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RESEARCH ARTICLE

The effects of sodium chloride stress on some biochemical characteristics and antioxidative enzymes activities in two sunflower (*Helianthus annuus* L.) genotypes

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ABSTRACT: Salinity is one of the most important non-biological stresses that affect plant growth and development. Effect of different levels of NaCl (0, 2, 4, 6 and 8 dS/m) were investigated on enzymatic and non-enzymatic activities in C64 and C68 oilseed sunflower genotypes at two times; 3 and 12 days after salinity stress application. Net photosynthesis rate, chlorophyll content and soluble proteins amount decreased by increasing salinity level but proline and malondialdehyde (MDA) contents increased. However, the changes in net photosynthesis in the two studied genotypes was different across time and do not follow statistically the same trend line. In genotype C86, the reduction of photosynthesis rate at all studied salinity levels was very high compared to normal condition (0 dS/m) after 3 days; especially at salinity levels of 2, 4 and 6 dS/m, while 12 days later, the decrease of photosynthesis rate was moderate at salinity levels of 2 and 4 dS/m but severe at 6 and 8 dS/m salinity levels. The highest amount of proline (31.36%) related to tolerant genotype and the lowest amount (7.72%) related to susceptible one was measured 12 days after 2 dS/m salt stress treatment. Considerable MDA was observed in both tolerant and sensitive genotypes 12 days post salt stress application; the highest amount (83%) was observed at 8 dS/m treatment. Catalase and ascorbate peroxidase activity increased with increasing salt intensity. The rate of increase in guaiacol peroxidase activity was higher in C86 genotype than C64. Chlorophyll a and total chlorophyll contents decreased in both sunflower genotypes under salinity stress. The lowest amount of total chlorophyll (8.6%) was observed in the salinity level of 8 dS/m in the sensitive line (C64). Results revealed the C64 and C68 selected genotypes from two our identified sunflower heterotic groups have different physiological response to salinity stress and C68 is more tolerant to salt stress than C64. So, they can be potentially used as parents in sunflower breeding programs to produce salt stress tolerant hybrids.

KEYWORDS: Antioxidant activity, Chlorophyll, Malondialdehyde, Net photosynthesis, Salinity stress, Sunflower

INTRODUCTION

Non-biological environmental stresses such as drought, salinity, cold, and heat have harmful effect on growth and fertility of crop plants [3]. Salinity stress is the second most important non-biologic stress after drought that decreases crop yield and performance [42]. Over 800 million hectares of agricultural land around the world are exposed to salinity (397 million hectares) or alkaline (434 million hectares) problems; equivalent to 20% of arable land and 50% of the worlds irrigated lands [35]. It is expected in the next 25 years 30% and by 2050, 50% of suitable agricultural land will be lost due to salinity [7]. Soil salinity and climate changes are steadily increasing

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in many parts of the world [36]. Na^+ and Cl^- are important cation and anion in saline soils and easily uptake by plant roots compared to other ions [14]. Because of this in salt research activities, the effects of salinity stress on plants mainly are investigated using NaCl [41,44]. Cultivated plants in arid and semi-arid regions are usually faced with dehydration risk and salinity stress. High NaCl concentrations causes osmotic stress and consequently ionic stress which is mainly due to high concentration of Na^+ and Cl^-. High intracellular Na^+ concentration inhibits K^+ uptake, which an essential element in various cellular processes. Osmotic stress reduces water uptake by plant roots and thus reduces photosynthetic activity and increase production of reactive oxygen species. Tolerance to salinity in plants depends on the relationship between different biochemical pathways involved in detoxification, ion balance and growth regulation [55]. First, the accumulated ROSs are refined and eliminated by synthesis of important components such as osmolytes [49] and antioxidants such as catalase, ascorbate peroxidase, guaiacol peroxidase [18,33,38]. At the same time, ionic equilibrium is achieved by Na^+ exclusion or accumulation and synthesis of compatible solutes. Finally, induction of expression of different genes leads to regrowth and high production in plants.

Sunflower (*Helianthus annuus* L.) is an annual plant with 2n = 2x = 34 chromosomes and it is one of the most important sources of vegetable oil in the world [43]. According to studies by Mass and Haffman [26], sunflower is a semi-tolerant plant with salinity tolerance threshold of 2.3 dS/m. Salinity stress affects many physiological and morphological processes of plants including sunflower [15]. Concentration of superoxide dismutase and catalase increased in sunflower with increasing salinity stress intensity [45,50]. In this study, the effect of salinity stress on activity of antioxidant enzymes and other biochemical factors was investigated in sunflower at seedling stage. The results can be important in selecting suitable traits for screening salt tolerant genotypes in breeding programs.

MATERIALS AND METHODS

Plant material and salinity stress

Two genotypes; C64 and C86 with different response to salinity stress (Supplementary file 1, Table 1) were selected [30]. The genotypes were selected according to the study of Morsali Aghajari et al. [30]. Responses of genotypes to different levels of NaCl including 0, 2, 4, 6 and 8 dS/m were evaluated in a completely randomized design with 3 replications. In the experiment, 60 plastic pots with a diameter of 12 cm and a height of 16 cm were selected and filled with field soil (loamy texture) (Table 1) and peat moss in the ratio of 3: 1. In each pot, three seeds were planted at a depth of 2 cm. The pots were placed in a growth chamber with conditions of 12 hours of light with an intensity of 200 $\mu Em^{-2}s^{-1}$, a maximum temperature of 28ºC and a minimum temperature of 12ºC. After germination and emergence of seedlings, one healthy seedling was kept in each pot and the rest of the seedlings were removed. Watering of pots was done three times a week and in two out of three times, watering was done with water containing 0.5 grams per liter of 20-20-20 fertilizer (N-P-K). After the seedlings reached to eight-leaf stage (45-day-old seedlings), different levels of salinity stress were applied to pots. To prevent osmotic stress, the NaCl application was done at two times; in morning and evening. By adding the appropriate amount of salt to the soil of the pots, the desired salinity stress (2, 4, 6 and 8 dS/m) was provided. During the experiment, the salinity of the pots was controlled by an EC meter. The desired variables were measured 3 and 12 days after salinity stress application. In the most of molecular studies, the enzymes activity and molecular traits are measured as presented in the range of present study. For character such as yield, it is necessary to wait long time. The experiment was performed in the research farm and laboratory of the Department of Plant Production and Genetics, Faculty of Agriculture, Urmia University, Iran.

Chlorophyll a, b, and net photosynthesis

Chlorophyll pigments were measured by a procedure described by Lichtenthaler and Wellburn [23]. About 0.1 g of fresh leaf were grounded in 5 ml of 100% acetone. The mixture was centrifuged at 2500 rpm for 10 min. The absorbance was read by UV/Vis spectrophotometer (WPA S2100, UK) at 662 and 645 nm. The following formulas were used to calculate chlorophyll a, chlorophyll b and total chlorophyll content:

Chla= 11.75 A662 – 2.350 A645
Chlb= 18.61 A645 – 3.960 A662
Chl total= Chla + Chlb

Net photosynthesis was measured using HCM-1000-WALZ apparatus (Germany). Full expanded leaves from the same positions in the pots were considered. The intensity of light and the temperature of the Cuvette

Table 1. Results of soil analysis used in the present experiment

pH	EC	OC	OM	CaCO3	Clay	Silt	Sand	Texture	K	P
-	dS/m	%	%	%	%	%	%	-	mg/kg	mg/kg
7.96	1.09	0.68	1.17	12.0	24	40	36	Loam	218.96	16

pH: potential of hydrogen; EC: electrical conductivity; OC: organic carbon; OM: organic matter; K: potassium; P: phosphorus.

chamber was fixed to 21500 mol μm^{-2} and 30-33˚C, respectively. The leaf area inside the chamber was 5 cm^2.

Proline

Extraction and determination of proline was performed according to the method described by Bates et al. [9]. Fresh leaves (0.04 g) were grounded with 3% sulfosalicylic acid. Extracts were placed in refrigerator for 72 hours at 4˚C. After 72 hours, the samples were centrifuged at 3000 rpm for 20 minutes. Then 2 ml of glacial acetic acid solution and 2 ml of ninhydrin reagent (including 20 ml of 6 M phosphoric acid, 30 ml of glacial acetic acid and 1.25 g of ninhydrin) were added. The samples were placed in water bath (100 °C) for 1 hour. They were rapidly cooled by ice and 4 ml of toluene was added to each sample and stirred them. Proline content was measured by a spectrophotometer at 520 nm.

Malondialdehyde

Malondialdehyde content was measured based on Heath and Packer [17] method. About 0.2 g of fresh leaf was grounded in 5 ml of 1% TCA. The homogenates were centrifuged at 8,000 rpm for 10 minutes, then, to one ml of supernatant 4 ml of 20% TCA and 0.5% TBA were added. Samples were placed in water bath (95 °C) for 30 minutes. They were cooled immediately in ice and then centrifuged at 8000 rpm for 5 minutes. Finally, the absorbance of the samples was measured at two wavelengths of 532 nm and 600 nm. Malondialdehyde (MDA) was calculated using the following formula**:**

MDA (µmol/g Fw) = [(A532- A600)] × 100.

Soluble protein

Soluble protein was measured based on Lowry et al. [25] method. 4 ml Tris-hydrochloric acid buffer (50 ml 0.2 N Tris, 17.2 g sucrose and 0.1 g Ascorbic acid in a final volume of 100 ml with distilled water, pH = 8) was added to 0.25 g of fresh leaf and they were placed in refrigerator during 24 hours. Then, they were centrifuged at 5000 rpm for 30 minutes. 4 ml of the solution (2 g Na_2CO_3, 0.4 g NaOH and 0.02 g Rachel salt were added in 100 ml distilled water and 1 ml of solution containing 0.5 g of crystalline $CuSO_4$, 5 H_2O in 100 ml of distilled water) was added to them. After 10 minutes, 1.5 ml of the diluted solution of Folin (1 ml of Folin and 9 ml of distilled water) was added and the samples placed in the dark place for 30 minutes. Finally, the absorbance of samples was measured at 660 nm by spectrophotometer. The amount of soluble proteins was determined using standard bovine serum albumin curve (BSA).

Catalase, Ascorbate Peroxidase and Guaiacol Peroxidase Activity

For enzyme activity, 0.1 g of powdered leaf was homogenized on ice with 1 ml of 100 mM potassium phosphate buffer (containing 0.1 mM EDTA and PVP, pH = 7.8). The extract was centrifuged at 1400 rpm for 30 minutes and then, catalase [1], ascorbate peroxidase [34] and guaiacol peroxidase [51] enzymes activities were assayed.

Data analysis

Statistical analysis of different characters was performed using SPSS software version 24 and SAS version 4.9. Before analysis of variance, basic ANOVA assumptions including homogeneity of variances and normal distribution of experimental errors were tested. Comparisons of means were performed using Student-Newman-Keuls (SNK) test at 5% probability level.

RESULTS

Changes in proline, soluble protein and malondialdehyde content

Analysis of variance revealed significant effect of genotype × stress interaction on proline content in sunflower (Table 2). Results showed that 3 days after salt stress application (8 dS/m), proline content increased significantly in C86 genotype compared to control plants. While, proline content in this genotype at other levels of salt stress was similar to control plants. In C64 genotype, proline content increased at salinity level of 4 and 6 dS/m compared to control plants, while at salinity level of 8

Table 2. Analysis of variance of proline, soluble protein, malondialdehyde content data in sunflower genotypes at different times after salinity stress application.

Source of variation	df (3 days)			Mean Square			df (12 days)			Mean Square		
	Proline	Protein	MDA	Proline	Protein	MDA	Proline	Protein	MDA	Proline	Protein	MDA
Salinity (S)	4	4	4	94.68*	46.06*	0.059	4	4	4	53.13	28.38	0.33*
Genotype (G)	1	1	1	5.2	30.15	0.033	1	1	1	0.16	16.46	0.055
G × S	4	4	4	35.11	3.04	0.005	4	4	4	297.29*	2.5	0.014
Error	13	17	17	25.31	12.2	0.031	18	13	18	1559.34	16.5	0.008

MDA: Malondialdehyde, df: degree of freedom.

Table 3. Analysis of variance of chlorophyll a, b and total content and net photosynthesis rate data in sunflower genotypes at different times after salinity stress application.

Source of variation	df (3 days)				Mean Square				df (12 days)				Mean Square			
	Chla	Chlb	Chlcol	NP	Chla	Chlb	Chlt	NP	Chla	Chlb	Chlt	NP	Chla	Chlb	Chlt	NP
Salinity (S)	4	4	4	4	53.75*	3.72	61.8	8.92**	4	4	4	4	21.65**	83.6***	146.93***	7.23*
Genotype (G)	1	1	1	1	77.42*	1.79	72.42	11.05*	1	1	1	1	22.96**	43.98*	147.29***	0.38
G × S	4	4	4	4	9.41	1.07	8.72	0.82	4	4	4	4	17.86**	12.07	46.82***	0.20
Error	17	17	15	10	15.13	3.04	26.31	1.75	17	18	17	10	2.18	7.35	8.96	1.53

Chla: Chlorophyll a; Chlb: Chlorophyll b; Chlt: Total chlorophyll; NP: Net photosynthesis, df: degree of freedom.

Table 4. Analysis of variance of ascorbate peroxidase, catalase and guaiacol peroxidase enzymes activity in sunflower genotypes at different times after salinity stress application.

Source of variation	df (3 days)			Mean Square			df (12 days)			Mean Square		
	APX	CAT	GPOX	APX	CAT	GPOX	APX	CAT	GPOX	APX	CAT	GPOX
Salinity (S)	4	4	4	0.03*	1.54**	0.000003	4	4	4	0.04	0.57	0.0002
Genotype (G)	1	1	1	0.001	0.174	0.000008	1	1	1	0.001	0.154	0.001**
G × S	9	4	4	0.0006	0.036	0.00003	4	9	9	0.009	0.05	0.0002**
Error	16	17	17	0.065	0.368	0.00002	14	14	18	0.16	0.327	0.00007

CAT :Catalase (EC 1.11.1.6), APX: Ascorbate peroxidase (EC 1.11.1.11), GPOX: Guaiacol peroxidase (EC 1.11.1.7), df: degree of freedom.

dS/m changes in proline content was not noticeable compared to control plants. In C86 genotype, 12 days after stress application (2, 4 and 8 dS/m), proline content increased significantly compared to control plants. So that, at salinity level of 2 dS/m proline content increased three times and at salinity levels of 4 and 8 dS/m its amounts increased 2 times more than that in control plants (Fig. 1a). Regarding to genotype C64, 12 days after stress application, at salinity levels of 4 and 6 dS/m proline content increased significantly compared to control plants. There was not any increase in the proline content at salinity level of 8 dS/m compared to control plants. At salinity level of 2 dS/m, proline content decreased in comparison to control plants (Figs. 1a, b). The results showed that malondialdehyde content was significantly different between salinity stress levels (Table 2).

Changes in net photosynthesis, chlorophyll a, b and total chlorophyll

Effect of salinity and time (days after stress application) on the rate of net photosynthesis was significant at 5% probability level (Table 3). Net photosynthesis decreased by increasing salinity in both studied genotypes (Figs. 2a and 2b) but its change was different depending on time after salt stress application. In C86 genotype, decrease in photosynthesis rate was very severe at 3 days after stress application in all levels of salinity stress, especially in salinity levels of 2, 4 and 6 dS/m compared to control plants. While, in time of 12 days after salinity stress application, decrease in photosynthesis rate was low at salinity level of 2 and 4 dS/m but it was high in higher levels of salinity stress (Fig. 2a). In genotype C64, photosynthesis rate decreased in time of 3 days after stress application especially at salinity level of 4 and 6 dS/m compared to control plants. At salinity levels of 2 and 8 dS/m, any significant changes were not observed in net photosynthesis rate compared to control plants. In time of 12 days after stress application, net photosynthesis rate severely decreased by increasing of intensity of salinity stress. Therefore, decrease of net photosynthesis rate in two studied genotypes did not follow the same pattern (Fig. 2b).

Analysis of variance revealed the significant effect of genotype, salinity and time (days after stress application) on chlorophyll content (Table 3). In both studied genotypes at 3 days after salinity stress Chlorophyll a

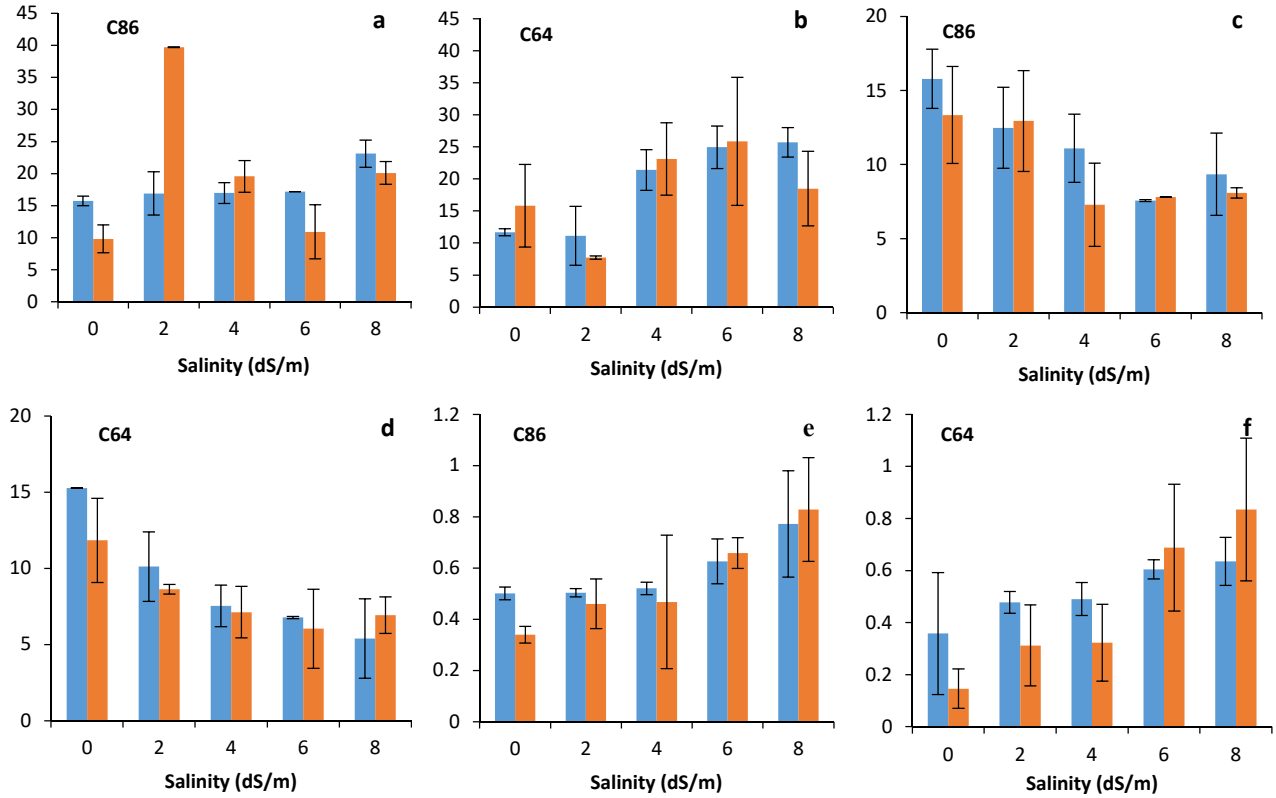

Figure 1. Effect of salinity stress on proline (a and b), soluble proteins (c and d) and malondialdehyde (e and f) content. The blue and red columns show changes at 3 and 12 days after salinity stress application, respectively.

content decreased compared to control by increasing the intensity of salinity stress (Figs. 2c and 2d). While decrease in chlorophyll a content in C64 genotype was higher than that in C86 genotype. In C86 genotype, in time of 12 days after salinity stress application chlorophyll a content decreased slightly only at salinity 6 dS/m but in C64 genotype, it decreased in all of salinity levels, especially at 8 dS/m.

In both studied genotypes chlorophyll b decreased at salinity levels of 2 and 8 dS/m compared to control both in 3 and 12 days after salinity stress application. At salinity level of 2 dS/m, chlorophyll b content highly decreased in C86 genotype in comparison to C64 genotype. Whereas, at salinity level of 8 dS/m the decrease in chlorophyll b content in C64 genotype was slightly higher than that in C86 genotype. Chlorophyll b decreased in both genotypes, especially in C64 genotype at salinity level of 8 dS/m, 12 days after salinity stress application (Fig. 2e).

Analysis of variance revealed significant effect of salinity × genotype interaction on total chlorophyll content (Table 3). Total chlorophyll content in both studied genotypes (C64 and C86) decreased in parallel with increasing the salinity stress intensity compared to control plants (Fig. 2g and 2h). However, decrease in total chlorophyll content at all of salinity levels, especially salinity level of 8 dS/m was higher in C64 genotype than that in C86 genotype (Fig. 2h).

Change in ascorbate peroxidase, catalase and guaiacol peroxidase enzymes activity

According to analysis of variance, the effect of salinity stress was significant on catalase and ascorbate peroxidase activity. Genotype, time and their interaction was significant on guaiacol peroxidase activity (Table 4). Results showed that by increasing salinity stress level, ascorbate peroxidase activity increased in both C64 and C86 genotypes at both sampling times. However, increase in ascorbate peroxidase activity was slightly occurred faster in C86 genotype than that in C64 genotype (Figs. 3a and 3b). Catalase activity increased in both genotypes in parallel with increasing salinity stress level but its increase was higher in C86 genotype than that in C64 genotype (Figs. 3c and 3d). Similar results were observed for guaiacol peroxidase enzyme (Figs. 2e and 2f).

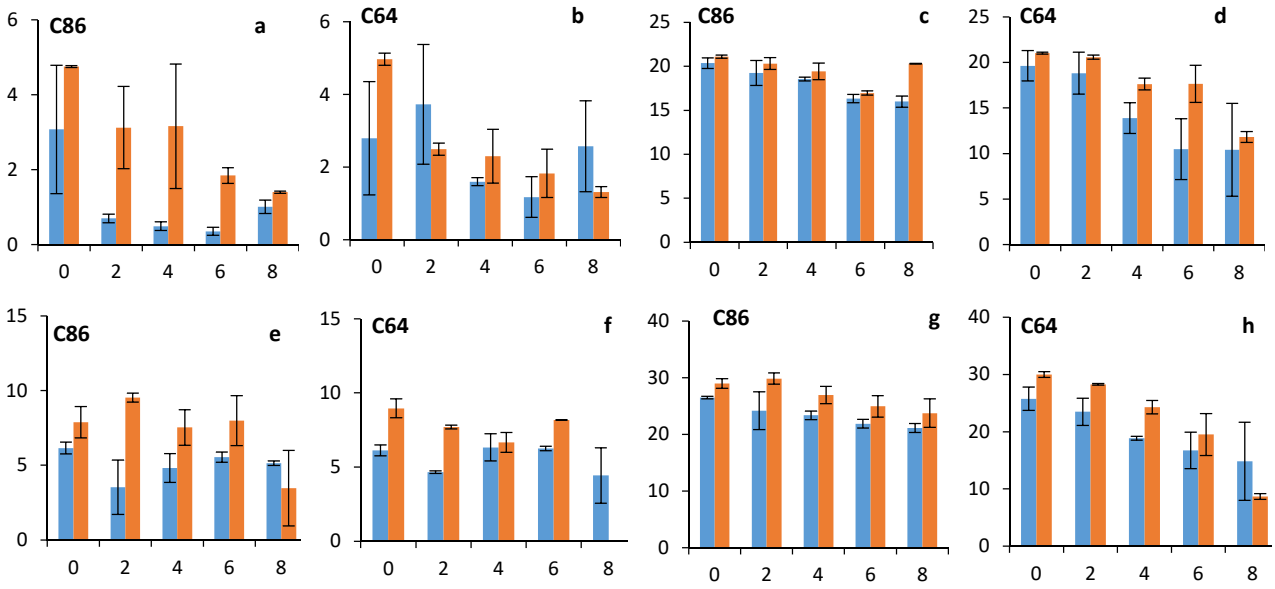

Figure 2. Effect of salinity stress (0 - 8 dS/m) on net photosynthesis (a and b), chlorophyll a (c and d), chlorophyll b (e and f) and total chlorophyll (g and h) content. The blue and red columns show changes at 3 and 12 days after salinity stress application, respectively.

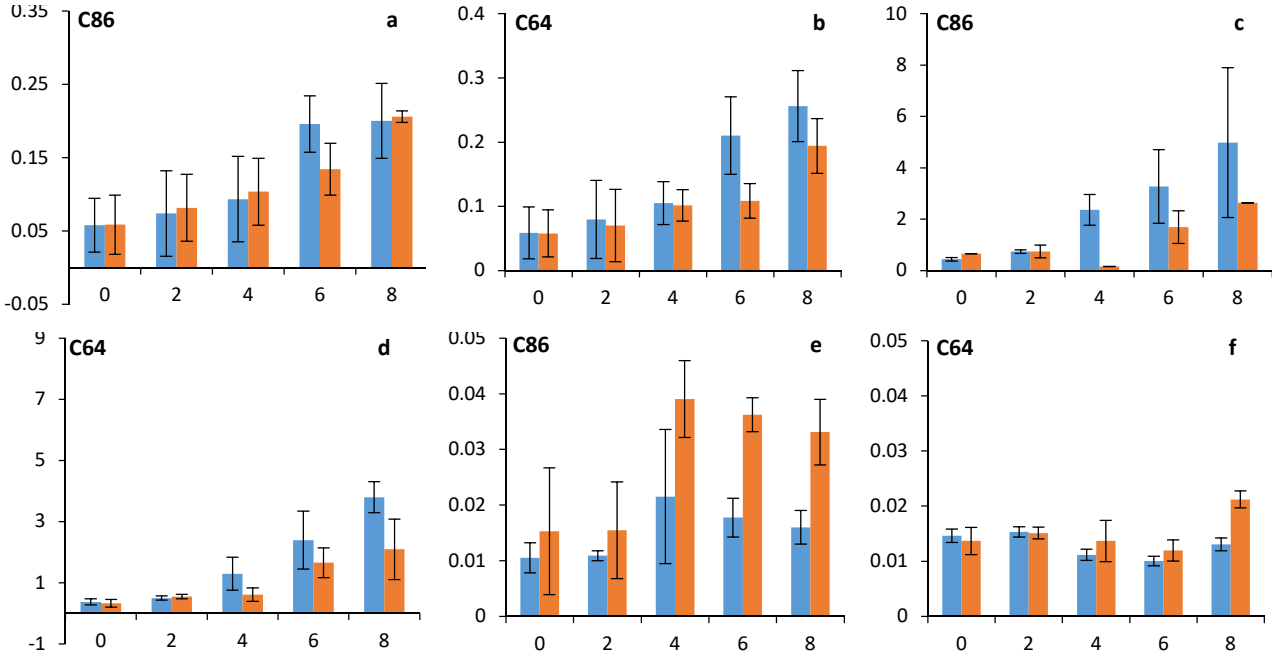

Figure 3. Effect of salinity stress (0 - 8 dS/m) on ascorbate peroxidase (A and B), catalase (C and D) and guaiacol peroxidase (E and F) activity. The blue and red columns show changes at 3 and 12 days after salinity stress application, respectively.

DISCUSSION

Malondialdehyde content increased by increasing the salinity levels in both sampling time (3 and 12 days after stress). However, increasing malondialdehyde content was more tangible and faster in C64 genotype than that in C86 genotype. In both genotypes, the most increased amount of malondialdehyde content was observed at salinity level of 8 dS/m, 12 days after stress application.

There are numerous reports about increasing of MDA in sunflower [40] and rapeseed [16]. In maize, prolonged salinity stress reduces the relative leaf water content and leaf water potential and increases the malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) content [20]. Results showed that variation pattern of proline content is different in two studied genotypes. It means that they show different responses to salinity stress.

Under salinity stress, proline is more than other amino acids produced and accumulated in plants [47]. Proline is highly effective in osmotic regulation and increasing membrane stability by reducing the effect of NaCl on cell membrane degradation [28]. Many researchers report that proline under saline stress increases water absorption and induce antioxidant mechanisms. In addition, it reduces the accumulation of toxic ions [46, 19,53]. There are many reports about increased level of proline in plants under salinity stress, including sunflower [40], rapeseed [8], wheat [2,4] and alfalfa [29]. Synthesis of proline has been increased in soybean in parallel with increasing salt stress intensity [5].

Unlike proline, soluble protein content decreased at 3 and 12 days after salinity stress application compared to control. Decrease in soluble protein content happened faster in genotype C64 than that in genotype C86. Salinity stress decreases RNA levels due to cytoplasmic RNAase activity, which results in decreased protein production [48].

Some researchers reported that water deficiency due to salinity stress leads to destruction of cell membranes and exhaust of membrane proteins. It is also possible the induced abscisic acid during salt stress inhibits protein synthesis [21]. Parida et al. [39] reported that the soluble protein content of leaf gradually decreased in parallel with increasing salt stress intensity. Salinity stress increases ROS production, which is highly toxic to cells and disrupts cellular redox homeostasis. Excess ROS in cells destroys protein and enzymes, and peroxide lipids and further cause damage to the electron transport system, the PSII system, and the structure of various membranes [22]. When signal of salinity stress transmit to leaf cells, they cause stomatal cells closure. So, stomatal conductance and ultimately net photosynthesis rate decrease. Ashraf and Harris [6] reported that photosynthetic apparatus included various components such as photosynthetic pigments, electron transfer system and CO_2 reduction pathways, so any damage to each one of them during salinity stress results in decrease the total photosynthesis rates in plants [6].

Decrease of chlorophyll content in plants under abiotic stresses may be due to increased activity of chlorophyll-degrading enzyme [11]. Some growth regulators such as abscisic acid and ethylene increase the activity of chlorophyll-degrading enzyme. Increased level of proline under abiotic stress conditions causes glutamate, a precursor of chlorophyll and proline, poorly participate in biosynthesis pathway of chlorophyll [13].

Based on the results, salinity stress decreased the amount of chlorophyll a, b and net photosynthesis that is in agreement with the study of Liu and Shi [24]. Liu and Shi [24] reported that the net photosynthesis rate, chlorophyll a and b contents in sunflower decreased by increasing salinity level from 0 to 10 dS/m. In canola, chlorophylls a and b contents significantly decreased in parallel wilt increasing NaCl concentrations [37]. Other studies have also found that salinity stress decreases chlorophyll content in tobacco [10] and radish [32].

Zheng et al. [54] reported that salinity stress reduced photosynthesis rate and stomatal conductance in wheat leaves. They also reported that high concentrations of salt in root environment reduces the ability of root to absorbing water. Following this, a chemical signal (abscisic acid hormone) is sent to aerial parts of plants and it makes the plants compatible to the environment by closing the stomata and reducing stomatal conductance and photosynthesis rate.

Increase in ascorbate peroxidase activity was slightly occurred faster in C86 genotype than that in C64 genotype. These results are in agreement with studies in rapeseed [16] and sunflower [50]. Catalase and guaiacol peroxidase enzyme activity increased in both genotypes in parallel with increasing salinity stress level but its increase was higher in C86 genotype than that in C64 genotype. These results are in agreement with recent studies in soybean [5,52] and tomato [12]. Resistance of plant to various environmental stresses is related to activity of antioxidant enzymes which responsible for the detoxification of free oxygen radicals [31]. During salinity stress, the induction of ROS occur which causes oxidative stress. Therefore, plants induce enzymatic and non-enzymatic mechanisms of antioxidants. In particular, the increased antioxidant enzymes include superoxide dismutase (SOD), catalase (CAT), peroxidase (POX), and ascorbate peroxidase (APX) whereas non-enzymatic antioxidants that increase are glutathione (GSH), ascorbate (ASC) and its derivatives, and photosynthetic side pigments such as carotenoids [27]. Antioxidants inhibit ROS. Therefore, they reduce strongly the salinity stress effects. Detoxification in a plant cell is a sequential process, first, SOD production in plants enrich, which leads to the conversion of superoxide (O_2) anion to H_2O_2 and then POX and CAT help break down toxic H_2O_2 in plant cells [53]. The results of this study regarding the

increase of catalase enzyme are in agreement with the study of Umar and Shaheed Siddiqui [50] in sunflower.

CONCLUSION

Net photosynthesis, chlorophyll content and soluble proteins decreased but proline and malondialdehyde content increased in sunflower genotypes in parallel with increasing salinity stress level. The activity of catalase and ascorbate peroxidase enzymes increased with increasing salinity stress level. The activity of guaiacol peroxidase was greater in C86 genotype than that in C64 genotype. Chlorophyll a and total chlorophyll content decreased in sunflower genotypes under salinity stress, but the amount of decrease in C64 genotype was more than that in C86 genotype. Therefore, it can be concluded that C64 and C68 genotypes have different physiological response to salinity stress and C68 is more tolerant to salt stress than C64.

Declarations

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Authors' contributions: Investigation, Data collection, Formal analysis: Fariba Morsali; Conceptualization, Methodology, Supervision: Reza Darvishzadeh; Writing – original draft: Mitra Razi.

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تأثیر تنش شوری بر فعالیت برخی از آنزیم‌های آنتی‌اکسیدان و پارامترهای بیوشیمیایی در لاین‌های آفتابگردان دانه روغنی (*Helianthus annuus* L.)

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چکیده

شوری یکی از مهمترین تنش‌های غیرزنده است که بر رشد و نمو گیاهان تأثیر می‌گذارد. اثر سطوح مختلف کلریدسدیم (۰، ۲، ۴، ۶ و ۸ دسی زیمنس بر متر) بر فعالیت آنزیمی و غیرآنزیمی در ژنوتیپ‌های آفتابگردان دانه روغنی C64 و C68 در دو زمان (۳ و ۱۲ روز پس از اعمال تنش شوری) بررسی شد. فتوسنتز خالص، کلروفیل و پروتئین‌های محلول با افزایش سطح شوری کاهش یافتند، اما محتوای پرولین و مالون دی آلدهید افزایش یافت. تغییرات میزان فتوسنتز خالص در دو ژنوتیپ مورد مطالعه در طول زمان متفاوت بود و از روند یکسانی پیروی نمی‌کند. در ژنوتیپ C86، سه روز بعد از تنش شوری کاهش میزان فتوسنتز در تمام سطوح شوری مورد مطالعه به ویژه سطوح شوری ۲، ۴ و ۶ دسی زیمنس بر متر در مقایسه با شاهد (۰ دسی زیمنس بر متر) بسیار بالا بود، در حالی که ۱۲ روز بعد از تنش شوری میزان فتوسنتز در سطوح شوری ۲ و ۴ دسی زیمنس بر متر به طور متوسط و در سطوح شوری بالا به طور شدید کاهش یافت. ۱۲ روز بعد از تنش شوری (۲ دسی زیمنس بر متر) بالاترین میزان پرولین (۳۱/۳۶٪) مربوط به ژنوتیپ مقاوم و پایین‌ترین میزان (۷/۷۲٪) آن مربوط به ژنوتیپ حساس بود. مالون دی آلدهید قابل ملاحظه ای در هر دو ژنوتیپ مقاوم و حساس ۱۲ روز پس از تنش مشاهده گردید، بالاترین مقدار آن (۸۳٪) در سطح شوری ۸ دسی زیمنس بر متر مشاهده شد. فعالیت کاتالاز و پراکسیداز آسکوربات با افزایش شدت شوری افزایش یافت. میزان افزایش فعالیت پراکسیداز گایاکول در ژنوتیپ C86 بیشتر از C64 بود. میزان کلروفیل a و کلروفیل کل در هر دو ژنوتیپ آفتابگردان تحت تنش شوری کاهش یافت. پایین‌ترین میزان کلروفیل کل (۸/۶٪) در سطح شوری ۸ دسی زیمنس بر متر و در لاین حساس (C64) مشاهده گردید. نتایج نشان داد که ژنوتیپ‌های C64 و C68 که از دو گروه هتروتیک شناسایی شده انتخاب شده بودند پاسخ فیزیولوژیکی متفاوتی به تنش شوری دارند و C68 نسبت به C64 تحمل بیشتری نسبت به تنش شوری دارد. بنابراین این ژنوتیپ‌ها می‌توانند بالقوه به عنوان والدین تلاقی در برنامه‌های اصلاحی ارقام هیبرید مقاوم به شوری استفاده شوند.

کلمات کلیدی: آفتابگردان، تنش شوری، فتوسنتز خالص، فعالیت آنتی اکسیدانی، کلروفیل، مالون دی آلدهید.