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Examination of the impact of salt weight on the cell reinforcement compound exercises on the youthful and old leaves of salsola (*Stenoptera*) and tomato (*Lycopersicon esculentum* L.)

Mahmut Doğan

Harran University, Turkey.

Abstract

We can define all negative factors affecting plant growth as stress. We can say that one of the most important problems among stress factors is salt stress. Although, many researches have been done regarding the effects of salt stress, especially the different responses to salt stress among young and old leaves are being investigated nowadays. For this purpose, responses to salt stress on young and old leaves of salsola (*Stenoptera*) plant which is considered halophyte, and tomato (*Lycopersicon esculentum* L.) which is considered glycophyte has been investigated. According to the results of the research, based on chlorophyl, MDA, ions (Na^+, K^+, Ca^{+2} and Cl^-) and enzyme (reduced glutathione (GR) ascorbate peroxidase (APX, EC 1.11.1.11) superoxide dismutase (SOD, EC 1.15.1.1) and catalase (CAT, EC 1.11.1.6) amounts, it was determined that young upper leaves gave positive responses compared to old lower leaves, and young upper leaves were more resistant.

Keywords: Salsola, tomato, salt stress, antioxidant enzymes.

INTRODUCTION

All the factors that inhibit plant growth are defined as stresses. Drought, saltiness, excess irrigation, high or low temperature, pH and heavy metals are common sources of stress. Those stresses create social and economic problems, especially in developing countries. Only 10% of the land that can be used for agriculture in the world isn't under the effect of any environmental stress element. For the rest 90%, the most common stress element is drought with 26%, followed by salt stress by
20% (Blum, 1985; Ashraf, 1994). Researches on the solutions for the nutrition problems arising from the fast population increase in the world, the aim is to create plant types that can be grown under unfavorable environmental conditions. Salt stress induces various biochemical and physiological responses in plants and affects almost all plant processes (Nemoto and Sasakuma, 2002; Megdiche et al., 2007). Salinity may cause hyperionic and hyperosmotic effects in plants leading to membrane disorganization, increase in reactive oxygen species (ROS) levels, and metabolic toxicity (Jaleel et al., 2007). High-salt stress disrupts the homeostasis in water potential and ion distribution at both the cellular and the whole plant levels (Errabii et al., 2007). Excess of Na^+ and Cl^- 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^+ ions and their compartmentation into vacuoles as

Abbreviations: MDA, malondialdehyde; **TBARS,** thiobarbituric acid reactive species; **TCA,** trichloroacetic acid; TBA, thiobarbituric acid; **ROS,** reactive oxygen species; **SOD,** superoxide dismutase; **APX,** ascorbate peroxidase; **CAT,** catalase; **GR,** glutathione reductase; **GST,** glutathione transferase.

well as accumulation of compatible solutes such as proline, glycinebetaine, and polyols (Errabii et al., 2007). In order to survive under stress conditions, plants are equipped with oxygen radical-detoxifying enzymes such as superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT), and glutathione reductase (GR). Oxidative stress is the result of ROS, such as superoxide, H_2O_2, and hydroxyl radicals, and causes rapid cell damage by triggering off a chain reaction. ROS scavenging is one among the common defense responses against abiotic stresses. Changes in antioxidants and protective molecules reflect the impact of environmental stresses on plant metabolism (Jaleel et al., 2007; Doğan et al., 2010a, b).

Salt-tolerant plants, besides being able to regulate the ion and water movements, should also have a better antioxidative system for effective removal of ROS and higher activities of SOD, APX and glutathione transferase (GST) enzymes of chloroplasts which probably play a key role in defense against oxidative damage (Wang et al., 2008). The mechanisms of salt tolerance, not yet clear, can be, to some extent, explained by stress adaptation effectors that mediate the ion homeostasis, osmolytic biosynthesis, toxic radical scavenging, water transport, and long-distance response coordination (Dalal and Khanna-Chopra, 2001; Jaleel et al., 2007). Undoubtedly, plant breeders have made a significant achievement in the past few years, which improves the salinity tolerance in a number of potential crops using artificial selection and conventional breeding approaches. However, most of the selection procedures have been based on differences in agronomic characters (Ashraf and Harris, 2004). Agronomic characters represent the combined genetic and environmental effects on plant growth and include 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 have suggested that selection is more convenient and practicable if the plant species possesses distinctive indicators of salt tolerance at the whole plant, tissue or cellular level (Ashraf and Harris, 2004). In 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; Daşgan et al., 2009).

Plant tissue culture allows to control the stress homogeneity and to characterize the cell behavior 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 Na^+, K^+, Ca^2, $^+Cl^-$ and proline contents, the rate of lipid peroxidation level in terms of malondialdehyde (MDA) and chlorophyll content, and the plant antioxidant systems (SOD, CAT, APX, and GR) in relatively tomato and salsola cultivars in order to evaluate the relative significance of these parameters in imparting tolerance to NaCl oxidative stress.

MATERIALS AND METHODS

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±2°C and 65±5% humidity under 16/8 light/dark, 13500 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 12 h, one day, two day, three day, four day, five day, six day and seven day in a media containing 0, 50, 75, 100, 125, 150 mg NaCl. At the end of the 7th day, the tissues harvested were kept in a deep freezer at – 80°C and homogenized into liquid nitrogen for the analyses.

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°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 HNO_3. The extract was filtered prior to analysis. Na+, K^+, Ca^{++} and Cl^- concentrations were determined using a ICP flame spectrophotometer (Perkim Elmer, OES, Optima 5300 DV), 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 deionizer 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 (Shimadzu AA-1208, Kyoto, Japan) 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). Leaves (0.2 g) were ground in 5 ml of trichloroacetic acid (TCA, 0.1%, w/v). The homogenate was centrifuged at 12000 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 (Shimadzu AA-1208, Kyoto, Japan). 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 AA-1208, Kyoto, Japan) spectrophotometer.

Enzyme assay

To determine the enzyme activities, 0.5 g of leaf tissues from control and treated plants were ground in liquid nitrogen and homogenized in 3 ml of buffer containing 50 mM KH_2PO_4 buffer (pH 7.0), 0.1 mM EDTA, and 1% PVPP (w/v). The homogenates

Table 1. Shows the chlorophyl amounts (µg/g fresh weight) on young upper and old lower leaves of salsola and tomato plants grown under salt stress of different concentrations (50, 75, 100, 125 and 150 mM NaCl) for a total of 7 days. Values are given as mean − SD of seven experiments in each group. Bar values are not sharing a common superscript differ significantly at P 0.*05* (DMRT)≦

Alsola	Control	50 mM	75 mM	100 mM	125 mM	150 mM
12.times on p	374±0.3	370±0.2	370±0.4	351±0.4	241±0.2	123±0.3
on p	370±0.4	367±0.3	365±0.3	350±0.3	338±0.3	118±0.3
7. day on p	389±0.3	310±0.3	295±0.3	294±0.2	201±0.3	92±0.2
on p	387±0.3	310±0.4	393±0.3	290±0.3	200±0.3	88±0.4
Tomates						
12.times on p	389±0.3	384±0.2	355±0.2	293±0.2	144±0.4	140±0.3
on p	390±0.3	282±0.3	354±0.4	290±0.4	137±0.3	128±0.2
7. day on p	389±0.3	375±0.4	324±0.3	265±0.4	132±0.3	124±0.3
on p	388±0.3	376±0.3	320±0.3	255±0.4	122±0.4	103±0.3

were centrifuged at 15,000 *g* for 15 min at 4°C, and the resulting supernatants were freshly used for determination of SOD, CAT, GR, and APX activities. The SOD (EC 1.15.1.1) activity was measured by the modified method of Beyer and Fridovich (1987). 3 ml of the reaction mixture contained 50 mM phosphate buffer (pH 7.8), 13 mM methionine, 60 *µ*M nitroblue tetrazolium (NBT), 0.1 mM EDTA, and 100 µl enzyme extract. The reaction was started by adding 60 *µ*M ribofl avine and placing the tubes under two 20-W cool white fluorescent lamps for 30 min. A complete reaction mixture without enzyme served as control. The reaction was stopped by switching off the light and putting the tubes into the dark. A nonirradiated completed reaction mixture served as a blank. The absorbance was recorded at 560 nm. One unit of SOD enzyme was defined as the amount that produces 50% inhibition of NBT reduction under the assay conditions and expressed as U SOD activity mg–1 protein. For determination of the CAT (EC 1.11.1.6) activity, the reaction mixture contained 50 mM KH_2PO_4 (pH 7), 13 mM H_2O_2, and 30 *µ*l enzyme extract. The decrease in absorbance of H_2O_2 was recorded at 240 nm for 3 min using a spectrophotometer (Shimadzu AA-1208, Kyoto, Japan). One unit of activity was defined as the amount of enzyme catalyzing the decomposition of 1 µmol H_2O_2 per min, calculated from the extinction coefficient (0.036 cm2/µmol) for H_2O_2 at 240 nm (Doğan et al., 2010a).

The GR (EC 1.6.4.2) activity was measured according to Foyer and Halliwell (1976). The assay medium contained 0.025 mm sodium phosphate buffer (pH 7.8), 0.5 mM GSSG, 0.12 mM NADPHNa4, and 100 *µ*l enzyme extract in a final assay volume of 1 ml. NADPH oxidation was determined at 340 nm. The activity was calculated using the extinction coefficient ε = 6.2 mM−1 cm−1 for GSSG. One unit of GR activity was defined as 1 mmol/ml GSSG reduced per min. The activity of APX (EC 1.11.1.11) was measured according to the method of Karabal et al. (2003). The reaction mixture consisted of 50 mM phosphate buffer (pH 6), 1.47 mM H_2O_2, 0.5 mM ascorbic acid, and 50 µl enzyme extract. The reaction was started by the addition of H_2O_2, and the oxidation of ascorbate was measured for 3 min at 290 nm. The enzyme activity was calculated from the initial rate of the reaction using the extinction coefficient 2.8 mM^{-1} cm^{-1} at 290 nm for ascorbate.

Statistical analysis

A randomized parcels experimental design was carried out with three replications in the factorial order. Time, one o f 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. Tables indicate mean values SE. Differences between the values for control and treated leaves were analysed by one-way ANOVA, taking P<0. *05* as significance level, according to LSD multiple range tests.

RESULTS

The young upper leaves of salsola and tomato plants grown under 7 different salt stress concentrations (50, 75, 100, 125 and 150 mM NaCl) were taken, and their chlorophyl, MDA, proline, ion and enzyme analyses were made. According to the analyses after the first 12 h, it was seen that chlorophyl amount on tomato and salsola lowered considerably after 100 mM NaCl in day 7 (Table 1). For salsola, in the 12th h it was calculated as 123 +/- 0.3 in upper leaves, 118±0.3 in lower leaves (µg/g F.W.). After day 7, it was 92±02 in upper leaves, 88±0.4 in lower leaves (µg/g F.W.). For tomato, in day 7 it was 124±0.3 in upper leaves, 128±0.2 in lower leaves, while it was 140±03 in upper leaves, 128±0.2 in lower leaves in 12th h (µg/g F.W.).

MDA percentage in salsola and tomato had increased substantially compared to stress duration and intensity. However, as the stress duration was short like 7 days, it followed a lowering trend up to 100 mM environment, and then increased. In salsola, in 12th h it was 46±0.3 in upper leaves and 51±0.4 in lower leaves, in 7th day it was 46±0.2 in upper leaves, 52±0.3 in lower leaves (µg/g F.W.). In tomato, in 12th h it was 46±0.2 in upper leaves and 56±0.3 in lower leaves, in 7th day it was 53±0.4 in upper leaves, 62±0.4 in lower leaves (µg/g F.W.) (Table 2). In proline analyses, initial proline amount was found to be lower in salsola compared to tomato. However, proline amount has steadily risen in salsola after the control in accordance with the amounts given up to 150 mM NaCl.

Table 2. Shows the MDA amounts (µg/g fresh weight) on young upper and old lower leaves of salsola and tomato plants grown under salt stress of different concentrations (50, 75, 100, 125 and 150 mM NaCl) for a total of 7 days. Values are given as mean – SD of seven experiments in each group. Bar values are not sharing a common superscript differ significantly at P ≤ 0.05 (DMRT).

Salsola	Control	50 mM	75 mM	100 mM	125 mM	150 mM
12.times on p	38±0.3	38±0.2	35±0.2	29±0.2	34±0.2	46±0.3
on p	39±0.3	38±0.4	33±0.4	31±0.3	38±0.3	51±0.4
7. day un p	38±0.3	37±0.2	32±0.3	26±0.2	33±0.2	45±0.2
on p	39±0.3	35±0.4	33±0.4	32±0.4	41±0.4	52±0.3
Tomates						
12.times on p	42±0.3	38±0.2	35±0.2	39±0.2	44±0.4	46±0.2
on p	43±0.3	37±0.3	38±0.4	41±0.3	48±0.3	56±0.3
7. day on p	43±0.2	40±0.2	32±0.4	36±0.4	43±0.3	53±0.4
on p	44±0.3	43±0.4	36±0.4	42±0.5	45±0.3	62±0.4

Table 3. Shows the proline amounts (µg/g fresh weight) on young upper and old lower leaves of salsola and tomato plants grown under salt stress of different concentrations (50, 75, 100, 125 and 150 mM NaCl) for a total of 7 days. Values are given as mean – SD of seven experiments in each group. Bar values are not sharing a common superscript differ significantly at P ≤ 0.05 (DMRT).

Salsola	Control	50 mM	75 mM	100 mM	125 mM	150 mM
12.times on p	22±0.3	23±0.2	25±0.2	29±0.2	34±0.3	44±0.2
on p	23±0.2	24±0.4	23±0.4	25±0.4	33±0.4	41±0.3
7. day on p	24±0.4	25±0.2	27±0.3	29±0.2	43±0.4	51±0.3
on p	25±0.3	26±0.4	31±0.5	36±0.3	41±0.4	42±0.4
Tomates						
12.times on p	40±0.3	37±0.2	35±0.2	39±0.2	44±0.4	62±0.3
on p	41±0.4	35±0.3	33±0.4	36±0.3	38±0.4	41±0.4
7. day on p	43±0.2	40±0.2	33±0.3	36±0.4	44±0.3	63±0.2
on p	42±0.3	40±0.4	34±0.3	33±0.3	39±0.3	43±0.3

In 12th h, the rates were 44±0.2 in upper leaves, 41±0.3 in lower leaves, in 7th day it was 51±0.3 in upper leaves, 41±0.4 in lower leaves. In tomato, in 12th h it was 62±0.3 in upper leaves and 41±0.4 in lower leaves, in 7th day it was 63±0.2 in upper leaves, 43±0.3 in lower leaves (µg/g F.W.) (Table 3).

Sodium (Na+) amounts have generally decreased both in salsola and in tomato control groups up to 150 mM NaCl. In salsola, in 150 mM NaCl environment and in 12th h it was 7±0.3 in upper leaves and 11±0.3 in lower leaves, in 7th day it was 9±0.3 in upper leaves, 11±0.3 in lower leaves (µg/mg D.W.). In tomato, in 12th h it was 9±0.3 in upper leaves and 12±0.4 in lower leaves, in 7th day it was 8±0.3 in upper leaves, 12±0.3 of Na+ in lower leaves (µg/mg D.W.) (Table 4).

Potassium (K^+) amounts have increased both in salsola and in tomato control groups up to 150 mM NaCl. In salsola, in 150 mM NaCl environment and in 12th h, it was 23±0.3 in upper leaves and 17±0.3 in lower leaves, in 7th day it was 25±0.6 in upper leaves, 19±0.3 µg/mg D.W. in lower leaves. In tomato, in 12th h it was 9±0.3 in upper leaves and 12±0.4 in lower leaves, in 7th day it was 8±0.3 in upper leaves, 12±0.3 (µg/mg D.W.) in lower leaves (Table 4).

Potassium (K+) amounts have increased both in salsola and in tomato control groups up to 150 mM NaCl. In salsola, in 150 mM NaCl environment and in 12th h, it was 23±0.3 in upper leaves and 17±0.3 in lower leaves, in 7th day it was 25±s0.6 in upper leaves, 19±0.3 µg/mg D.W. in lower leaves. In tomato, in 12th h it was 9±0.3 in upper leaves and 12±0.4 in lower leaves, in 7th day it was 8±0.3 in upper leaves, 12±0.3 (µg/mg D.W.) in lower leaves (Table 4).

Potassium (K^+) amounts have decreased both in salsola and in tomato control groups up to 150 mM NaCl. In salsola, in 150 mM NaCl environment and in 12th h, it was 11±0.3 in upper leaves and 22±0.3 in lower leaves, in 7th day it was 10±0.3 in upper leaves, 19±0.3 (µg/mg

Table 4. Shows the Na^+, K^+, Ca^{+2} and Cl^- amounts (µg/g fresh weight) on young upper and old lower leaves of salsola and tomato plants grown under salt stress of different concentrations (50, 75, 100, 125 and 150 mM NaCl) for a total of 7 days. Values are given as mean±SD of seven experiments in each group. Bar values are not sharing a common superscript differ significantly at *P 0≤05* (DMRT).

Salsola (Na+)	Control	50 mM	75 mM	100 mM	125 mM	150 mM
12.times on p	23±0.3	18±0.2	15±0.2	13±0.2	10±0.3	7±0.3
on p	22±0.3	17±0.2	16±0.4	14±0.2	12±0.4	11±0.3
7. day on p	23±0.4	17±0.2	13±0.3	12±0.3	11±0.4	9±0.3
on p	22±0.3	17±0.3	14±0.4	13±0.3	11±0.3	11±0.3
Tomates						
12.times on p	22±0.4	22±0.2	15±0.2	12±0.2	14±0.3	9±0.3
on p	21±0.3	23±0.3	18±0.4	16±0.5	13±0.4	12±0.3
7. day on p	23±0.2	21±0.2	14±0.4	12±0.4	12±0.3	8±0.3
on p	23±0.3	22±0.3	14±0.3	13±0.3	13±0.4	12±0.3
Salsola (K^+)						
12.times on p	18±0.3	18±0.2	15±0.2	19±0.2	20±0.3	23±0.3
on p	17±0.3	19±0.3	17±0.3	17±0.3	16±0.4	17±0.3
7. day on p	18±0.2	18±0.3	16±0.3	18±0.1	21±0.4	25±0.6
on p	18±0.3	17±0.3	16±0.3	17±0.3	18±0.3	19±0.3
Tomates (K^+)						
12.times on p	12±0.1	13±0.2	15±0.2	19±0.2	21±0.3	22±0.3
on p	12±0.3	12±0.3	13±0.3	15±0.3	17±0.4	18±0.2
7. day un p	13±0.2	12±0.2	14±0.4	17±0.4	22±0.3	26±0.5
on p	13±0.2	12±0.3	14±0.3	15±0.3	16±0.3	18±0.3
Salsola (Ca^{+2})						
12.times un p	28±0.3	26±0.2	22±0.2	19±0.2	14±0.3	11±0.3
on p	27±0.2	24±0.4	23±0.3	23±0.3	22±0.4	22±0.3
7. day on p	26±0.4	25±0.2	22±0.4	17±0.4	13±0.4	10±0.3
on p	24±0.3	23±0.3	23±0.3	21±0.3	21±0.2	19±0.3
Tomates (Ca^{+2})						
12.times on p	22±0.3	18±0.2	15±0.2	13±0.2	14±0.1	11±0.3
on p	23±0.3	22±0.3	22±0.3	21±0.4	19±0.3	19±0.3
7. day on p	23±0.2	19±0.2	16±0.3	14±0.3	13±0.1	10±0.4
on p	22±0.4	22±0.3	20±0.3	20±0.3	19±0.3	18±0.3
Salsola (Cl^-)						
12.times on p	18±0.3	18±0.2	15±0.2	19±0.2	14±0.4	12±0.4
on p	18±0.3	18±0.3	19±0.3	18±0.4	17±0.4	17±0.3
7. day on p	18±0.3	17±0.2	13±0.2	16±0.4	13±0.4	11±0.3
on p	19±0.3	18±0.3	17±0.3	17±0.3	15±0.3	15±0.3
Domates (Cl-)						
12.times on p	22±0.2	18±0.2	25±0.2	19±0.2	14±0.3	12±0.3
on p	23±0.3	22±0.3	22±0.3	21±0.3	20±0.3	19±0.3
7. day on p	23±0.2	20±0.2	23±0.3	16±0.4	13±0.4	11±0.3
0n p	22±0.5	22±0.4	23±0.4	21±0.4	20±0.2	20±0.4

D.W) in lower leaves. In tomato, in 12th h it was 11±0.3 in upper leaves and 19±0.3 in lower leaves, in 7th day it was 10±0.4 in upper leaves, 18±0.3 (µg/mg D.W.) in lower leaves (Table 4).

Chlorine (Cl^-) amounts has decreased both in salsola and in tomato control groups up to 150 mM NaCl. In salsola, in 150 mM NaCl environment and in 12th h, it was 12±0.4 in upper leaves and 17±0.3 in lower leaves,

Table 5. Shows the SOD, CAT, GR and APX amounts (µg/g fresh weight) on young upper and old lower leaves of salsola and tomato plants grown under salt stress of different concentrations (50, 75, 100, 125 and 150 mM NaCl) for a total of 7 days. Values are given as mean ± SD of seven experiments in each group. Bar values are not sharing a common superscript differ significantly at P 0.*05* (DMRT).

Domates (SOD)	Control	50 mM	75 mM	100 mM	125 mM	150 mM
12.times on p	142±0.3	138±0.2	130±0.2	123±0.2	112±0.4	105±0.4
on p	144±0.4	140±0.4	128±0.4	119±0.4	108±0.4	97±0.4
7. day on p	143±0.2	140±0.2	126±0.4	120±0.4	111±0.3	96±0.4
on p	144±0.4	141±0.3	126±0.4	121±0.4	114±0.3	88±0.4
Salsola (CAT)						
12.times on p	38±0.3	38±0.2	35±0.2	29±0.2	17±0.4	17±0.4
on p	39±0.4	38±0.3	34±0.4	24±0.4	17±0.4	12±0.4
7. day on p	37±0.3	32±0.2	26±0.4	23±0.3	17±0.4	16±0.3
on p	38±0.4	33±0.4	25±0.4	17±0.4	14±0.4	10±0.4
Tomates (CAT)						
12.times on p	42±0.3	38±0.2	30±0.2	27±0.2	24±0.4	22±0.4
on p	42±0.4	36±0.3	28±0.3	28±0.4	20±0.4	16±0.4
7. day on p	40±0.2	32±0.2	26±0.3	23±0.4	21±0.4	21±0.3
on p	41±0.4	33±0.4	24±0.3	20±0.4	20±0.4	18±0.3
Salsola (GR)						
12.times on p	123±0.3	118±0.2	115±0.2	112±0.2	104±0.3	98±0.4
on p	122±0.4	117±0.3	113±0.4	109±0.3	102±0.5	92±0.4
7. day on p	123±0.3	118±0.2	111±0.4	103±0.4	103±0.3	92±0.3
on p	123±0.3	118±0.3	108±0.3	108±0.3	100±0.3	88±0.3
Tomates (GR)						
12.times on p	142±0.3	138±0.2	135±0.2	129±0.3	114±0.4	107±0.4
on p	143±0.3	138±0.3	133±0.3	125±0.3	110±0.3	102±0.3
7. day on p	140±0.2	141±0.2	126±0.3	122±0.4	113±0.3	106±0.4
on p	141±0.3	142±0.3	133±0.4	119±0.3	116±0.4	100±0.4
Salsola (APX)						
12.times on p	128±0.3	118±0.2	115±0.2	119±0.2	104±0.4	94±0.4
on p	128±0.3	119±0.3	112±0.3	114±0.3	105±0.4	90±0.3
7. day on p	128±0.3	115±0.2	111±0.3	112±0.4	100±0.4	88±0.3
on p	127±0.3	120±0.3	110±0.3	105±0.4	100±0.3	82±0.3
Tomates (APX)						
12.times on p	132±0.3	133±0.2	135±0.3	127±0.2	114±0.3	104±0.5
on p	133±0.3	130±0.4	130±0.4	122±0.3	111±0.4	98±0.4
7. day on p	135±0.2	131±0.2	121±0.2	120±0.4	110±0.3	101±0.4
on p	136±0.4	133±0.3	117±0.3	110±0.3	103±0.3	92±0.3

in 7th day it was 11±0.3 in upper leaves, 15±0.3 µg/mg D.W. in lower leaves. In tomato, in 12th h it was 12±0.3 in upper leaves and 19±0.3 in lower leaves, in 7th day it was 11±0.3 in upper leaves, 20±0.3 (µg/mg D.W.) in lower leaves (Table 4).

Based on enzyme rates, salsola and tomato plants' changes under 7 days of salt (50, 75, 100, 125 and 150 mM NaCl) were like this. Comparing superoxide dismutase rate with control, both salsola and tomato had decreases. In 150 mM NaCl environment in day 12, salsola upper leaves had 92±0.3, lower leaves had 88±0.4, and in day 7 those rates were 82±0.3 for upper leaves, 71±0.4 (µmol/g F.W.) for lower leaves. For tomato in 150 mM NaCl environment in day 12, upper leaves had 105±0.4, lower leaves had 97±0.4, in day 7 those rates were 96 ±0.4 for upper leaves, 88±0.4 (µmol/g F.W.) for lower leaves (Table 5).

There was a general decrease in Catalase. In salsola on 150 mM NaCl environment, in day 12 upper leaves had rates of 17±0.4, lower leaves had 12±0.4 rates, those

were 16±0.3 in upper leaves and 10±0.4 (µmol/g F.W.) in lower leaves. In tomato on 150 mM NaCl environment, in day 12 upper leaves had rates of 22±0.4, lower leaves had 16±0.4 rates, those were 21±0.3 in upper leaves and 18±0.4 (µmol/g F.W.) in lower leaves.

Glutathione reductase rates in salsola in 150 mM NaCl environment in day 12 had rates of 98 ±0.4 in upper leaves, 92±0.4 in lower leaves, in day 7 those were 92±0.3 in upper leaves and 88±0.3 in lower leaves. In tomato in 150 mM NaCl environment in day 12 had rates of 22±0.4 in upper leaves, 16±0.4 in lower leaves, in day 7 those were 21±0.3 in upper leaves and 18±0.3 (µmol/g F.W.) in lower leaves (Table 5). As seen in the results, GR amounts generally decrease with the intensity and duration of stress, but it has had a more distinct trend on salsola.

Ascorbate peroxidase rate for salsola in 150 mM NaCl environment in day 12 had rates of 94± 0.4 in upper leaves and 90±0.3 in lower leaves, in day 7 those were 88±0.3 in upper leaves and 82±0.3 (µmol/g F.W.) in lower leaves. For tomato in 150 mM NaCl environment in day 12 had rates of 104±0.5 in upper leaves and 98±0.4 in lower leaves, in day 7 those were 101 ±0.4 in upper leaves and 92±0.3 (µmol/g F.W.) in lower leaves (Table 5). According to the results, APX rates were very high in salsola, and had a decreasing trend in tomato.

DISCUSSION

In this study, we investigated the effects of salt stress on antioxidant enzymes and its possible role, how the enzyme activity changes in young upper leaves and old lower leaves with aging and salt stress in Salsola (*stenoptera*) and tomato (*L. esculentum* L.) plant growth.

In this manner, evaluation of the findings on chlorophyl, lipid peroxidation product (MDA), proline, ion and enzyme analyses, determining the amount of salt stress and its role on lower and upper leaves in time, enzyme responses of plants to salt stress with other parameters was our goal.

Salt stress application has created considerable changes in leaves' cholorophyl levels. According to the observed relation between salt stress and the chlorophyl levels of leaves, stress related leaf loss has increased substantially. In a study involving two types of tomatoes, wild L. *hirsitum* plant that has tolerance against salt stress had considerable chlorophyl increase alongside stress, meanwhile local type (*L. esculentum*) that is sensitive to salt stress didn't have any statistically significant changes (Murillo et al., 2005; Islam et al., 2007; Parvaiz et al., 2008; Dalal and Khanna-Chopra, 2001). The literature mentioned above is in agreement with our results. According to this, our findings show controls having high chlorophyl, decrease in salt stress as time progresses, disruption in pigments in time, decrease in organic components as a result of photosynthesis that changes the pH of cell vacuole juice (Table 1). Because of the reasons mentioned above, although young upper leaves had decreased chlorophyl amounts, old lower leaves had higher chlorophyl amounts. Generally, young leaves with high chlorophyl amounts were more resistant to stress. In limited studies on the changes in the amount of MDA, a product of lipid peroxidation in salt stress, MDA is shown to be increasing. In this manner, it was found that in studies on corn and cucumber, there was the increase in lipid peroxidation was the most characteristic change related to salt stress (Shen et al., 1999a; Hodges et al., 1999; Chen et al., 2000; Shalata et al., 2001; Munne-Bosch and Penuelas, 2003; Doğan, 2004; Ben-amor et al., 2006; Doğan et al., 2010a, b). According to Kendall and McKersie (1989), active O_2 radicals created under stress conditions result in lipid peroxidation in membranes and this causes damages in membranes. Parallel to the studies above, our study shows that high average lipid peroxidation values on salsola and tomato leaves showed that those two plants could be affected by environmental stress factors more and the production of free radicals could be higher. In comparison of control plants, the increase in salt stress and lipid peroxidation is seen to decrease substantially with the elimination of stress. Lipid peroxidation decrease in stress resistant young leaf tissues shows that the resistance of membranes was preserved by one or more mechanisms (Table 2).

According to the results of the proline analysis, proline, an amino acid which can be found abundantly under stress conditions, participate in detoxification of free O_2 radicals (Bohnert and Sheveleva, 1998; Patel *et al*., 2008). It is shown that tolerance against stress increases as proline increases with stress in many plants. For example, a positive relationship was found between leaf proline amount and tolerance increase against salt stress in winter colza and winter wheat (Stefl et al., 1978), halophyte (Popp and Albert, 1981), tomato (Doğan, 2004). According to Kushad and Yelenosky (1987), absolute proline amount was more important than the increase percentage of proline. As a result, tolerance against stress in young leaves increase with proline increase, proline amount decreases with the removal stress, so it can be of importance in plants' resistance to oxidative stress related to salt stress. Stefl *et al*., (1978) has said that increased tolerance in plants against stress is related to the increase of proline and arginine or other unidentified components (Table 3). Evaluating salsola and tomato genotypes according to their ion concentrations, plants generally have some amount of sodium (Na^+), some more potassium (K^+), calcium (Ca^{++}) and chlorine (Cl^-) before being subjected to salt stress. Evaluating according to sodium (Na^+) contents, this element is known to be able to move both in phloem and in xylem in plants (Marschner, 1997). In recent studies, it is thought that high amounts of Na^+ ions taken with the

increase of saltiness disrupts the ion balance of plants and create a toxic effect (Botella et al., 1997). Having a negative effect on the ion balance of plants, and preventing the intake of basic elements used in metabolic actions, this is thought to be able to create some physiological problems (Gorham et al., 1985b). Mutlu (2003) says that the root, body and leaves of cauliflower plants grown under salt stress have increased Na^+ content differing according to the level and duration of salt stress, and the increase is affecting the growth of the plants, either directly or indirectly. Na^+ is seen to be high in upper and lower leaves in the first days of stress, and its decrease in the following days gives the impression of a disruption in the ion balance (Table 4).

It is said that potassium (K^+) has an important role in the protection of plants against salt stress (Marschner, 1995). This situation has been explained as "growth-dilution" by Marchner (1995), Lahet et al. (2003) and Walia et al. (2005). Also, increase in K^+ is known to increase with the age of the leaf because of K^+'s activeness in the phloem (Sparks, 1977; Uriu and Crane, 1977; Ghoulam et al., 2002; Chattopadhayay et al., 2002; Murillo-Amador et al., 2005). While some of the studies mentioned above support some of our findings, some follow a different route. K^+ amount in increase after day 4 of stress especially in young leaves, and maximize in day 7. Potassium is moves fast especially from young leaves and growing parts to body and fruits. Accordingly, increase in K^+ concentration is expected under stress conditions, and we can say that it can have a protective effect as it has a role in enzyme activation (Table 4).

As the movement of calcium is minimal in phloem (Subbarao et al., 1990; Marschner, 1995; Murillo et al., 2005) it is thought that the level change in leaves during the trial was low. However increase in Ca^{++} intake after the maturity period of the leave as a result of the stress which is seen as the result of the decrease in Ca^{++} levels in young leaves, decreases in a strict and definite manner (Doğan et al., 2009; Rengel, 1992). Ca^{++} amount decreased in young leaves in relation with stress duration and concentration compared to control, this may be explained by the facts that Ca^{++} does not affect the cell wall diffusion in young leaves, cell wall diffusion is lost in old leaves with high Ca^{++} content, so elective diffusion is lost in time (Table 4).

Other investigators found that levels of said enzyme can differ with salt stress (Yelenosky and Yu, 1992). According to this, SOD, GR activities increase with stress (El-Saht, 1998; Khan et al., 2008), catalase enzyme activity which has a definitive role in antioxidative defense system decreases in Arabidopsis thaliana (Kubo et al., 1999; Munne-Bosch and Penuelas, 2003; Sairam et al., 2005), rice seeds aren't affected by it (Oidaire et al., 2000). In the study on rice seeds, SOD activity has increased slowly and steadily after salt stress (Oidaire et al., 2000). In a study of Lee and Lee (2000) on cucumber plant, Sod activity in leaves was found to be increasing during the salt application and calculated activity was the same as in the control plant. It was found that tomato with salt stress tolerance had a more effective antioxidative enzyme system compared to sensitive ones (Sala, 1998; Doğan, 2004; Doğan *et al*., 2010a,b). The reason that modern tomato (*L. esculentum*) created using the culture of the wild tomato type (*L. perivianum*) adapted well to stress is found to be related with prevention of the creation of active oxygen radicals more effectively (Bruggerman et al., 1999; Mittova et al., 2002). Catalizing the dismutation of superoxide radical ($O2_2$) of superoxide dysmutase (SOD) to H_2O_2 and O_2 (Bowler et al., 1992; Scandalious, 1993; Çakmak et al., 1993; Çakmak, 1994) has made superoxide concentration low and stable and thus minimizing the creation of hydroxyl radicals by Haber-Weiss reaction (Elstner, 1982) has been reported. That's because it is affected from water insufficiency and growing pale, thus having a decreasing effect on photosynthesis. Thus, CO_2 assimilation rates of leaves decreased significantly in relation to the decrease in the water inside leaves was shown in wheat (Evans, 1983), spinach (Evans and Terashima, 1988) and corn (Wond et al., 1985; Sinclair and Horie, 1989). Decreased SOD levels in salsola and tomato in relation with the salt stress duration arises from the fact that photosynthetic actions happen in leaf chloroplasts, enzyme activity would decrease if we consider that superoxide dismutase take place in chloroplasts (Jackson et al., 1978). Although, SOD levels decreased both in young and old leaves, SOD levels were higher in older leaves compared to young leaves (Table 5).

There are many studies which state that stress has a preventive effect on catalase activity. It is stated that cell destruction based on salt stress has a distinctive role in decreasing catalase activity. According to Prasad (1997), Shalata et al. (2001), catalase has a distinctive role on protection against salt stress. Prasad (1997) has found that catalase provides the best relationship with salt tolerance among the enzymes he has worked with. In our study, tolerance to salt stress changed with time, and has caused catalase activity to decrease. The results show that catalase activity changes are more distinctive in young leaves. Because enzyme concentration found in the upper young leaves is more than the older leaves in the lower region. Based on catalase activity, we can say that it is an indication of salt stress tolerance of the young leaves in upper region (Table 5).

There are important studies mentioning that salt stress affect GR levels differently. For example, it was found that GR activity increases with stress in *Arabidopsis thaliana* (Kubo et al., 1999; Chattopadhayay et al., 2002). In a study on cucumber, it was shown that GR activity increased gradually with stress application, but the enzyme activity here was below known enzyme levels in the plant, (Walker and MeKersie, 1993; Doğan, 2004; Doğan et al., 2010a,b), but decreased in seeds (Fadzillah et al., 1996; Chattopadhayay et al., 2002). According to

the GR decrease in salt stress application, we can say that the defense mechanism of the system has activated. The decrease in GR levels under salt stress, as in SOD, shows that detoxification capacity of GR can be increased by some unknown mechanisms (Table 5).

Ascorbate peroxidase activity is found to be increasing in rice seeds after salt stress application (Oidaire et al., 2000). Less specific for ascorbic acid and not chloroplast and localizing mainly in cell walls and cytoplasm, ascorbate peroxidase (APX) (Asada, 1992; Hernandez and Almonsa, 2002) has significantly decreased under stress in trials. In the study, it was seen that APX activity in salsola and tomato leaves has decreased with salt stress (Scebba et al., 1998; Ben-Amor et al., 2006). The significant reduction of ascorbate peroxidase activity under stress and its increase when stress has ended is another result which shows that tolerance against stress has been affected positively (Table 5). According to Gaspar et al. (1985) and Mittova et al. (2004), high ascorbate peroxidase activity may show the high level of H_2O_2 production in cell walls and/or cytosol it is localized in. So we can say that, related to decreases in APX activity, H_2O_2 production may be low where this enzyme is located. Another explanation may be like this; APX activity reduction with stress may be related to its detoxification (reduction) capacity being below the oxidation capacity of H_2O_2.

In conclusion, it is shown that salt stress enzyme activity has a protective role against oxygen radicals and contributes to cell toughness. There is a positive correlation between resistance to oxidative damage and is thought to be resulting from the reduction in enzyme activity that causes a reduction in protein amount. It has been determined that plants close stomas and minimize their photosynthesis activities in response to salt stress, stoma movements are related to many physiological and biochemical events. In order to hold water level in plants in a certain level in response to salt stress, it is known that plants lower their osmotic potentials, proline, chlorophyl and MDA levels change differently in response to salt stress. It has been determined that chlorophyl level drops, proline and MDA amounts increase, every plant gets affected differently and give different responses to stress. Enzyme levels generally decrease in response to stress, but it was seen that young upper leaves have higher amounts of enzyme compared to old lower leaves. In light of the findings above, we can say that younger leaves are more resistant to stress.

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