and to elevated chlorophyll concentration (Ashraf 2004). High salinity reduced the leaf chlorophyll content in all genotypes, which might be due to the increased activity of chlorophyllase (Megdiche et al. 2007). A reduction in total chlorophyll contents was observed in the NaCl-stressed *Catharanthus* plants when compared to control. The reduction in leaf chlorophyll under salinity was attributed to the destruction of the chlorophyll pigments and the instability of the pigment protein complex (Jaleel et al. 2007). Some researchers reported chlorophyll content as one of the parameters of salt tolerance in crop plants. Others observed higher chlorophyll degradation in a sodium chloride-sensitive pea cultivar as compared to a tolerant one (Sairam et al. 2005). Our results suggest that in the absence of stress, chlorophyll concentration in leaf tissue differed significantly ( $P < 0.01$ ) among the cultivars and was higher in salt-resistant than in salt-sensitive cultivars. The exposure to NaCl induced a significant decrease in chlorophyll concentration (Table 6) both in salt-resistant and salt-sensitive cultivars; yet, chlorophyll concentration was more decreased in salt-sensitive than in salt-resistant cultivars.

The exposure to NaCl induced significantly an increase in MDA concentration in both salt-resistant and salt-sensitive cultivars. However, MDA concentration was higher in salt-sensitive cultivars. The level of MDA produced during peroxidation of membrane lipids, is often used as an indicator of oxidative damage. The lower level of lipid peroxidation in salt-resistant cultivars compared to salt-sensitive cultivars suggests that it may have a better protection against oxidative damage under salt stress. In our study, we found that salt-sensitive cultivars accumulate MDA than salt-resistant cultivars (Table 6). There are reports of higher increase in the amount of MDA with the increase in salt stress in the salt-sensitive cultivar as compared to tolerant cultivar of rice and in roots of *Lemna minor* (Khan and Panda 2008). The better NaCl stress tolerance in salt-tolerant cultivars as compared to salt-sensitive cultivars observed during present investigation may be due to the restriction of damage of cellular membranes with lower MDA and  $H_2O_2$  content. Higher  $H_2O_2$  accumulation and lipid peroxidation in sensitive cultivars of pea and rice were reported earlier.

Salinity-induced increase in lipid peroxidation was also reported by some researchers (Sairam et al. 2005).

Proline is one of the most important osmoprotectant in plants. Under salt stress most plant species exhibit a remarkable increase in their proline content (Patel and Pandey 2008, Dasgan et al. 2009). In our experiments we also observed a similar behaviour in the leaves of tomato seedlings. Supporting findings come from other leguminous plants (alfalfa, soybean and pea) and sugar beet where salt stress resulted in extensive proline accumulation. Likewise, in tomato salt tolerance was attributed to the degree of plant to accumulate osmoprotectants, like proline. Proline is generally assumed to serve as a physiologically compatible solute that increases as needed to maintain a favorable osmotic potential between the cell and its surroundings (Patel and Pandey 2008, Dasgan et al. 2009). Nevertheless, the levels of proline in leaves of salt-sensitive plants under salt stress were higher than in salt-resistant cultivars. Also some researchers report a negative correlation between proline accumulation and salt tolerance in tomato and *Aegiceras corniculatum* (Parvaiz and Satyawati 2008). However, accumulation of proline in rice plants grown under salt stress was due to salt injury and not as an indication of salt tolerance (Parvaiz and Satyawati 2008). Similarly, accumulation of proline in two sorghum genotypes contrasting in salt tolerance suggested that proline accumulation was a reaction to salt stress and not a plant response associated with tolerance (Parvaiz and Satyawati 2008). In another experiment, researchers showed that under salt stress, higher concentration of proline was accumulated in sensitive rice cultivars than in tolerant genotypes (Parvaiz and Satyawati 2008).

As a conclusion, the salt-resistant cultivars accumulated less  $Na^+$  and  $Cl^-$  than the salt-sensitive cultivars. Salt-tolerant cultivars have a mechanism to select  $K^+$  in the presence of  $Na^+$ , and this ability is related to maintaining high  $K^+$  contents under salt stress. Only the salt-tolerant cultivars maintained higher  $Ca^{2+}$  contents in all tissues of the plant. Chlorophyll concentration was more decreased in salt-sensitive cultivars than in salt-resistant cultivars. Increased proline content may have an important role in maintaining tolerance to salt stress. Higher  $H_2O_2$  accumulation and lipid peroxidation in sensitive cultivars are a result of salt stress. These features (ion accumulation, chlorophyll, proline and MDA contents) as can be used as identifiers for tolerance to salt.



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