

Evidence for Differential Lipid Peroxidation and Antioxidant Enzyme Activities in *Sesamum indicum* L. Genotypes under NaCl Salinity

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ABSTRACT

As sesame is a somewhat neglected crop, information concerning its response to salinity is lacking. The effects of NaCl stress were studied on seven genotypes of *Sesamum indicum* L. The 6-weeks-old sesame seedlings were treated with different NaCl concentrations (0, 30, and 60 mM). The NaCl caused significant decreases in fresh and dry mass of all genotypes; however responses of genotypes to the salt were significantly different. Based on the data obtained for fresh and dry mass accumulation, the genotypes were divided into two groups: i.e. salt-tolerant (Ardestan, Varamin, and Darab) and salt-sensitive (Naz-Takshakheh, Naz-Chandshakheh, Yekta and Oltan). Lipid peroxidation was recorded to be the lowest in cv. 'Varamin', 'Darab', and 'Ardestan' (tolerant group), whereas it was recorded to be maximum in genotypes 'Naz-Takshakheh', 'Naz-Chandshakheh', 'Oltan' and 'Yekta' (sensitive group) in the 60 mM NaCl treatment. In both 30 and 60 mM NaCl treatments, the activities of SuperOxide Dismutase (SOD), Catalase (CAT), Ascorbate Peroxidase (APX) and Glutathione Reductase (GR) were found to be higher in the tolerant group, compared to the sensitive group of genotypes. The accumulation of proline in the sesame leaves under saline conditions was higher in tolerant group, compared to the sensitive genotypes. It seems that in the salt-tolerant genotypes of sesame, increases in antioxidant enzymes activities and proline accumulation, along with a lower lipid peroxidation at cellular membranes, led to a higher level of tolerance to the salt.

Keywords: Lipid peroxidation, Proline, Salt stress, Sesame.

INTRODUCTION

Salinity has long been identified as one of a major constraint to the productivity of agricultural crops worldwide. Nearly 80 million hectares of arable lands of the world are estimated to be affected by salt (Li *et al.*, 2010). Salinity effects are more noticeable in arid and semiarid regions, due mainly to the acceleration of salinity by a deficit of precipitation and high temperature coupled with a high evaporation demand (Azevedo Neto *et al.*, 2006). Different types of salts including Na₂SO₄, MgSO₄, CaSO₄, MgCl₂, KCl, and Na₂CO₃, more or less exist in

agricultural lands, each of which can lead to the salinity stress. However, NaCl is generally the most deleterious and prevalent salt, at least in Iran (Azizpour *et al.*, 2010). Salinity suppresses plant growth through reduction in water availability, sodium ion accumulation and ionic imbalances, leading to cellular and molecular damages (Munns and Tester, 2008; Ehsanzadeh *et al.*, 2009). As a consequence of these primary effects, salt stress also leads to the secondary oxidative damage by inhibition of the CO₂ assimilation, exposing chloroplasts to excessive excitation energy (Parvaiz, and Satyawati, 2008).

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Oxidative stress increases the generation of Reactive Oxygen Species (ROS), such as superoxide radicals ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2) and hydroxyl radicals (OH^{\bullet}) from triplet chlorophyll (Sairam *et al.*, 2002; Meloni *et al.*, 2003). These cytotoxic ROS's are highly reactive and when the ability of plant for scavenging is less than the ROS production, they can seriously disrupt normal metabolism through oxidative damages to the photosynthetic pigments, proteins, nucleic acids and lipids (Leshem *et al.*, 2007; Abogadallah, 2010). To alleviate the ROS-induced oxidative damage, plants possess an effective antioxidant defense network. This antioxidative defense system includes two components: low-molecular mass antioxidants (glutathione, ascorbate and carotenoids) as well as antioxidative enzymes, such as SuperOxide Dismutase (SOD), Catalase (CAT), Ascorbate Peroxidase (APX), Guaiacol Peroxidase (GPX) and Glutathione Reductase (GR) (Demiral and Türkan, 2005; Tunc-Ozdemir *et al.*, 2009). SOD is the main $O_2^{\bullet -}$ scavenger and its enzymatic action catalyzes the dismutation of $O_2^{\bullet -}$ radicals to molecular O_2 and H_2O_2 (Meloni *et al.*, 2003). Further, the accumulation of H_2O_2 is restricted through the action of CAT or by the ascorbate glutathione cycle, where APX uses ascorbate as an electron donor to reduce H_2O_2 to water. Finally, GR catalyzes the NADPH dependent reduction of Oxidized Glutathione (GSSG) to the Reduced Glutathione (GSH) (Bor *et al.*, 2003; Abogadallah, 2010). Many authors have reported that the extent of oxidative cellular damage in plants exposed to salinity is controlled by the capacity of the antioxidant enzymes and their resistance to the oxidative damage may, at least in part, be involved in salt stress tolerance (Sairam *et al.*, 2002; Vaidyanathan *et al.*, 2003; Azevedo Neto *et al.*, 2006). In various plant species, changes in lipid peroxidation-induced MalonDiAldehyde (MDA) content have been observed because of oxidative stress (Vaidyanathan *et al.*, 2003; Li *et al.*, 2010).

Sesame (*Sesamum indicum* L.) is one of the oldest crops in the world, and has been under cultivation in Asia for over 5000 years (El Naim *et al.*, 2010). Sesame oil is rich in natural antioxidants and the antioxidative compounds are helpful in preserving the stability of sesame seed and its oil (Farhoosh *et al.*, 2013). Beside food, sesame is widely used in pharmaceuticals and in biofuel production (Koca *et al.*, 2007).

Sesame has been distributed and cultivated in warm and subtropical regions of the world, where soil salinization is a common concern. However, it could be named as a partially neglected crop in terms of research on its physiology under stresses such as salinity. Bazrafshan and Ehsanzadeh (2014) have indicated that salinity adversely affects photosynthetic and growth attributes of sesame, though, information on antioxidative defense systems of this oilseed crop against salt stress conditions is scarce. In the present study, an attempt was made to assess and compare some physiological responses of seven sesame genotypes in response to the NaCl levels which typically might be encountered in saline soils of warm and semiarid regions.

MATERIALS AND METHODS

Plant Material, Growth, and Treatment Conditions

Sufficient seeds of seven sesame genotypes, 'Naz-Takshakhe', 'Naz-Chandshakhe', 'Ardestan', 'Varamin', 'Yekta', 'Darab' and 'Oltan' (Table 1) were sown in the 20×30×5 cm plastic trays containing washed sand, at the Isfahan University of Technology, Isfahan, Iran, in 2010. Seeds were irrigated daily with a half strength Hoagland's solution. Four healthy 5-week-old seedlings from each genotype were transferred to the containers containing four liters of aerated full Hoagland's nutrient solution. Salt treatment started when plants were 40 days old. While no salt was added to the control and the 30 mM NaCl level was

Table 1. Genotypes used in the present study and their reported agronomics.

Genotype	Local region	Branching status	Relative height	Seed color	Lodging status	Siliques status	Maturity status
Naz-Takshakhe	Northern Iran	low	tall	white	intermediate	intermediate	early
Ardestan	Central Iran	high	tall	brown	sensitive	sensitive	late
Naz-Chandshakhe	Northern Iran	high	tall	white	intermediate	sensitive	early
Yekta	Karaj	low	tall	white	resistant	intermediate	Semi-early
Varamin	Central Iran	low	tall	brown	resistant	intermediate	Semi-late
Darab	Southern Iran	high	intermediate	brown	intermediate	sensitive	Semi-late
Oltan	North-east Iran	high	tall	brown	sensitive	intermediate	Semi-early

applied in one step, the 60 mM NaCl level was applied to the containers in two 30 mM NaCl steps with a 2-day interval. The nutrient solution was renewed biweekly and water lost through evapotranspiration was compensated by daily addition of deionized water. The experiment was carried out in a temperature-regulated greenhouse. The mean values of temperature, air relative humidity and photosynthetically active radiation were 25°C, 65% and 400 $\mu\text{mol m}^{-2} \text{s}^{-1}$, respectively.

Growth Parameters

The plants were harvested 6 weeks after the initiation of salt treatment and separated into shoots and roots. After measuring Fresh Masses of Shoots (SFM) and Roots (RFM), the samples were dried at 70°C for 72 hours and then weighed to obtain the dry masses (SDM and RDM, respectively). Before drying, two randomly chosen fresh leaves from each container were excised, weighed, frozen in liquid nitrogen, lyophilized, and kept in a freezer (−80°C) for further analyses.

Preparation of Extracts and Enzyme Assays

In order to determine the enzyme activity and estimate the lipid peroxidation, frozen leaf samples (0.20 g) were grinded to a powder in liquid nitrogen and extracted with 4 mL of ice-cold extraction buffer (100 mM phosphate buffer, pH 7.0). The extracts were centrifuged for 30 minutes at 20,000×g and the resulting supernatants were used as the crude extract for enzyme activity and lipid peroxidation assays. All extraction operations were carried out at 4°C.

Total CAT (EC 1.11.1.6) activity was measured according to Beers and Sizer (1952), with minor modifications. The reaction solution consisted of 50 μL of the enzyme extract, 100 mM phosphate buffer (pH 7.0), 0.1 μM EDTA, and 20 mM H_2O_2 .



in a total volume of 1.5 mL. The enzyme activity was estimated by the decrease in absorbance of H_2O_2 at 240 nm as a consequence of H_2O_2 consumption. The decrease of H_2O_2 was monitored by reading the absorbance at the latter wave length, using a UV-visible spectrophotometer (model M 36, Beckman, CA, USA), and quantified by its molar extinction coefficient ($36 \text{ mM}^{-1} \text{ cm}^{-1}$) and the enzyme activity was expressed as $\mu\text{mol H}_2\text{O}_2 \text{ min}^{-1} \text{ g}^{-1} \text{ DM}$.

Total SOD (EC 1.15.1.1) activity was determined by measuring its ability to inhibit the photochemical reduction of NitroBlue Tetrazolium chloride (NBT), as described by Giannopolitis and Ries (1997). The reaction solution consisted of 50 μL of the enzyme extract, 50 mM phosphate buffer (pH 7.8), 0.1 μM EDTA, 13 mM methionine, 75 μM nitrobluetetrazolium and 2 μM riboflavin in a total volume of 1.5 mL. After adding riboflavin, the test tubes were shaken manually and placed under fluorescent lighting from two 20W lamps. The reaction was allowed to proceed for 15 min, after which the lights were switched off. Reduction in NBT was estimated by reading the absorbance of the reaction mixture at 560 nm, and one unit of SOD activity (U) was defined as the amount of enzyme required to cause 50% inhibition of the NBT reduction and the results were expressed as $\text{U mg}^{-1} \text{ DM}$.

Total GR activity (EC 1.6.4.2) was assayed as described by Foyer and Halliwell (1976), with minor modifications. The reaction mixture (1.0 mL) consisted of 100 mM phosphate buffer (pH 7.8), 0.1 μM EDTA, 0.05 mM NADPH, 3 mM GSSG and 50 μL enzyme extract. The reaction was started by the addition of GSSG and the NADPH oxidation rate was monitored at 340 nm for 1 min. Enzyme activity was determined using the molar extinction coefficient for NADPH ($6.2 \text{ mM}^{-1} \text{ cm}^{-1}$) and expressed as $\mu\text{mol NADPH min}^{-1} \text{ g}^{-1} \text{ DM}$.

Total APX (EC 1.11.1.1) activity was assayed according to Nakano and Asada (1981). The reaction solution (1.5 mL) contained 50 mM phosphate buffer (pH 6.0),

0.1 μM EDTA, 0.5 mM ascorbate, 1.0 mM H_2O_2 and 50 μL enzyme extract. The enzyme activity was assayed by monitoring the decrease in absorbance at 290 nm, as a consequence of oxidation of ascorbate. The reaction was started by the addition of H_2O_2 and the ascorbate oxidation was measured at 290 nm for 1 minute. Enzyme activity was quantified using the molar extinction coefficient for ascorbate ($2.8 \text{ mM}^{-1} \text{ cm}^{-1}$) and the results were expressed in $\mu\text{mol H}_2\text{O}_2 \text{ min}^{-1} \text{ g}^{-1} \text{ DM}$.

Lipid Peroxidation and Leaf Proline Content

Since MDA content is a measure of lipid peroxidation and MDA is a product of lipid peroxidation by thiobarbituric acid reaction, the level of lipid peroxidation was measured in terms of ThioBarbituric Acid Reactive Substances (TBARS) content of the leaf samples as described by Heath and Packer (1968). Leaf samples of 0.5 g were homogenized in 10 mL of 0.1% TriChloroacetic Acid (TCA). The homogenate was centrifuged ($15,000\times g$) for 5 minutes. To 2 mL of aliquot of the supernatant, 4 mL of 0.5% ThioBarbituric Acid (TBA) in 20% TCA was added. The mixture was heated at 90°C for 30 minutes and then quickly cooled in an ice bath. After centrifuging at $10,000\times g$ for 10 min to remove suspended turbidity, absorbance of the supernatant was recorded at 532 nm. The value for nonspecific absorption at 600 nm was subtracted. The TBARS content was calculated using its absorption coefficient of $155 \text{ mM}^{-1} \text{ cm}^{-1}$ and the results were expressed as $\text{nmol MDA g}^{-1} \text{ DM}$.

Total leaf proline was extracted by the method of Bates *et al.* (1973). Leaf samples (0.5 g) were homogenized in 10 mL of 3% aqueous sulfosalicylic acid and the homogenate was filtered through filter paper. Two mL of the filtered extract was reacted with 2 mL of acid ninhydrin and 2 mL of glacial acetic acid in a test tube for 1 hour under a temperature of 100°C , and the

reaction terminated by placing the tube on an ice bath. The reaction mixture was extracted using 4 mL of toluene and vortexing. The chromophore containing toluene was aspirated from the aqueous phase, warmed to room temperature and the absorbance was read at 520 nm using toluene as a blank. Proline concentration was determined using the calibration curve and expressed as $\mu\text{mol proline g}^{-1} \text{DM}$.

Statistical Analysis

The factorial experiment was conducted using a randomized complete block design with three replications. All data are reported as mean \pm SE. The data were analyzed using a Statistical Analysis Software Version 9.1 (SAS Institute Inc., Cary, North Carolina, USA). Analyses Of Variances (ANOVA) were conducted on all data. Based on the data obtained for SFM and SDM, the genotypes were divided into two groups: i.e. salt-tolerant (Ardestan, Varamin and Darab) and salt-sensitive (Naz-Takshakheh, Naz-Chandshakheh, Yekta and Oltan). Orthogonal independent comparisons were conducted within and between these groups and for their interaction with salt treatment as well. The means were separated using Fisher's protected Least Significant Differences (LSD). Differences were considered significant at 0.05 level of probability.

RESULTS

Growth Parameters

RFM and RDM were significantly affected by salt, genotype, and tolerant *vs* sensitive, while SFM and SDM were significantly affected by salt, genotype, tolerant *vs* sensitive, and interaction effect of group \times salt (Table 2). Although the 30 mM NaCl level was sufficient to exert significant decreases on the root and shoot attributes of sesame, increasing the NaCl level to 60 mM

Table 2. A synopsis of Analysis Of Variance (ANOVA) Root Fresh Mass (RFM), Shoot Fresh Mass (SFM), Root Dry Mass (RDM), Shoot Dry Mass (SDM), MalonDiAldehyde content (MDA), Catalase (CAT), Ascorbate Peroxidase (APX), Glutathione Reductase (GR), SuperOxide Dismutase (SOD) of seven *S. indicum* genotypes when 40-days-old seedlings were allowed to grow for 6 weeks under normal or saline conditions.

Source of variation	df	Mean square									
		RFM	SFM	RDM	SDM	Proline	MDA	CAT	APX	GR	SOD
Replication	2	3.59 ^{ns}	37.728 ^{ns}	0.057 ^{ns}	0.370 ^{ns}	0.573 ^{ns}	11.791 ^{ns}	0.007 ^{ns}	1.939 ^{ns}	0.0008 ^{ns}	25.751 [*]
Salt treatment	2	62.758 ^{***}	6316.241 ^{***}	0.890 ^{***}	70.369 ^{***}	50.457 ^{***}	327.613 ^{***}	0.627 ^{***}	71.118 ^{***}	0.073 ^{***}	108.409 ^{***}
Genotype	6	4.473 ^{***}	390.647 ^{***}	0.043 ^{**}	4.218 ^{***}	1.353 [*]	31.132 ^{***}	0.580 ^{***}	10.881 ^{**}	0.011 ^{***}	37.377 ^{***}
Sensitive group	3	0.143 ^{ns}	14.725 ^{ns}	0.011 ^{ns}	0.325 ^{ns}	1.042 ^{ns}	12.123 [*]	0.194 ^{ns}	11.653 [*]	0.013 ^{**}	37.444 ^{**}
Tolerant group	2	2.090 ^{ns}	215.640 ^{ns}	0.012 ^{ns}	1.859 ^{ns}	1.292 [*]	4.366 ^{ns}	0.076 ^{ns}	8.424 ^{ns}	0.001 ^{ns}	2.020 ^{ns}
Between group	1	22.224 ^{***}	1868.428 ^{***}	0.228 ^{***}	20.616 ^{***}	2.404 [*]	141.691 ^{***}	0.515 ^{***}	13.483 [*]	0.021 ^{**}	31.948 ^{***}
Genotype \times Salt treatment	12	0.735 ^{ns}	93.546 ^{ns}	0.012 ^{ns}	0.902 ^{ns}	0.330 ^{ns}	11.690 ^{***}	2.750 ^{***}	1.839 ^{ns}	0.001 ^{ns}	107.890 ^{***}
Sensitive \times Salt treatment	6	1.558 ^{ns}	20.650 ^{ns}	0.007 ^{ns}	0.390 ^{ns}	0.505 ^{ns}	4.520 ^{ns}	0.364 ^{**}	2.158 ^{ns}	0.001 ^{ns}	23.068 [*]
Tolerant \times Salt treatment	4	0.889 ^{ns}	64.008 ^{ns}	0.024 ^{ns}	0.347 ^{ns}	0.194 ^{ns}	1.397 ^{ns}	0.009 ^{ns}	1.892 ^{ns}	0.001 ^{ns}	0.882 ^{ns}
Groups \times Salt treatment	2	1.852 ^{ns}	371.309 ^{**}	0.001 ^{ns}	3.545 [*]	0.075 ^{ns}	53.784 ^{***}	1.976 ^{***}	0.776 ^{ns}	0.003 [*]	120.078 ^{***}
Error	40	0.894	60.39	0.011	0.862	0.439	4.330	0.067	2.808	0.001	5.541

* $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$, ns= Non-significant.



led to rather substantial decreases in SFM and SDM by 61% and 65%, and RFM and RDM by 67% and 74%, respectively, compared to the control (Table 3). The response of sesame genotypes to the salt was significantly different for SFM, SDM, RFM and RDM (Table 2). The greatest RFM, RDM, SFM and SDM were recorded in genotypes 'Ardestan', 'Varamin' and 'Darab' (all representing the tolerant group) at both 30 and 60 mM levels of salt (Table 3). On the other hand, genotypes 'Naz-Takshakhe', 'Naz-Chandshakhe', 'Oltan' and 'Yekta' (all representing the sensitive group) indicated smaller RFM, RDM, SFM and SDM when subjected to NaCl (Table 3). Although the two groups did not differ significantly in their RFM (Figure 1-a), SFM (Figure 1-c) and SDM (Figure 1-d) under the control conditions, the sensitive group (i.e. 'Naz-Takshakhe', 'Naz-Chandshakhe', 'Oltan' and 'Yekta') showed greater decreases in their RFM, RDM, SFM and SDM when grown under 30 and 60 mM NaCl levels, compared to the tolerant group (Figure 1-b).

Antioxidant Enzyme Activities

CAT activity was significantly affected by salt, genotype, tolerant *vs* sensitive, and interaction effect of group \times salt (Table 2). CAT activity increased in all sesame genotypes, except for 'Oltan', at the 30 mM level of salt, compared to the control. CAT activity in 'Oltan', 'Naz-Chandsakhe' and 'Naz-Takshakhe' was lowered with progressive salinity, relative to the control. CAT activity for the rest of the genotypes increased at the 60 mM level of salinity and the greatest increases were recorded in the genotype 'Darab' followed by 'Ardestan' and 'Varamin' (all representing the tolerant group) (Figure 2-a). The result of contrast comparisons indicated that although CAT activity of the two groups was not significantly different when grown at the control and 30 mM levels of NaCl, CAT activity in the tolerant group was nearly two

times greater than that found in the sensitive group at 60 mM (Figure 3-a).

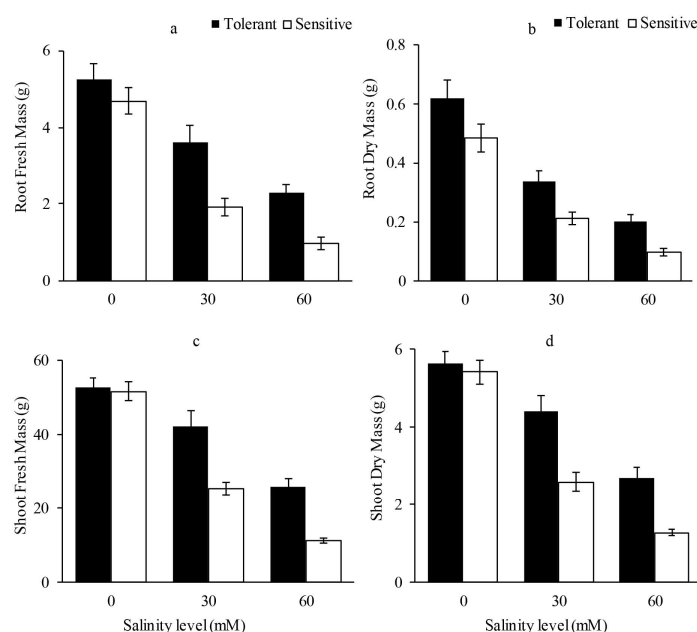
Leaf SOD activity was significantly affected by salt, genotype, tolerant *vs* sensitive, and interaction effect of group \times salt (Table 2). Leaf SOD activities of all sesame genotypes, with the exception of 'Yekta', increased when treated with 30 mM of NaCl. Leaf SOD activity was lowered in 'Naz-Chandshakhe' (13.56%), 'Naz-Takshakhe' (10.67%), and 'Oltan' (8.03%), whereas it increased in 'Darab' (77.60%), 'Varamin' (73.38%), 'Ardestan' (71.66%) and 'Yekta' (32.87%), with increase in NaCl concentration to 60 mM compared to the non-saline control (Figure 2-b). Although, the two groups of sesame genotypes did not differ in their SOD activity at the control and 30 mM NaCl levels, mean SOD activity of tolerant group was 64% higher than the sensitive group at the 60 mM (Figure 3-b).

The GR activity was significantly affected by salt, genotype, tolerant *vs* sensitive, and interaction effect of group \times salt (Table 2). Leaf GR activity for all genotypes significantly increased at the 60 mM level of salt, relative to the control (Figure 2-c). However, the extent of increase was greater in genotypes 'Darab', 'Yekta', and 'Varamin', compared to the remaining genotypes. Mean GR activity of the two groups was not significantly different at the control and 30 mM levels of salt, but that of the tolerant group was 22.8% higher than the sensitive group at 60 mM (Figure 3-c).

The APX activity in the sesame leaves was significantly affected by salt, genotype, sensitive group, and tolerant *vs* sensitive (Table 2). All sesame genotypes indicated increases in their APX activity with increases in the salt level (Figure 2-d). However, the greatest APX activities at the 30 and 60 mM NaCl levels were recorded in genotypes 'Varamin', 'Naz-takshakhe', 'Oltan' and 'Darab'. On the other hand, the smallest APX activities were recorded in genotypes 'Naz-Chandshakhe', and 'Yekta', compared to the remaining genotypes in both sensitive and tolerant groups.

Table 3. Effect of different salinity levels on Root Fresh Mass (RFM), Shoot Fresh Mass (SFM), Root Dry Mass (RDM), and Shoot Dry Mass (SDM) of *S. indicum* genotypes.^a

Genotypes	Salinity (mM)				
		RFM (g)	SFM (g)	RDM (g)	SDM (g)
Naz-Chandshakhe	0	4.16±0.87	54.16 ± 6.93	0.49 ± 0.08	5.68±0.73
	30	2.14 ± 0.46	23.16±2.94	0.21±0.002	2.25± 0.485
	60	0.83±0.17	10.93±1.05	0.07± 0.005	1.26 ± 0.13
Ardestan	0	4.30±0.52	53.93±3.94	0.49±0.08	5.69±0.56
	30	3.83±0.77	43.03±3.62	0.35±0.06	4.67±0.47
	60	1.88±0.19	27.20 ±4.68	0.26±0.024	2.75±0.61
Naz-Takshakhe	0	5.00±0.70	50.16±5.80	0.58±0.15	5.38±0.84
	30	1.60±0.32	24.36±2.57	0.20±0.04	2.26±0.36
	60	0.88±0.16	10.70±0.70	0.08±0.01	1.36±0.23
Yekta	0	4.96±0.86	49.93±5.45	0.45±0.08	5.29±0.60
	30	2.00±0.61	30.83±5.48	0.21±0.07	3.43±0.63
	60	1.09±0.61	12.88±1.32	0.13±0.04	1.41±0.19
Varamin	0	5.80±0.79	54.46±4.42	0.75±0.10	5.84 ±0.49
	30	3.96±0.52	52.13±1.80	0.36±0.04	5.11±0.08
	60	3.03±0.17	27.66±3.48	0.17±0.03	2.96±0.46
Darab	0	5.63±0.72	50.26±5.94	0.61±0.10	5.42±0.64
	30	3.12±0.99	31.83±9.18	0.30±0.07	3.48±0.94
	60	1.97±0.27	23.23±4.26	0.17±0.03	2.35±0.51
Oltan	0	4.65±0.48	52.50±5.35	0.41±0.07	5.35±0.64
	30	1.90±0.55	23.17±1.69	0.22±0.05	2.44±0.08
	60	1.08±0.13	10.43±0.91	0.11±0.01	1.07±0.05

^a Each value is mean±SE (n= 21).**Figure 1.** Root Fresh Mass (RFM) (a), Root Dry Mass (RDM) (b), Shoot Fresh Mass (SFM) (c) and Shoot Dry Mass (SDM) (d) of sensitive (Naz-Takshakhe, Naz-Chandshakhe, Oltan, Yekta) and tolerant (Varamin, Darab, Ardestan) genotypes of *S. indicum* when grown for 6-weeks under 0, 30 and 60 mM NaCl. Data are the mean±SE (n= 9 for tolerant; n= 12 for sensitive).

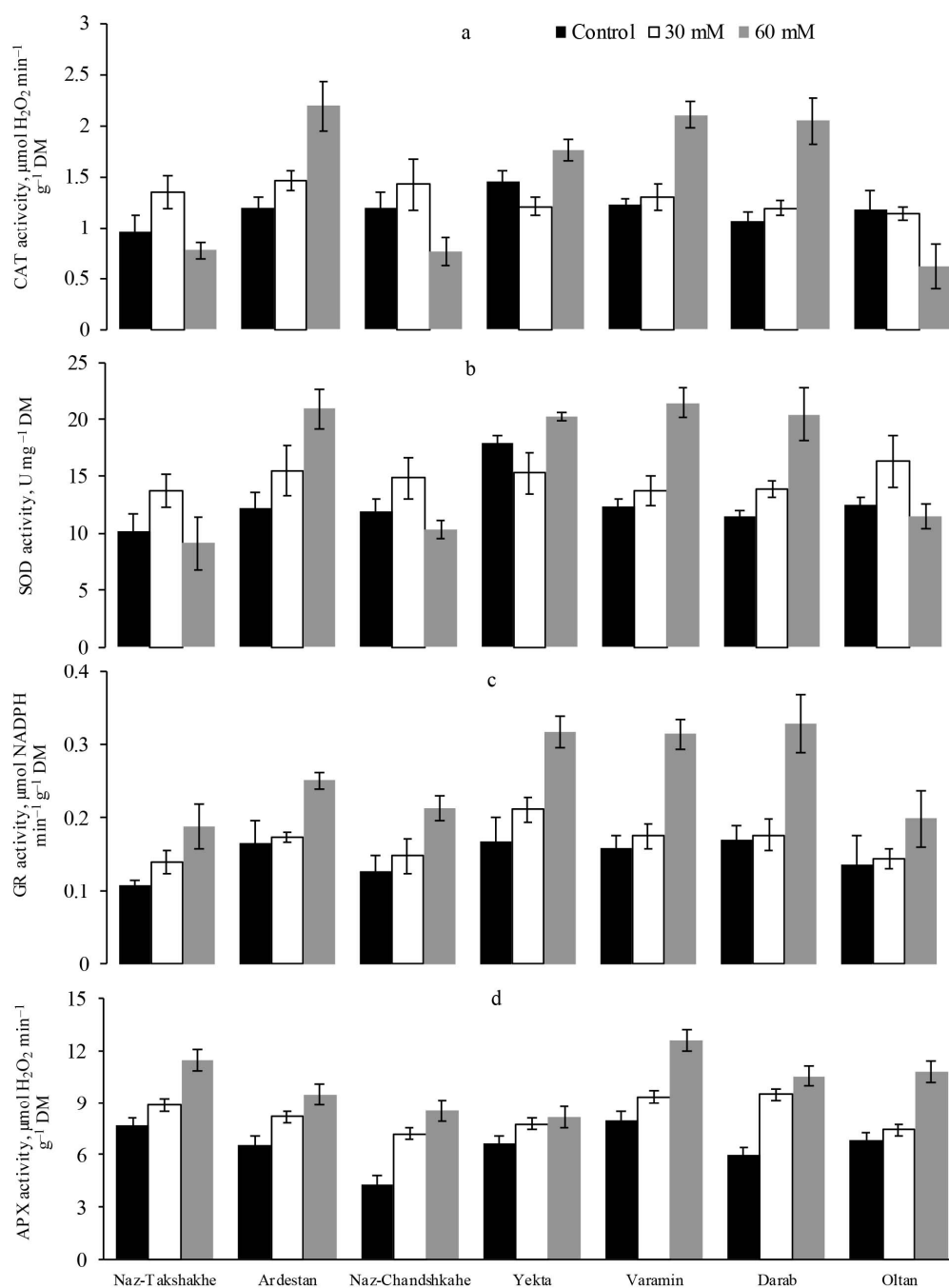


Figure 2. Catalase (CAT) (a), SuperOxide Dismutase (SOD) (b), Glutathione Reductase (GR) (c) and Ascorbate Peroxidase (APX) (d) of seven genotypes of *S. indicum* when grown for 6-weeks under 0, 30 and 60 mM NaCl. Data are the mean \pm SE ($n=3$).

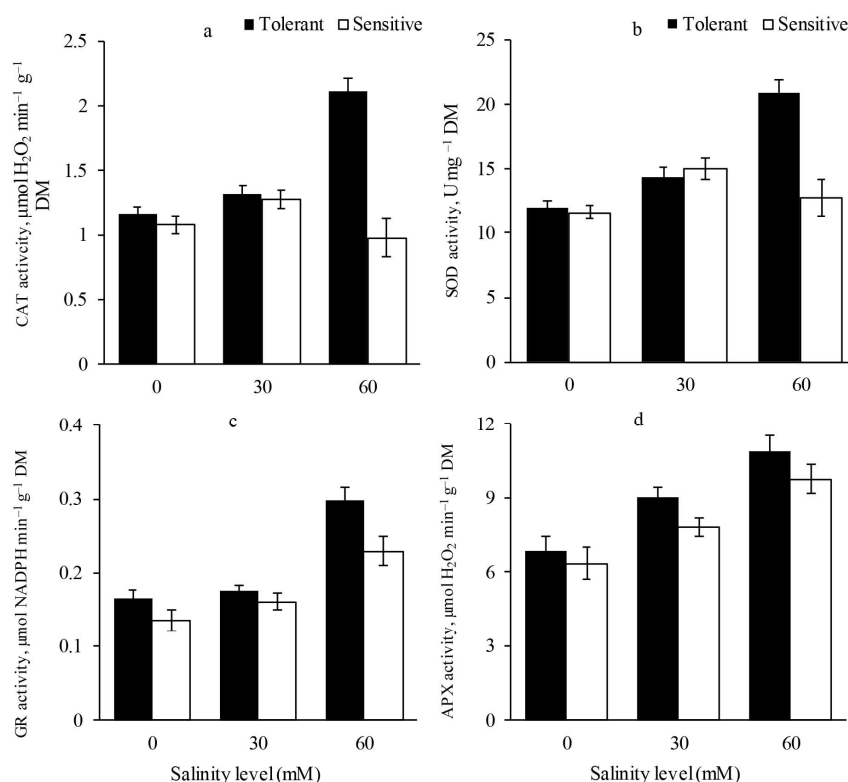


Figure 3. Catalase (CAT) (a), SuperOxide Dismutase (SOD) (b), Glutathione Reductase (GR) (c) and Ascorbate Peroxidase (APX) (d) of sensitive (Naz-Takshakhe, Naz-Chandshakhe, Oltan, Yekta) and tolerant (Varamin, Darab, Ardestan) genotypes of *S. indicum* when grown for 6-weeks under 0, 30 and 60 mM NaCl. Data are the mean±SE (n= 9 for tolerant; n= 12 for sensitive).

Leaf Proline Content

Leaf proline content was significantly affected by salt, genotype, tolerant group, and tolerant vs sensitive (Table 2). Averaged over genotypes, the 60 mM level of NaCl led to a 95% increase in the mean free proline content of the sesame leaves, relative to the non-saline control (data not shown). The greatest accumulation of proline at the 60 mM level of NaCl was recorded in the leaves of genotypes 'Varamin' and 'Ardestan' from the tolerant group and 'Yekta', from the sensitive group, whereas the smallest proline accumulation was observed in 'Naz-Takshakhe' and 'Naz-Chandshakhe', from the sensitive group, compared to the rest of genotypes (Figure 4). There were some indications of higher

level of proline in tolerant group under both saline and non-saline conditions, though these differences were not proven significant (Figure 6-a).

Membrane Lipid Peroxidation

The leaf MDA content of sesame, as a measure of lipid peroxidation, was significantly affected by salt, genotype, sensitive group, tolerant vs sensitive, and interaction effects of genotype×salt and group×salt (Table 2). The leaf MDA content for genotypes 'Naz-Chandsakhe' and 'Naz-Takshakhe' increased significantly at the 30 mM of NaCl compared to the control, whereas for the rest of genotypes it remained unaffected (Figure 5). Leaf MDA content at the 60 mM level of NaCl increased

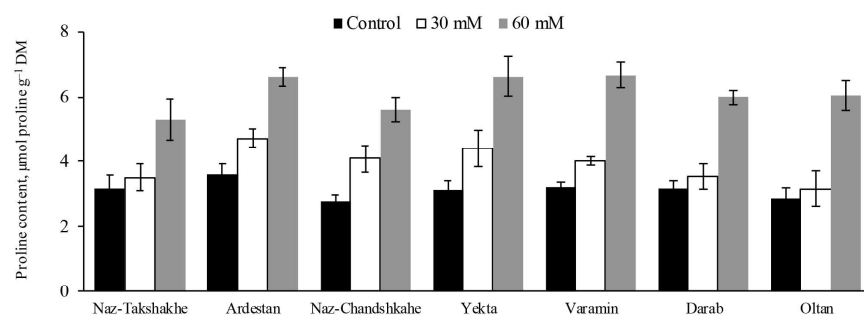


Figure 4. Proline content of seven genotypes of *S. indicum* when grown for 6-weeks under 0, 30 and 60 mM NaCl. Data are the mean \pm SE (n= 3).

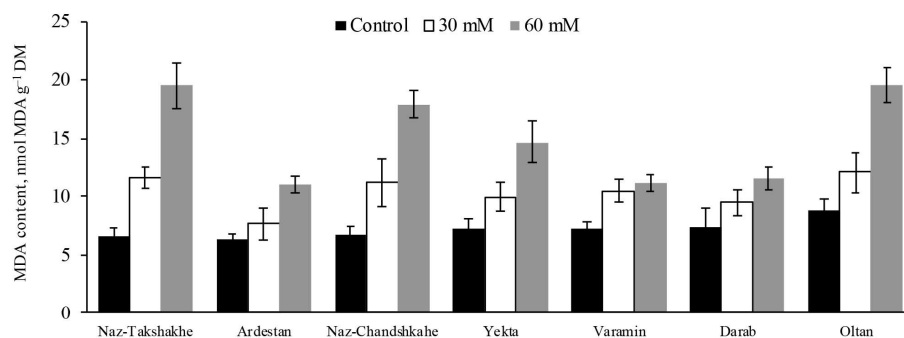


Figure 5. Malondialdehyde content of seven genotypes of *S. indicum* when grown for 6-weeks under 0, 30 and 60 mM NaCl. Data are the mean \pm SE (n= 3).

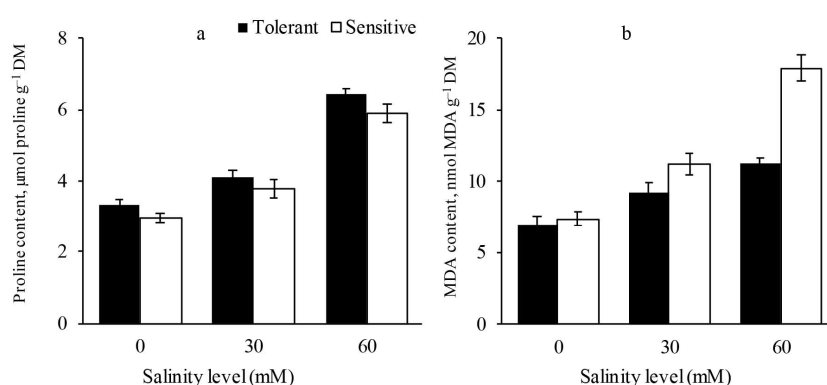


Figure 6. Proline (a) and malondialdehyde content (b) of sensitive (Naz-Takshakhe, Naz-Chandshakhe, Oltan, Yekta) and tolerant (Varamin, Darab, Ardestan) genotypes of *S. indicum* when grown for 6-weeks under 0, 30 and 60 mM NaCl. Data are the mean \pm SE (n= 9 for tolerant; n= 12 for sensitive).

significantly in all of the sesame genotypes, relative to the control and 30 mM treatment. However, the magnitude of the increases was greater in 'Naz-Takshakhe' (194%), 'Naz-Takshakhe' (166%), 'Oltan' (123%), and 'Yekta' (96%) (all representing the sensitive group), compared to the rest of genotypes. On the other hand, minimal increases in lipid peroxidation were observed in genotype 'Varamin' followed by 'Darab' and 'Ardestan' (all representing the tolerant group). Although increasing NaCl level from 0 to 60 mM led to increases in lipid peroxidation in both groups, the magnitude of the increase was remarkably greater (59%) in the sensitive group, compared to the tolerant (Figure 6-b).

DISCUSSION

Inhibition of shoot and root growth is a common response to salinity and plant growth attributes are the most important agricultural criteria of salt stress tolerance (Munns and Tester, 2008; Azizpour *et al.*, 2010, Bazrafshan and Ehsanzadeh, 2014). In order to obtain a quantitative measure of relative salt stress tolerance of the sesame genotypes, SFM, RFM, SDM and RDM were measured under the effect of NaCl treatment. Overall, the latter growth attributes decreased in all genotypes when subjected to 30 and 60 mM NaCl, proving an adverse effect of relatively mild salt levels (i.e. 30 mM \approx 3 dS m⁻¹) on growth attributes of this oilseed crop. However, among the sesame genotypes studied in the present work, genotypes 'Varamin', 'Ardestan' and 'Darab' indicated the greatest SDM and RDM in the presence of NaCl (Table 3) and, therefore, were categorized as salt-tolerant. Although information is lacking on sesame, the adverse effect of salinity on plant biomass has earlier been observed in a number of diverse crops species, e.g. cotton (Meloni *et al.*, 2003), linseed (Nasir Khan *et al.*, 2007), bean (Kaymakanova and Stoeva, 2008), maize (Tuna *et al.*, 2008), tetraploid wheat

(Ehsanzadeh *et al.*, 2009), pea (Maksimović *et al.*, 2010), alfalfa (Farissi *et al.*, 2014) and sorghum (Kafi *et al.*, 2013). According to Munns and Tester (2008), salinity reduces the ability of plants to uptake water and this, subsequently, leads to a reduction in growth rate, along with a suite of metabolic changes. There are also reductions in plant biomass attributes under stressful conditions because of a reduced photosynthetic activity per unit leaf area (Kaymakanova and Stoeva, 2008), additional cost to exclude or compartmentalize salts within the cells, and also the salt-induced damage to the plant cells and tissues (Maksimović *et al.*, 2010).

In addition to rather direct impacts that salt stress may exert on plants, salinity may impose, through reduction in the photosynthetic capacity, indirect ROS-induced cellular damages to the cells (Abogadallah, 2010). Hence, salt stress tolerance may depend, at least in part, on the enhancement of the antioxidative defense system that is, often, brought about by the antioxidant compounds and several antioxidative enzymes.

SOD is one of the most important antioxidant enzymes and is the first line of cellular defense against the oxidative stress (Demiral and Türkan, 2005; Tuna *et al.*, 2008). SOD plays an important role in modulating the relative amount of O₂^{•-} and H₂O₂ in plants and, hence, performs a key role in the defense mechanism against ROS toxicity (Meloni *et al.*, 2003). In the present study, the 60 mM level of NaCl suppressed the SOD activity in 'Oltan', 'Naz-Takshakhe' and 'Naz-Chandshakhe' (i.e. salt-sensitive group), whereas it led to notable increases in the activity of the latter enzyme in the salt-tolerant group, i.e. 'Ardestan', 'Darab' and 'Varamin' (Figure 2-b). The observed depression in SOD activity in the salt-sensitive genotypes could be regarded as the lack of an ability of these genotypes to scavenge O₂^{•-} radicals, which could lead to cellular damage and suppression of plant growth. Many studies have found positive correlations between salt stress tolerance and the level of SOD



activity in different plant species (Bor *et al.*, 2003; Liang *et al.*, 2003; Koca *et al.*, 2007).

H₂O₂, a product of SOD activity in plant cells, is toxic and in order to become harmless to the cell, it must undergo detoxification by conversion to H₂O in further reactions. In plant cells, different antioxidative enzymes may take part in regulating the intracellular level of H₂O₂, though CAT, APX and GR are considered the most prevalent ones. CAT is the principal scavenger of the oxidant H₂O₂ in peroxisomes and eliminates H₂O₂ by its direct decomposition to water and molecular oxygen (Abogadallah, 2010). Increase in CAT activity is thought to be an adaptive mechanism possibly helping to overcome the ROS-induced damage to the cells (Tunc-Ozdemir *et al.*, 2009). In the present study, in contrast to the salt-sensitive group, where CAT activity decreased or remained unchanged when plants were exposed to 30 and 60 mM levels of NaCl (Figure 3-a), increase in CAT activity (Figure 2-a) of the salt-tolerant group is suggestive of presence of an effective scavenging mechanism to remove H₂O₂. Increases in CAT activity under saline conditions in salt-tolerant cultivars have been reported by some researchers working on sesame (Koca *et al.*, 2007) and other plant species (Sairam *et al.*, 2002; Bor *et al.*, 2003; Liang *et al.*, 2003; Tuna *et al.*, 2008).

The role of APX and GR in the H₂O₂ scavenging in the ascorbate-glutathione cycle of plant cells has been well established (Demiral and Türkan, 2005). The elevated levels of GR activity could increase the GSH/GSSG ratio, which is required for regeneration of ascorbate and activation of several CO₂ fixing enzymes in the chloroplasts (Abogadallah, 2010), ensuring NADP⁺ availability to accept electrons from the photosynthetic electron transport chain (Reddy *et al.*, 2004). Moreover, ascorbate is the most common reducing substrate for H₂O₂ detoxification in plant cells and APX uses ascorbate to reduce the H₂O₂ to water (Noctor *et al.*, 2002). In the present study, the NaCl-induced enhancement in GR and

APX activities seemed to be a common response in all sesame genotypes studied (Figures 2-c and -d). However, genotypes 'Varamin', 'Darab', 'Yekta' and 'Ardestan' in general, and the salt-tolerant group in particular, (Figure 3-c) indicated the greatest levels of GR activities at least at the 60 mM level of NaCl. In contrast to the GR activity, where the two groups behaved differently, the APX activity in the salt-sensitive and salt-tolerant groups did not appear much different (Figure 3-d). These results are in agreement with those of Farooq and Azam (2006) and Sairam *et al.* (2002) who reported increases in GR and APX activity in different salt-treated wheat cultivars.

It has been suggested that the accumulation of amino acids following stress conditions is correlated to the plant cell damage. The amino acid proline is one of the prevalent osmolytes that are commonly found in high concentrations when plants are exposed to salt stress (Munns and Tester, 2008). The exact role of proline with regard to plant's response to environmental stresses is rather controversial (Flowers *et al.*, 2010). Proline accumulation in plant cells might be due to an increase in proteolysis or a decrease in protein synthesis (Ashraf and Harris, 2004). Anyhow, the accumulation of proline under stress conditions can protect the cell by stabilizing sub cellular structures (e.g. proteins and enzymes) and buffering the cellular redox potential (Demiral and Türkan, 2005). Besides its role as an osmolyte, proline can also confer enzyme protection and increase membrane stability under various conditions (Nasir Khan *et al.*, 2007). In our study, salinity led to increases in leaf proline content in all genotypes at 60 mM and to a lesser extent at 30 mM level (Figure 4). Proline has been shown to accumulate in response to salinity in a number of plant species (Azevedo Neto *et al.*, 2006; Koca *et al.*, 2007; Nasir Khan *et al.*, 2007; Li *et al.*, 2010). Since proline accumulation in the sesame genotypes was well-correlated to their growth attributes and production of this free amino acid in the

salt-tolerant genotypes was more notable (Figure 4), it seems that it plays some protective roles against salt stress in sesame, at least in the genotypes used in this experiment.

Lipid peroxidation has been associated with cell damages caused by different biotic and abiotic stresses and is often used as an indicator of salt-induced oxidative damage to the cellular membranes (Katsuhara *et al.*, 2005). The MDA content tended to increase significantly at both 30 and 60 mM levels of salt in all sesame genotypes studied, but genotypes representing the salt-tolerant group, i.e. 'Varamin', 'Darab' and 'Ardestan', indicated a smaller tendency for MDA accumulation in the presence of NaCl, in general, and at the 60 mM level of salt, in particular (Figures 5 and 6-b). The latter discrepancy in MDA level between the two groups of sesame genotypes was, more or less, in proportion to the correspondent antioxidant enzyme activities particularly at the 60 mM level of NaCl. In previous reports on sesame (Koca *et al.*, 2007) and other crop species such as castor bean (Li *et al.*, 2010), maize (Azevedo Neto *et al.*, 2006), rice (Vaidyanathan *et al.*, 2003) and barley (Liang *et al.*, 2003) negative correlations between MDA content and level of tolerance to salt stress have been emphasized.

In conclusion, the addition of NaCl to the growing medium resulted in a number of stress responses. Antioxidant enzymes such as CAT, SOD, APX and GR were, presumably, crucial in partial relief of sesame genotypes from ROS-induced damage. On the contrary to the MDA content, proline accumulation was more notable in a set of genotypes whose growth attributes were less affected by the salt stress. Therefore, we found clear indications of physiological differences between sesame genotypes in their response to salt stress; genotypes such as 'Ardestan', 'Varamin' and 'Darab' seemed to rely at least in part on their antioxidant activity and osmolytes production to tolerate the moderate NaCl levels applied in this study. It remains to be

seen whether the probable differences in grain yield and agronomic performance of the latter sesame genotypes under field conditions, in response to NaCl, will be consistent with the present findings or not.

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شواهدی برای تفاوت در پراکسیداسیون چربی و فعالیت آنزیمهای آنتی اکسیدانی ژنوتیپهای کنجد تحت سطوح مختلف شوری

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چکیده

از آنجا که کنجد گیاهی تا حدودی فراموش شده است اطلاعات علمی چندانی در مورد پاسخ آن به شوری وجود ندارد. در این آزمایش تاثیر تنش شوری بر هفت ژنوتیپ کنجد مورد بررسی قرار گرفت. بدین منظور گیاهچه های شش هفته ای کنجد تحت تاثیر سطوح مختلف کلرید سدیم (۰، ۳۰ و ۶۰ میلی مولار) قرار گرفتند. کلرید سدیم باعث کاهش معنی داری در وزن تر و خشک تمامی ژنوتیپها شد. با این وجود واکنش ژنوتیپها به شوری از نظر وزن تر و خشک به طور معنی داری با یکدیگر متفاوت بود. براساس میزان تجمع ماده تر و خشک تحت سطوح تنش ژنوتیپها به دو گروه متحمل (اردستان، ورامین و داراب) و حساس به شوری (ناز تک شاخه، ناز چندشاخه، یکتا و اولتان) دسته بندی شدند. کمترین میزان پراکسیداسیون چربی در سطح ۶۰ میلی مولار شوری مربوط به ژنوتیپ اردستان، ورامین و داراب (گروه



متحمل) بود. درحالیکه بیشترین میزان پراکسیداسیون چربی در این سطح در ژنوتیپهای نازتک شاخه، ناز چندشاخه، یکتا و اولتان (گروه حساس) مشاهده شد. در این آزمایش فعالیت آنزیمهای آنتی اکسیدانی سوپراکسیددسموتاز، کاتالاز، آسکوربات پراکسیداز و گلوتاتیون ردوکتاز تعیین و اندازه گیری شد. فعالیت تمامی این آنزیمها در هر دو سطح ۳۰ و ۶۰ میلی مولار کلرید سدیم در گروه متحمل به شوری نسبت به گروه حساس به شوری بالاتر بود. میزان تجمع پرولین در برگ تحت شرایط تنش در گروه متحمل نسبت به بقیه ژنوتیپها به طور معنی داری بیشتر بود. به نظر می رسد در گروه ژنوتیپهای متحمل افزایش در فعالیت آنزیمهای آنتی اکسیدانی و میزان تجمع پرولین برگ به همراه کاهش کمتر پراکسیداسیون چربی درغشای سلولی منجر به افزایش تحمل این ژنوتیپها به شوری شد.