Morphological and biochemical changes in response to salinity in sunflower (*Helianthus annus* L.) cultivars

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Abstract

This study was conducted to evaluate the alterations of some morphological and biochemical parameters of sunflower cultivars ('08-TR-003', 'TR-3080' and 'TARSAN-1018') under salt stress. For this aim, the seedling of sunflower cultivars was irrigated with tap water as a control, and with salinised water with 50, 150 and 250 mM NaCl for 30 days under controlled conditions. Salinity caused an apparent reduction in morphological parameters (plant height, leaf area, fresh weight, dry matter and water content) in all cultivars. Salt stress significantly ($P<0.01$) reduced the activity of glutathione reductase (GR) and ascorbate peroxidase (APX) activities in all sunflower cultivars expect for superoxide dismutase (SOD) activity. According to our results, SOD seems to play a key role in the antioxidative process in salt treated sunflower plants. Proline and malondialdehyde contents were significantly ($P<0.05$) increased under salt stress in all cultivars. Among the cultivars, 'TR-3080' had greater values in terms of morphological (plant height, leaf area, fresh weight, water content) and biochemical [GR, APX and SOD (secondly) activities and proline contents] parameters. In the light of these findings, cv. 'TR-3080' seems to be less affected by salt stress.

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Introduction

Biotic and abiotic stresses are the main problems of agricultural systems. Salinity is also considered as a major abiotic stress and a significant factor affecting crop production all over the world (about 7% of arable lands of the world are under salinity pressure) and especially in arid and semi-arid regions (Bajehbaj, 2010). Throughout semi-arid regions of temperate climates, the most important oil-yielding crop is sunflower (*Helianthus annuus* L.) (Jabeen and Ahmad, 2012). Sunflower (*Helianthus annuus* L.), a species of the Asteraceae family, is an important annual economic (edible or oil-producing) oilseed crop that is ranked as the 4th important vegetable oil crop after soybean, palm oil and rapeseed (Wen-Zhi *et al.*, 2014; Achakzai *et al.*, 2015; Bakhoum and Sadak, 2016). It is grown around the world and is a popular crop in countries that have salt affected soils (Masor, 2011). The soil salinity level was the main limiting factor for vegetative growth of sunflower (Ma *et al.*, 2016). Sunflower (*Helianthus annuus* L.) has been rated as moderately salt-resistant with no significant yield reduction up to 4.8 dS m^{-1}, and variability for salt resistance has been detected within this crop (Ceccoli *et al.*, 2012; Machekposhti *et al.*, 2017).

Salinity adversely affects important physiological processes and biochemical mechanisms, causes severe loss in crop productivity worldwide (Per *et al.*, 2017) and can cause some biochemical changes in plant cell such as losing of cell turgor and the accumulation of reactive oxygen species (ROS) (Nxele *et al.*, 2017). ROS including hydrogen peroxide (H_2O_2), superoxide anions ($O_2^{\bullet-}$), hydroxyl radical ($OH^{\bullet}$) and singlet oxygen ($^1O_2$) are by-products of physiological metabolisms, and are precisely controlled by enzymatic and non-enzymatic antioxidant defense systems (You and Chan, 2015). The main antioxidant enzymes include superoxide dismutase (SOD), catalase, ascorbate peroxidase (APX), glutathione reductase (GR) and the activities of these enzymes are generally increased in plants under oxidative stress conditions (Bela *et al.*, 2015; Vighi *et al.*, 2017). One of the other cellular responses to saline conditions is the alteration of metabolism and production of compatible solutes (osmolytes) such as proline, which are distributed among different organisms (Mansour and Ali, 2017). The accumulation of proline (Pro) is one of the striking metabolic responses of plants to salt stress (Per *et al.*, 2017). One of the cell parts where stress-related oxidative damage has the most effect is the cell membrane. As a result of oxidative damage, lipid peroxidation occurs in cell membranes and the permeability of the membrane is damaged. Lipid peroxidation can be measured with the help of malondialdehyde (MDA), which is a byproduct of this process (Koç, 2015). There are limited reports (Rios-Gonzalez *et al.*, 2002) on the morphological and biochemical responses of sunflower to soil salinity. Therefore, this study was

conducted to evaluate the alterations of some morphological and biochemical parameters of different sunflower cultivars under salt stress.

Materials and methods

Plant material

Oil type sunflower cultivars '08-TR-003', 'TR-3080' and 'TARSAN-1018' obtained from *Trakya Agricultural Research Institute* were used in the study.

NaCl experiments, planting and plant development

For each NaCl concentration and genotype, four replicates were tested, and there was one plant per replication. 500 mL pots were used in all experiments and one seed was sown in each pot. After sowing, each pot was irrigated with 50 mL tap water. After one week from sowing, pots were irrigated with 50 mL tap water containing different NaCl concentrations (0-control, 50, 150 and 250 mM) for four weeks. Tap water without NaCl was used as a control. All experiments were conducted in the controlled growth chamber for incubation at 24°±1°C under cool white fluorescent light (27 mmol m^{-2} s^{-1}) with a 16 h light/8 h dark photoperiod.

Morphological characters

After sowing, seeds were irrigated with 50 mL tap water for 10 days. First NaCl treatments began after these 10 days. Plantlets were watered with tap water (50 mL) containing different NaCl concentrations (0-control, 50, 150 and 250 mM) every two days. The application continued for 30 days, and then measurements were made. Fresh weight (g) of leaves was measured with a precision scale. Dry weights were measured after drying samples at 70ºC for 48 h in an oven. Water content was calculated with the following formula:

$$Water\ content\ (g) = Fresh\ weight\ of\ leaf\ (g) - Dry\ weight\ of\ leaf\ (g) \quad (1)$$

Percentages of dry matter and water content were measured with the following formulas:

$$Dry\ matter\ (\%) = \frac{Dry\ weight\ of\ leaf\ (g)}{Fresh\ weight\ of\ leaf\ (g)} x100 \quad (2)$$

$$Water\ content\ (\%) = \frac{Dry\ weight\ of\ leaf\ (g) - Fresh\ weight\ of\ leaf\ (g)}{Fresh\ weight\ of\ leaf\ (g)} x100 \quad (3)$$

Biochemical observation

Measurement of antioxidant enzymes

To determine the enzyme changes in plants under salt stress, approximately 1 g of fresh leaf samples in liquid nitrogen were ground up in porcelain mortars and homogenised with 10 mL of a 50-mM phosphate buffer solution containing 0.1 mM of Na-EDTA (pH 7.6). Homogenised samples were centrifuged at 15,000 rpm for 15 min and the resultant precipitates were used in enzyme analyses. Samples were kept at +4°C until the enzyme analyses were performed. For the enzyme measurements, final volumes were obtained using the buffer solution.

Superoxide dismutase activity

Superoxide dismutase activity was determined by using the method proposed by Cakmak and Marschner (1992), and Cakmak (1994), based on the reduction of nitro blue tetrazolium chloride (NBT) by O_2- under light. All the solutions were added into the reaction medium: first, 0.1 mM of Na-EDTA containing 50 mM (pH: 7.6) phosphate (P) buffer, then, the enzyme extract (25 to 100 µL) followed by 0.5 mL of 50 mM Na_2CO_3 (pH of 10.2), 0.5 mL of 12 mM of L-methionine, 0.5 mL of 75 µm of p-NBT and 10 µm of riboflavin were each added into the medium so that the final volume of the medium was 5 mL. The samples were kept under light for 15 min and measurements were carried out at 560 nm.

Ascorbate peroxidase activity

Ascorbate peroxidase activity was measured by using the method proposed by Cakmak and Marschner (1992), and Cakmak (1994), based on the oxidation of ascorbate at 290 nm (E = 2.8 mM cm^{-1}). By following the method, the final volume of the reaction medium was adjusted to 1 mL by adding 0.1 mM of EDTA containing a 50-mM phosphate buffer (pH of 7.6), 0.1 mL of 10 mM of EDTA containing 12 mM of H_2O_2, 0.1 mL of 0.25 mM of L-ascorbic acid and enzyme extract into the medium, and then the ascorbate concentration was measured at 290 nm using spectrophotometry.

Glutathione reductase activity

Glutathione reductase activity was measured with the method proposed by Cakmak and Marschner (1992), and Cakmak (1994), based on the oxidation of NADPH at 340 nm (E = 6.2 mM cm^{-1}). By following the method, the final volume of the reaction medium was adjusted to 1 mL by adding 0.1 mM of EDTA containing a 50-mM phosphor buffer (pH of 7.6), 0.1 mL of 0.5 mM of oxidised glutathione, 0.1 mL of 0.12 mM of NADPH and enzyme extract into the medium, and then the NADPH oxidation was measured at 340 nm.

Measurement proline and lipid oxidation (malondialdehyde content)

The proline assay was based on the method of Bates *et al.* (1973), which uses 3% sulfosalicylic acid for grinding the fresh plant samples. The ninhydrin reagent was added to the tubes containing the ground sample, which were then placed in a water bath at 100°C for 1 h. After cooling, 4 mL of toluene were added to the samples. The samples were measured at 520 nm. MDA was determined according to Lutts *et al.* (1996). Briefly, 5 mL trichloroacetic acid (0.1%) were added to the sample of 200 mg fresh leaves, and then centrifuged at 12,500 rpm for 20 min; 3 mL supernatant were taken from 5 mL extracts. Three milliliters of 0.1% thiobarbituric acid in 20% trichloroacetic acid (w/v) were added to an equal amount of each of the supernatants. The A-absorbance of the samples was measured using a spectrophotometer at 532 and 600 nm.

Statistical analysis

The experimental design was completely randomised design with four replications. Each treatment was arranged in 500 mL pots containing 5 plants. For all investigated parameters, Analysis of Variance was performed by using the *SPSS for Windows* computer software. Means of treatments were compared with Duncan's multiple range test by using 'MSTAT-C' computer software. Data given in percentages were subjected to arcsine ($\sqrt{X}$) transformation before statistical analysis (Snedecor and Cochran, 1967).

Results and discussion

The obtained results presented in Tables 1-4 show that, salt stress significantly reduced all growth characters (plant height, leaf area, fresh weight, dry matter and water contents) of the three sunflower cultivars ('08-TR-003', 'TR-3080' and 'TARSAN-1018'). Results of two-way ANOVA in the characters of plant height and leaf surface area showed that there were statistically significant ($P<0.01$) interactions between cultivars and salt concentrations while interaction between cultivars and salt concentrations in fresh weight was significant at 0.05 level (Table 1). From the mean data, it is apparent that cv. 'TR-3080' had greater plant height (21.33 cm) and fresh weight (7.47 g) than the other cultivars at the highest NaCl concentration (250 mM) (Table 2). Leaf area is a good indicator of water and salinity stress (Kumar *et al.*, 2014). In the present study, salt stress significantly ($P<0.01$) reduced leaf area of the three sunflower cultivars ('08-TR-003', 'TR-3080' and 'TARSAN-1018') (Figure 1). The highest value (45.46 mm^2, 43% decrease when compared to control) was registered in cv. 'TR-3080' at the highest NaCl concentration (250 mM). 34.42 mm^2 and 26.32 mm^2 were recorded in cv. '08-TR-003' and 'TARSAN-1018' with 49% and 65% decreasing rate, respectively (Table 2). Adverse effects of salt stress on leaf area of sunflower have also been reported by several authors (Rivelli *et al.*, 2010; Achakzai *et al.*, 2015; Khan *et al.*, 2016). Data related to fresh weight showed that the results dramatically decreased by increasing salt concentrations in all cultivars. The highest results regarding fresh weight were recorded at all salt concentrations from cv. 'TR-3080' (Table 2). Lower fresh weight at higher salt concentrations was due to decreasing water absorption (Prado *et al.*, 1995). It was reported that fresh weight increase was based on cell enlargement due to water intake, cell vacuolation and turgor-driven wall expansion (Dale, 1988).

There were statistically significant interactions between cultivars and salt concentrations on dry matter content, dry matter percentage and water content percentage at 0.01 level (Table 3). Since no interaction was determined between cultivars and salt concentrations in water content, the effects of these factors was analysed separately (Table 3). Both factors (cultivars and salt concentrations) affected water content significantly at 0.01 level. In term of dry matter, the highest value was registered in cv. 'TARSAN-1018' with 1.25 g (13% decrease compare to control) at 250 mM NaCl. The dry matter of cv. 'TR-3080' and '08-TR-003' was 1.03 g (50%

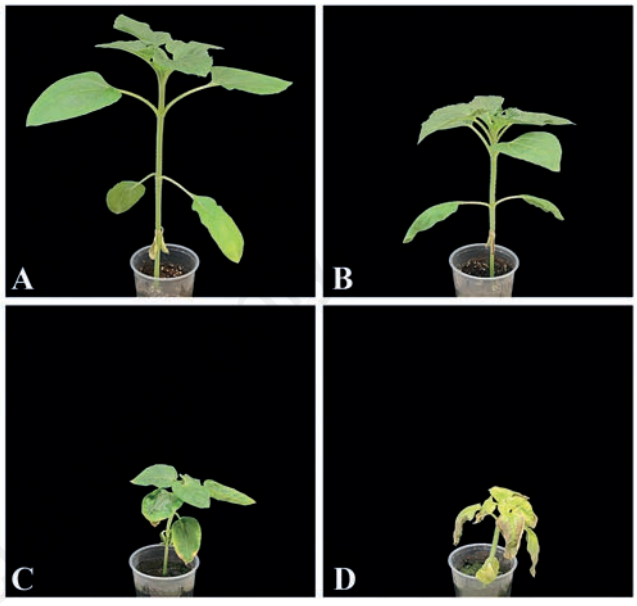

Figure 1. The effect of different salt concentrations on seedling growth of sunflower cv. 'TARSAN 1018'. A) 0 mM (control); B) 50 mM; C) 150 mM; and D) 250 mM.

Table 1. Results of two-way ANOVA of the effect of different cultivars and salt concentrations on plant height, leaf surface area and fresh weight.

Source		Plant height (cm)				Leaf surface area (mm^2)				Fresh weight (g)			
	df	Sum of squares	Mean square	F	Sig.	Sum of squares	Mean square	F	Sig.	Sum of squares	Mean square	F	Sig.
Corrected model	11	840.3	76.4	32.9	0.000	8981.5	816.5	101.1	0.000	370.8	33.7	54.4	0.000
Intercept	1	17,689.0	17,689.0	7626.4	0.000	111,915.9	111,915.9	13,862.3	0.000	3765.7	3765.7	6077.5	0.000
Cultivar	2	296.2	148.1	63.8	0.000	1035.9	518.0	64.2	0.000	31.0	15.5	25.1	0.000
Salt	3	484.9	161.6	69.7	0.000	7642.5	2547.5	315.5	0.000	330.1	110.0	177.6	0.000
Cultivar * Salt	6	59.3	9.89	4.3	0.005	303.1	50.5	6.3	0.000	9.7	1.6	2.6	0.043
Error	24	55.7	2.3			193.8	8.1			14.8	0.6		
Total	36	18,585.0				121,091.2				4151.4			
Corrected total	35	896.000				9175.3				385.7			

Table 2. The effect of different cultivars and salt concentrations on plant height, leaf surface area, fresh weight in sunflower.

Cultivars	NaCl Cont. (mM)	Plant height (cm)				Leaf surface area (mm^2)				Fresh weight (g)			
		0	50	150	250	0	50	150	250	0	50	150	250
'TARSAN 1018'		24.83^{bc}	21.50^{cd}	19.17^{de}	12.83^{f}	75.33^{ab}	62.67^{cd}	44.79^{e}	26.32^{g}	13.56^{b}	10.52^{c}	7.22^{d}	4.48^{e}
'TR-3080'		33.33^{a}	26.00^{b}	24.00^{bc}	21.33^{cd}	81.17^{a}	69.00^{bc}	57.71^{d}	45.46^{e}	15.89^{a}	11.26^{c}	9.82^{c}	7.47^{d}
'08-TR-003'		23.33^{bc}	22.00^{cd}	21.00^{cd}	16.67^{e}	67.13^{c}	57.00^{d}	48.08^{e}	34.42^{f}	14.79^{ab}	10.44^{c}	9.97^{c}	7.31^{d}

[a-e]Values in a row and in a column (for water content in g) followed by the different letters are significantly different at the 0.01 level.

decrease) and 0.62 g (68%decrease), respectively (Table 4). According to Nobre *et al.* (2010) salinity affects the plant dry matter production because the high salt concentrations at the root zone decrease water availability. Sunderland (1960) reported that dry weight content of the leaf depended on cell division and new material synthesis. However, it was reported that one of the reasons of dry matter decrease in sunflower cultivars was salt stress (Rivelli *et al.*, 2010; Maia *et al.*, 2016; Khan *et al.*, 2016). The highest water content was observed in the cv. 'TR-3080' (6.44 g, 53% decrease compare to control) at 250 mM NaCl. The water content of cv. '08-TR-003' and 'TARSAN-1018' was 6.06 g (54% decrease) and 3.06 g (66% decrease) at 250 mM NaCl, respectively (Table 2). Jabeen and Ahmad (2012) stated that imposition of salt stress also had adverse effects on relative leaf water content in sunflower. Overall, decrease in growth is one of the most commonly observed symptoms in plants grown in saline environments and has been well documented some sunflower cultivars (Steduto *et al.*, 2000; Rios-Gonzales *et al.* 2002; Hussain *et al.*, 2012). Water content in g decreased significantly by increasing salt concentrations. The highest water was noted in control treatment, while the lowest values were obtained from 250 mM NaCl treatment. Lower levels of all parameters at higher NaCl concentrations could be attiributed to the decreasing amount of water absorption from the soil and consequently, to a reduced uptake of solutes due to lower osmotic pressure of the roots. The inhibition of growth under water stress conditions hinders cell division and elongation (Hsia, 1973). Osmotic stress hinders cell wall extension (Van Volkenburg and Boyer, 1985). Karmoker and Van Steveninck (1979) stated that stress-induced growth reduction could be due to changes in membrane permeability and water absorption.

Statistical analysis showed that there were statistically significant ($P<0.01$) interactions between cultivars and salt concentrations in proline and lipid peroxidation (MDA) contents, activity of ascorbate peroxidase and superoxide dismutase. In glutathione reductase activity, no interaction was observed between cultivars and salt concentrations. That was because the effect of these factors was analysed separately. The effects of cultivars and salt concentrations on glutathione reductase activity were found statistically significant at 0.01 level (Table 5). Our results show that increasing the NaCl concentration significantly reduced the activity of GR and APX activities in all sunflower cultivars except for SOD activity compared to control (Table 6). GR is a potential enzyme of the ASH-GSH cycle and plays an essential role in the defense system against ROS by sustaining the reduced status of GSH (Gill and Tuteja, 2010). The highest activity of GR was observed in 'TR-3080' (102.78 mmol min^{-1} mg^{-1} FW, 8.51% decrease compared to control) at highest NaCl concentration (250 mM). However, GR activities were 96.00 and 94.00 $unit^{-1}min^{-1}$ mg FW in 'TARSAN1018' and '08-TR-003', respectively. The percentage decreases were 17.64% and 8.05% at 250 mM NaCl concentration in 'TARSAN1018' and '08-TR-003', respectively. The first enzyme of the ascorbate-glutathione cycle, APX, plays a vital role in the elimination of H_2O_2 (Vighi *et al.*, 2017). At 250 mM NaCl level, the highest activity of APX (2730.96 mmol min^{-1} mg^{-1} FW) was recorded in cv. 'TR-3080' in control treatment. However, APX activities were 1759.73 and 1532.99 mmol min^{-1} mg^{-1} FW in '08-TR-003' and 'TARSAN1018', respectively. Accelerated salt stress reduced APX activities by 35.57% and 12.39%, in '08-TR-003 and 'TARSAN1018', respectively. In an oxidative defense system, SOD has an important role in the first dismutation ROS. Unlike the other enzymes, our results show that SOD activity increases with increasing NaCl concentrations in all cultivars (Table 6). The high-

Table 3. Results of two-way ANOVA of the effect of cultivars and salt concentrations on the contents of dry matter and water.

Source	df	Dry matter content (g)				Dry matter content (%)				Water content (g)				Water content (%)			
		Sum of squares	Mean square	F	Sig.	Sum of squares	Mean square	F	Sig.	Sum of square	Mean square	F	Sig.	Sum of square	Mean square	F	Sig.
Corrected model	11	5.6	0.5	22.8	0.000	93.5	8.5	5.9	0.000	296.8	27.0	52.1	0.000	93.5	8.5	5.9	0.000
Intercept	1	68.2	68.3	3040.2	0.000	16,905.1	16,905.1	11,686.0	0.000	2820.3	2820.3	5449.7	0.000	168,060.7	168,060.7	116,175.1	0.000
Cultivar	2	0.3	0.2	6.8	0.005	8.6	4.3	3.0	0.070	25.5	12.7	24.6	0.000	8.6	4.3	3.0	0.070
Salt	3	3.7	1.2	54.5	0.000	17.8	5.9	4.1	0.017	264.4	88.1	170.3	0.000	17.8	5.9	4.1	0.017
Cultivar * Salt	6	1.6	0.3	12.3	0.000	67.1	11.2	7.7	0.000	6.9	1.2	2.2	0.075	67.1	11.2	7.7	0.000
Error	24	0.5	0.02			34.7	1.4			12.4	0.5			34.7	1.4		
Total	36	74.4				17,033.4				3129.5				168,188.9			
Corrected total	35	6.2				128.3				309.2				128.2			

Table 4. The effect of different cultivars and salt concentrations on dry matter and water content in sunflower.

Cultivars	NaCl Cont. (mM)	Dry matter content (g) 0	50	150	250	Dry matter content (%) 0	50	150	250	Water content (g) 0	50	150	250	Mean	Water content (%) 0	50	150	250
'TARSAN 1018'		1.98^{a}	1.36^{bc}	1.03^{c}	0.62^{d}	14.62^{ab}	12.97^{bcd}	14.32^{abc}	13.59^{abc}	11.58	9.16	6.19	3.86	7.69^{b}	85.38^{d}	87.03^{abc}	85.68^{bcd}	86.41^{bcd}
'TR-3080'		2.06^{a}	1.57^{b}	1.07^{c}	1.03^{c}	13.00^{bcd}	13.95^{abc}	10.89^{cd}	13.81^{abc}	13.83	9.69	8.75	6.44	9.67^{a}	87.00^{abc}	86.05^{bcd}	89.11^{ab}	86.19^{bcd}
'08-TR-003'		1.45^{b}	1.50^{b}	1.60^{b}	1.25^{bc}	9.81^{d}	14.35^{abc}	16.08^{ab}	17.18^{a}	13.34	8.94	8.37	6.06	9.17^{a}	90.19^{a}	85.65^{bcd}	83.92^{cd}	82.82^{d}
	Mean									12.91^{a}	9.26^{b}	7.77^{c}	5.45^{d}					

[a-d]Values in a row and in a column (for water content in g) followed by the different letters are significantly different at the 0.01 level.

est SOD activity (85.46 mmol min^{-1} mg^{-1} FW, 78.04% increase compared to control) was registered in cv. 'TARSAN1018' under high salt stress (250 mM). However, the lowest activity of SOD (80.21 mmol min^{-1} mg^{-1} FW) was obtained from 'TR-3080' at the same salt concentration. SOD activities increased by 78.0%, 42.1% and 43.4% in 'TARSAN1018', 'TR-3080' and '08-TR-003', respectively. Many changes have been detected in the activities of antioxidant enzymes in plants under salinity. The activity of antioxidant enzymes was reported to increase under saline conditions in the case of safflower (Siddiqi *et al.*, 2011; Çulha Erdal and Çakirlar, 2014) and sunflower (Rios-Gonzales *et al.*, 2002; Jabeen and Ahmad, 2012). According to our results, SOD seems to be more sensitive in the antioxidative process of salt stressed sunflower plants and more active in cv. "TARSAN1018".

Proline concentration has been often suggested as an indicator of osmotic stress (Khalil *et al.*, 2016). Proline accumulation under salt stress has been correlated with salt tolerance (Mansour and Ali, 2017). In this investigation, the findings showed that salt stress (from 0 mM to 250 mM NaCl) considerably enhanced the leaf free proline contents of all sunflower cultivars (Table 6). However, the cultivars differed significantly in proline content. Among the cultivars, 'TR-3080' accumulated considerably more proline (16.70

Table 5. Results of two-way ANOVA of the effect of cultivars and salt concentrations on proline content, activities of glutathione reductase (GR), ascorbate peroxidase (APX), lipid peroxidase (MDA) and superoxide dismutase (SOD).

Source		Proline				GR				APX			
	df	Sum of squares	Mean square	F	Sig.	Sum of squares	Mean square	F	Sig.	Sum of squares	Mean square	F	Sig.
Corrected model	11	1294.3	117.7	281.4	0.000	2343.9	213.1	9.4	0.000	4,939,877.0	449,079.7	389.2	0.000
Intercept	1	3736.7	3736.7	8937.4	0.000	389,024.6	389,024.6	17,116.1	0.000	191,431,344.8	191,431,344.8	165,914.4	0.000
Cultivar	2	6.8	3.4	8.1	0.002	1062.5	531.3	23.4	0.000	268,088.7	134,044.4	116.2	0.000
Salt	3	1173.0	391.0	935.2	0.000	1017.4	339.1	14.9	0.000	3,701,717.5	1,233,905.8	1069.4	0.000
Cultivar * Salt	6	114.5	19.1	45.7	0.000	264.0	44.0	1.9	0.116	970,070.8	161,678.5	140.1	0.000
Error	24	10.0	0.4			545.5	22.7			27,692.0	1153.8		
Total	36	5041.0				391,913.9				196,398,912.9			
Corrected total	35	1304.3				2889.4				4,967,568.1			

Source		MDA				SOD			
	df	Sum of squares	Mean square	F	Sig.	Sum of squares	Mean square	F	Sig.
Corrected model	11	174.8	15.9	125.5	0.000	15,501.0	1409.2	95.9	0.000
Intercept	1	2789.1	2789.1	22017.5	0.000	224,046.0	224,046.0	15,245.3	0.000
Cultivar	2	56.3	28.1	222.1	0.000	786.9	393.5	26.8	0.000
Salt	3	100.9	33.6	265.5	0.000	13,015.0	4338.3	295.2	0.000
Cultivar * Salt	6	17.7	2.9	23.5	0.000	1699.2	283.2	19.3	0.000
Error	24	3.0	0.1			352.7	14.7		
Total	36	2966.9				239,899.8			
Corrected total	35	177.9				15,853.7			

Table 6. The effect of cultivars and salt concentrations on the activity of antioxidant enzymes (GR, APX and SOD) (unit/min./mg fresh weight) and proline content (µmol/g fresh weight) with lipid peroxidation (MDA, µmol/g fresh weight) in sunflower.

Cultivars		Proline				GR					APX			
	NaCl Cont. (mM)	0	50	150	250	0	50	150	250	Mean	0	50	150	250
'TARSAN 1018'		1.11^{h}	10.39^{f}	14.99^{c}	12.99^{d}	116.56	114.11	103.33	96.00	107.50^{a}	2676.82^{ab}	2514.38^{c}	2345.18^{d}	1532.99^{h}
'TR-3080'		1.96^{h}	5.65^{g}	18.90^{a}	16.70^{b}	112.33	109.89	107.22	102.78	108.06^{a}	2626.06^{b}	2494.08^{c}	2348.56^{d}	2233.50^{e}
'08-TR-003'		1.03^{h}	11.31^{ef}	14.82^{c}	12.40^{de}	102.22	99.67	89.22	94.00	96.28^{b}	2730.96^{a}	2538.07^{c}	1871.40^{f}	1759.73^{g}
Mean						110.37^{a}	107.89^{a}	99.92^{b}	97.59^{b}					

	MDA				SOD			
NaCl Cont. (mM)	0	50	150	250	0	50	150	250
'TARSAN 1018'	6.50^{h}	7.66^{fg}	7.47^{fg}	9.13^{cd}	48.00^{h}	72.15^{de}	95.82^{b}	85.46^{c}
'TR-3080'	8.56^{de}	11.23^{b}	9.73^{c}	12.68^{a}	56.45^{gh}	77.33^{cd}	128.00^{a}	80.21^{cd}
'08-TR-003'	4.79	8.27^{ef}	7.34^{g}	12.10^{a}	58.18^{fg}	65.63^{ef}	96.00^{b}	83.44^{c}

[a-h]Values in a row and in a column (for glutathione reductase activity) followed by the different letters are significantly different at the 0.01 level.

µmol/g FW, 752.04% increase compared to control) than the other sunflower cultivars ('TARSAN1018' with 12.99 µmol/g FW, 1070.27% increase compared to control and '08-TR-003' with 12.40 µmol/g FW, 1103.88% increase compared to control) under saline conditions. The results of this study are also in agreement with Shahbaz *et al.* (2011), Jabeen and Ahmad (2012) and Bakhaum and Sadak (2016), who emphasised that salt stress markedly enhanced free proline contents in sunflower cultivars. However, our result showed that there is a negative relationship of morphological traits (plant height, leaf area, fresh weight, dry matter and water contents) with proline accumulation in all sunflower cultivars (Table 6). Khalil *et al.* (2016), stated that the negative relationship with morphological traits indicated that proline concentration may not be related with enhancing growth in sunflower but could increase survivability under stress contributing to the osmotic adjustment, and could participate in rapid recovery.

Lipid molecules, specifically unsaturated lipids, are sensitive to oxidation by ROS (Rasool *et al.*, 2013). Membrane lipid peroxidation is often used as a marker of an adverse effect of oxidative stress (Ozturk *et al.*, 2012). In the present study, the results of sunflower cultivars showed that with increasing levels of salt stress, MDA content increased (Table 6). The lowest MDA content (9.13 µmol/g FW, 40.46% increase compared to control) was record in cv. 'TARSAN-1018', while the highest value (12.68 µmol/g FW, 48.13% increase compared to control) was registered in cv. 'TR-3080' at 250 mM NaCl concentration. For cv. '08-TR-003', MDA content was 12.10 µmol/g FW with a 152.6% increase. The findings are in agreement with those obtained by Jabeen and Ahmad (2012) in sunflower. In particular, SOD constitutes the end product of peroxidation of membrane lipids and is the first line of defense against ROS (Hussain *et al.*, 2016). Our result indicated that the lowest MDA content (9.13 µmol/g FW) and the highest activity (85.46 mmol min^{-1} mg^{-1} FW) of SOD were obtained from cv. 'TARSAN-1018'. The reason of the lowest MDA content in cv. 'TARSAN-1018' may due to the high activity of SOD.

Conclusions

The results from this investigation allow to conclude that there are differences in the response to salt stress among sunflower cultivars. In term of morphological (plant height, leaf area, fresh weight, water content) and biochemical [GR, APX and SOD (secondly) activities and proline contents] parameters, cv. 'TR-3080' seems to be less affected from salt stress. Interestingly and unlike other study about the relationship between salt stress and antioxidant enzyme activities, we observed a decreasing activity of the two antioxidant enzymes (GR and APX) under salt stress conditions. On the other hand, similarly with the other studies in the literature, an increased activity was detected for SOD in all cultivars. As concerns these findings, cv. 'TR-3080' seems to be less affected by salt stress.

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