Impact of salt stress on photosystem II efficiency and antioxidant enzyme activities of safflower (*Carthamus tinctorius* L.) cultivars

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Abstract: This study was conducted to determine the tolerance of safflower (*Carthamus tinctorius* L.) cultivars (Dinçer, Remzibey-05, and Yenice) against salt stress based on some physiological and biochemical parameters at the vegetative stage. Eighteen-day-old plants were subjected to salt stress [0 (control), 75, 150, 225, and 300 mM NaCl concentrations] for 12 days, which led to a significant decrease in growth parameters (stem growth, fresh weight, and relative water content) and the photosynthetic pigment contents of the safflower cultivars. The photochemical activities of photosystem II of the cultivars were negatively affected by salinity, especially at the highest concentration (300 mM). Salt stress decreased K^+ content and K^+/Na^+ ratio while it increased Na^+ content. Malondialdehyde and free proline contents in the leaves of cultivars increased gradually in proportion to the increase of NaCl concentration. Analysis of antioxidant enzyme activities showed that these enzymes responded differently to the NaCl concentrations. Dinçer, with higher antioxidant enzyme activities, had a more effective response than the other cultivars. Considering growth and biochemical and chlorophyll fluorescence parameters with the endogenous defense system, Dinçer had a higher withstanding capacity against salinity than the other cultivars.

Key words: Antioxidative response, *Carthamus tinctorius* L., photochemical activity, salt stress

1. Introduction

Salinity is a major abiotic stress on both irrigated and nonirrigated lands, inhibiting plant growth and crop productivity (Gondim et al., 2012). Throughout the world, more than 800×10^6 ha of lands are salt-affected (Türkan and Demiral, 2009), and, every year, 2×10^6 ha of the world's agricultural lands are disrupted by salinity (Tuteja, 2007). Sodium chloride (NaCl) is one of the most important components of salt and saline soils, which is caused by Na^+, composing a major part of all the salt-affected soils worldwide (Pessarakli and Szabolcs, 1999).

In the rhizosphere, increasing salinity influences plant growth and development by limiting the intake of water and nutrients from the soil. The salinity response of plants occurs in 2 phases. The osmotic effect is the first phase, which causes a reduction of usable water outside the roots (http://ucce.ucdavis.edu/files/repositoryfiles/ca3810p38-72376.pdf). In the second phase, which is known as the ionic effect, increasing Na^+ and Cl^- levels compete with nutrients such as K^+, Ca^{+2}, and NO_3^- and inhibit nutrient uptake or induce imbalances (Hu and Schmidhalter, 2005). In many studies it was reported that salinity affected plant nutrients (Na^+, K^+, Ca^{+2}, Mg^+, and Cl^-), growth, membrane integrity, osmotic adjustment,

photosynthetic activity, and antioxidant activity (M'rah et al., 2006; Çiçek and Çakırlar, 2008; Ejaz et al., 2012; Shahzad et al., 2012).

Photosynthesis, which is one of the primary metabolic processes in plant growth and production, is adversely affected by salinity in various ways, such as the inhibition of CO_2 intake with stomatal closure (Degl'Innocenti et al., 2009), the reduction of photosynthetic pigment amount (Qados, 2011), and damage to photosynthetic structures [photosystems I and II (PSI and PSII), electron transport proteins etc.] (Sudhir et al., 2005). Another reason for restricted photosynthetic activity is the generation of reactive oxygen species (ROS; $O_2^{.-}$, $^1O_2$, H_2O_2, and OH^-). Under salt stress, these ROS can cause damage to membranes and other essential macromolecules such as pigment, proteins, DNA, RNA, and lipids (Ashraf and Ali, 2008). To scavenge ROS and remove oxidative stress, plants have a well-developed complex antioxidant defense system including enzymatic [superoxide dismutase (SOD), ascorbate peroxidase (APX), glutathione reductase (GR), peroxidase (POD), etc.] and nonenzymatic (ascorbate, glutathione, carotenoids, etc.) antioxidant processes (Sekmen Esen et al., 2012).

Plants tend to cope with salt stress while synthesizing and accumulating osmoprotective compounds such as

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proline, glycinebetaine, or polyols, which are known as compatible solutes (Hussain et al., 2008). These compatible solutes protect plants from stress in different ways, including cellular osmotic adjustment, detoxification of ROS, protection of membrane integrity, and stabilization of enzymes/proteins (Vijayan, 2009).

Safflower, which has high oleic acid and linoleic acid, is one of the world's oldest oil seed crops (Baydar and Gökmen, 2003; Coşge et al., 2007) and its content of linoleic acid is higher than those of hazel, olive, and other oil seed plants (http://www.hort.purdue.edu/NEWCROP/AFCM/safflower.html). Hence, safflower is used in many areas, such as the food, medicinal, and dye industries (http://www.ampc.montana.edu/briefings/briefing58.pdf), and also in the production of biodiesel (Zareie et al., 2013). Although safflower has been cultivated in a small area in the world, it is one of the important alternative oil crops (Coşge et al., 2007), especially in dry lands, because of its tolerance to cold, drought, and salinity.

In Turkey, safflower seeds are mostly sown in spring and grown in summer. In this period, salinity adversely affects the growth and seed yield capacity (Siddiqi et al., 2011). For this reason, the determination of tolerant genotypes and tolerance mechanisms of safflower cultivars against salinity stress has attracted attention. Most studies of safflower cultivars under salinity stress focused on germination and seedling stages (Francois and Bernstein, 1964; Kaya et al., 2003; Siddiqi et al., 2007) or the reproductive stage (Siddiqi et al., 2011; Aymen et al., 2012; Fraj et al., 2013). On the other hand, physiological research at the vegetative stage of safflowers exposed to salinity is inadequate (Hosseini et al., 2010; Tayefi-Nasrabadi et al., 2011). The aim of this study was to obtain a better understanding of the salt tolerance of safflower cultivars at the vegetative stage by analyzing plant growth, photochemical activity, and membrane integrity with protective endogenous systems (proline and antioxidant enzymes activities).

2. Materials and methods

2.1. Plant materials, growth, and treatment conditions
The seeds of 3 safflower (*Carthamus tinctorius* L.) cultivars (Dinçer, Remzibey-05, and Yenice) were obtained from the Central Research Institute for Field Crops, Turkey. The seeds were surface-sterilized with 5% (v/v) sodium hypochlorite (NaOCl) solution for 3 min. They were then washed and imbibed in distilled water for 2 h. After incubation, 5 seeds were sown in plastic pots (14 cm in diameter and 13 cm in height) filled with perlite. They were watered every other day with modified half-strength Hoagland's solution (Hoagland and Arnon, 1950; Nagy and Galiba, 1995). In each pot, 5 plants were grown in a controlled growth room, with a temperature regime of 25

± 1 °C, a 16-h photoperiod, 60 ± 5% humidity, and 200 μmol m^{-2} s^{-1} light intensity.

On the 18th day after sowing, salt treatment was initiated. Pots of each cultivar were randomly divided into 5 groups, 1 of which served as the control while the others were subjected to salt stresses. Salinized culture solutions were prepared by adding various amounts of NaCl (75, 150, 225, and 300 mM) to the half-strength Hoagland culture solution. Control and NaCl-stressed plants were grown in the growth chamber under the same conditions for another 12 days. Accordingly, plants were harvested on the 30th day after sowing to provide suitable analyses.

2.2. Growth parameters
At the end of the experiment, shoot lengths (distance from perlite surface to node of newly emerging leaf) of safflower seedlings were measured (mm plant^{-1}) and 3 plants representing each treatment were harvested to determine fresh weight (g FW^{-1}). The water status of the leaves [2 leaf disks (R = 0.5 cm) of each treatment and 3 replications] was evaluated by calculating relative water content (RWC) (Farrant, 2000).

2.3. Chlorophyll a fluorescence measurements
Chlorophyll *a* fluorescence measurements were performed with a portable, modulated fluorescence monitoring system (FMS 2; Hansatech Ltd., UK) on randomly selected leaves of the safflower cultivars (6 replicates). Following at least 30 min of dark adaptation, the minimum chlorophyll *a* fluorescence (F_O) was determined using a measuring beam of 0.2 μmol m^{-2} s^{-1} intensity. A saturation pulse (1 s of white light of 7500 μmol m^{-2} s^{-1} intensity) was used to obtain the maximum fluorescence (F_M) after a dark-adapted state was reached. The maximal quantum efficiency of PSII of dark-adapted plants (F_V/F_M) was calculated using ($F_M - F_O$) / F_M. F_V is known to be the variable fluorescence ($F_V = F_M - F_O$). Light-induced changes in chlorophyll *a* fluorescence following actinic illumination (300 μmol m^{-2} s^{-1}) were recorded prior to the measurement of F'_O (minimum chlorophyll *a* fluorescence in light-saturated state) and F'_M (maximum fluorescence in light-saturated state). The quantum efficiency of PSII open centers in the light-adapted state, referred to as ΦPSII, ($F'_M - F_S$ / F'_M), was determined from F'_M and F_S (steady-state fluorescence in the light-saturated state) values. The quantum efficiency of excitation energy trapping of PSII (F'_V / F'_M) was calculated according to Genty et al. (1989). Later, the actinic light was shut off and the minimum fluorescence in the light-adapted state (F'_O) was determined by illuminating the leaves with far-red light (7 μmol m^{-2} s^{-1}). Electron transport rate (ETR) was determined by multiplying the quantum efficiency by incident photon flux density and an average factor of 0.84 for leaf absorbance and dividing by a factor of 2 to account for the sharing of absorbed photons between the 2 photosystems (PSI and PSII) [ETR = (F'_M

$– F_S) / (F'_M) \times PAR \times 0.84 \times 0.5]$ (Genty et al., 1989). The photochemical quenching, $qP = (F'_M – F_S) / (F'_M – F'_O)$, and the nonphotochemical quenching, $NPQ = F_M – F'_M / F'_M$, were calculated according to Genty et al. (1989).

2.4. Pigment analysis

To determine the content of chlorophylls ($a+b$) and total carotenoids ($x+c$), fresh leaf samples (0.1 g) were extracted in 10 mL of 100% acetone. The absorbance of extracts was then measured at 470, 644.8, and 661.6 nm using a Shimadzu Mini-1240 UV-Vis spectrophotometer. The contents of chlorophylls ($a+b$) and carotenoids ($x+c$) were determined using adjusted extinction coefficients (Lichtenthaler, 1987).

2.5. Ion content

To determine the content of K^+ and Na^+ (mg g DW^{-1}), 10-mg dry leaf samples were extracted according to Weimberg (1987). The ion contents were defined using an atomic absorption spectrophotometer (PerkinElmer 2280) and the K^+/Na^+ ratio was calculated from the content of K^+ and Na^+.

2.6. Malondialdehyde content

The level of malondialdehyde (MDA) content was determined according to the method of Esterbauer and Cheeseman (1990). Fresh leaf tissue (0.1 g) was homogenized in 0.1% TCA at 4 °C and centrifuged at 10,000 rpm for 15 min. To determine the content of MDA, 0.1 M Tris-HCl (pH 7.6) and TCA-TBA-HCl (trichloroacetic acid-thiobarbituric acid-hydrochloric acid) reagent were added to the supernatant. This solution was boiled for 45 min in a water bath and then centrifuged at 10,000 rpm for 5 min. The absorbance was read at 532 and 600 nm and calculated using the extinction coefficient 155 mM^{-1} cm^{-1}.

2.7. Proline content

The free proline content was quantified using the method of Bates et al. (1973), whereby 0.5 g of fresh leaves from each treatment with 3 replicates was homogenized in 3% aqueous sulfosalicylic acid and the homogenate was filtrated with filter paper. After addition of acid ninhydrin and glacial acetic acid, the mixture was kept in a water bath at 100 °C for 60 min. Toluene was then added to the reaction mixture and the absorbance was read at 520 nm. Finally, a proline standard curve was used to determine the content of free proline.

2.8. Detection of activities of antioxidant enzymes

Fresh leaf samples (of 0.5 g with 3 replicates) were ground with liquid nitrogen and soluble protein was extracted by homogenizing in related buffer. The protein concentrations from leaf extracts were determined according to Bradford (1976).

The homogenates were homogenized in 1 mL of buffer containing 9 mM Tris-HCl buffer (pH 6.8) and 13.6% glycerol, and the SOD (EC 1.15.1.1) activity was

determined according to Beyer and Fridovich (1987). One unit of SOD is defined as the amount of enzyme that causes 50% decrease of the SOD-inhibited nitroblue tetrazolium reduction.

APX (EC 1.11.1.11) activity was assayed according to the method of Wang et al. (1991) and the reaction mixture contained 50 mM Tris-HCl (pH 7.2) buffer, 2% PVP, 1 mM Na_2EDTA, and 2 mM ascorbate. The enzyme activity was calculated from the initial rate of the reaction using the extinction coefficient, ε, of ascorbate (ε = 2.8 mM cm^{-1}) at 290 nm.

GR (EC 1.6.4.2) activities were determined according to the method of Rao et al. (1995). The reaction mixture contained 100 mM potassium phosphate buffer (pH 7.0), 2% PVP, and 1 mM Na_2EDTA. The enzyme activity was calculated from the initial rate of the reaction after subtracting the nonenzymatic initial oxidation rate using the extinction coefficient of NADPH (ε = 6.2 mM cm^{-1}) at 340 nm.

Guaiacol POD (EC 1.11.1.7) activity was based on the determination of guaiacol oxidation (ε = 26.6 mM cm^{-1}) at 470 nm by H_2O_2. The reaction mixture contained 100 mM potassium phosphate buffer (pH 7.0), 20.1 mM guaiacol, 12.3 mM H_2O_2, and enzyme extract in a 3-mL volume (Bergmeyer, 1974).

2.9. Statistical data analysis

The experiments were performed in a completely randomized design with 3 replicates and SPSS was used to determine the differences between the cultivars and their treatments. To correct the variability of data and check the validity of results, all data were subjected to analysis of variance, and to detect differences between cultivars and treatments, the least significant difference (LSD) was calculated at the 5% level.

3. Results

3.1. Growth parameters

The effects of salt stress on some growth parameters (length and fresh weight of shoot and RWC of leaves) are shown in Table 1. Shoot length of all cultivars gradually decreased for all NaCl applications; at the highest NaCl application (300 mM), the reduction was more than 50% compared to the controls. Among the cultivars, the shoot length of Dinçer was less affected by salinity. Similar results were determined in the shoot fresh weights of cultivars. With the 75 mM NaCl application, the fresh weight of shoots was decreased by 41% and 44% in Remzibey-05 and Yenice, respectively, whereas the reduction was only 14% in Dinçer. RWC also decreased with increasing salt concentrations. More than 10% reduction of RWC was established in Yenice at 150 mM and higher NaCl concentrations, whereas the same reduction level was determined at 225 and 300 mM NaCl levels in Remzibey-05 and Dinçer.

Table 1. Effects of salt stress on the shoot elongation, fresh matter production, and relative water content (RWC) of leaves of 3 safflower cultivars.

Cultivars	Salt level (mM)	Length of shoot (mm plant^{-1})	Fresh weight of shoot (g plant^{-1})	RWC of leaves (%)
Dinçer	Control	234.5* ± 4.6	4.33** ± 0.25	83.8** ± 0.1
	75	199.7 ± 5.4	3.71 ± 0.10	81.2 ± 1.1
	150	188.6 ± 6.4	2.48 ± 0.10	78.0 ± 0.5
	225	151.5 ± 6.6	2.15 ± 0.16	71.5 ± 0.6
	300	100.2 ± 14.1	1.15 ± 0.02	59.0 ± 1.5
Remzibey-05	Control	240.3* ± 8.4	7.03** ± 0.22	83.2** ± 1.5
	75	196.7 ± 4.5	4.13 ± 0.04	78.6 ± 3.5
	150	175.3 ± 3.3	3.01 ± 0.12	76.7 ± 2.7
	225	140.6 ± 9.8	2.81 ± 0.05	73.2 ± 2.1
	300	86.9 ± 14.0	1.02 ± 0.21	51.6 ± 1.4
Yenice	Control	241.6* ± 10.3	6.19** ± 0.21	79.0** ± 0.8
	75	208.5 ± 5.6	3.51 ± 0.10	70.8 ± 0.3
	150	156.1 ± 5.5	3.04 ± 0.14	69.5 ± 0.8
	225	115.8 ± 9.0	2.3 ± 0.02	63.7 ± 0.2
	300	66.5 ± 11.5	2.11 ± 0.11	47.53 ± 0.6
LSD 5%		20.4	0.56	5.9

*: Each value represents the mean ± SE (n = 15).
**: Each value represents the mean ± SE (n = 3).

3.2. Chlorophyll a fluorescence measurements

It was determined that no significant changes took place in the chlorophyll *a* fluorescence of safflower cultivars at the lower salt concentrations (75 and 150 mM NaCl) (Figures 1A–1D and 2A–2D) compared to their control groups, except for F_M values (Figure 1B). In the dark-adapted leaves, the value of F_O decreased in Dinçer and Yenice at 225 and 300 mM NaCl compared to the control (Figure 1A). The F_M values of cultivars progressively decreased in salt-treated leaves starting from 150 mM NaCl (Figure 1B). The F_V/F_M, ΦPSII, F'_V/F'_M, and ETR parameters were reduced depending on the increase of NaCl concentration, but these reductions were only significant at the highest salt treatment (300 mM NaCl) for Dinçer (35%, 39%, 37%, and 39%, respectively) and Remzibey-05 (64%, 67%, 62%, and 67%, respectively) (Figures 1C, 1D, 2A, and 2B). In addition, these reductions were significant for Yenice under 225 and 300 mM NaCl. Except for the comparison between 75 and 225 mM salt treatments, for all salt treatments, there was no significant change in qP and NPQ parameters of Dinçer. Reductions in qP and NPQ parameters were significant for Remzibey-05 at the highest salt treatment and Yenice at 225 and 300 mM NaCl applications (Figures 2C and 2D).

3.3. Pigment analysis

Application of NaCl affected pigment contents of safflower cultivars (Figure 3). In general, the chlorophyll content was significantly reduced for all cultivars (Figure 3A). Among the 150 mM and higher NaCl concentrations, alteration of chlorophyll content was not important for Dinçer and the reductions were about 77% of the control with those treatments. Similar to chlorophyll content, the carotenoid contents of cultivars declined significantly with all salt applications (Figure 3B).

### 3.4. Ion concentrations and K$^+$/Na$^+$ ratio

The effects of salt stress on K$^+$ and Na$^+$ contents and K$^+$/Na$^+$ ratio are shown in Table 2. While the reduction of K$^+$ content of Remzibey-05 was significant only at the highest salt concentration (300 mM NaCl), in Dinçer and Yenice the K$^+$ contents were reduced markedly with all NaCl treatments, except for 75 mM NaCl for Dinçer. The Na$^+$ content increased with increasing salt treatments and the
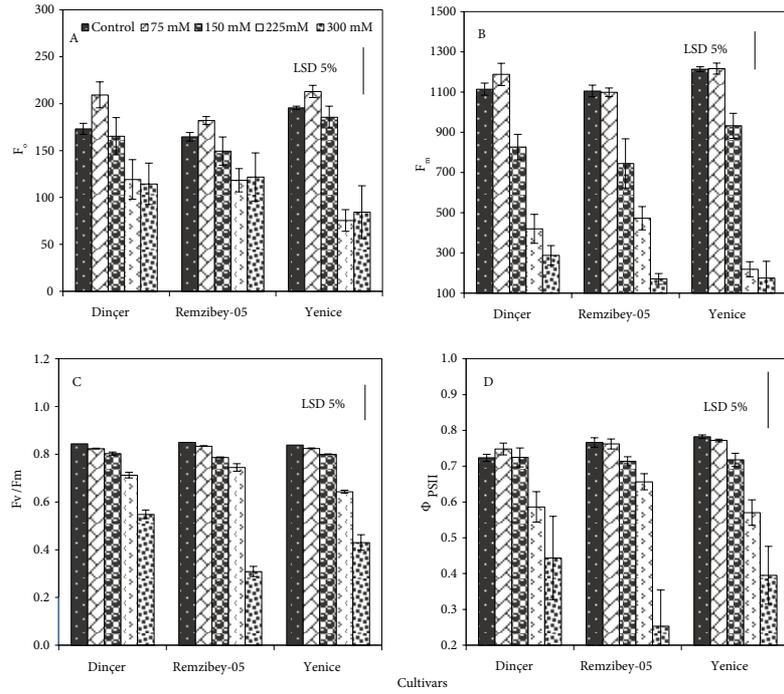
Figure 1. F_O minimum fluorescence (A), F_M maximum fluorescence (B), F_V/F_M potential quantum yield of PSII (C), and ΦPSII efficiency of open reaction centre of light adapted state (D) of safflower cultivars subjected to different salt stresses (n = 6).
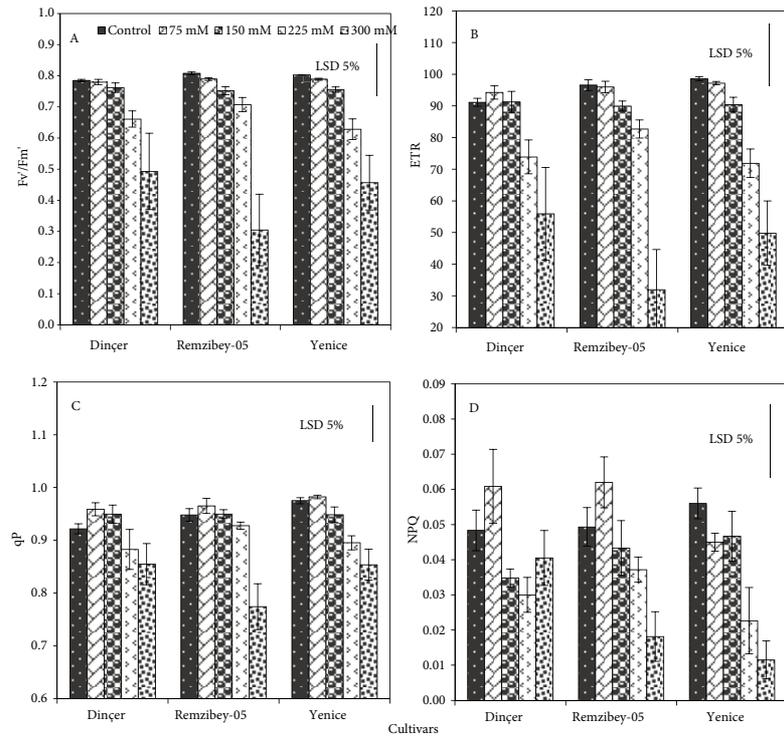
Figure 2. F'_V/F'_M quantum efficiency of excitation energy trapping of PSII of light-adapted leaves (A), ETR electron transport rate (B), qP photochemical quenching (C), and NPQ nonphotochemical quenching (D) of safflower cultivars subjected to different salt stresses (n = 6).
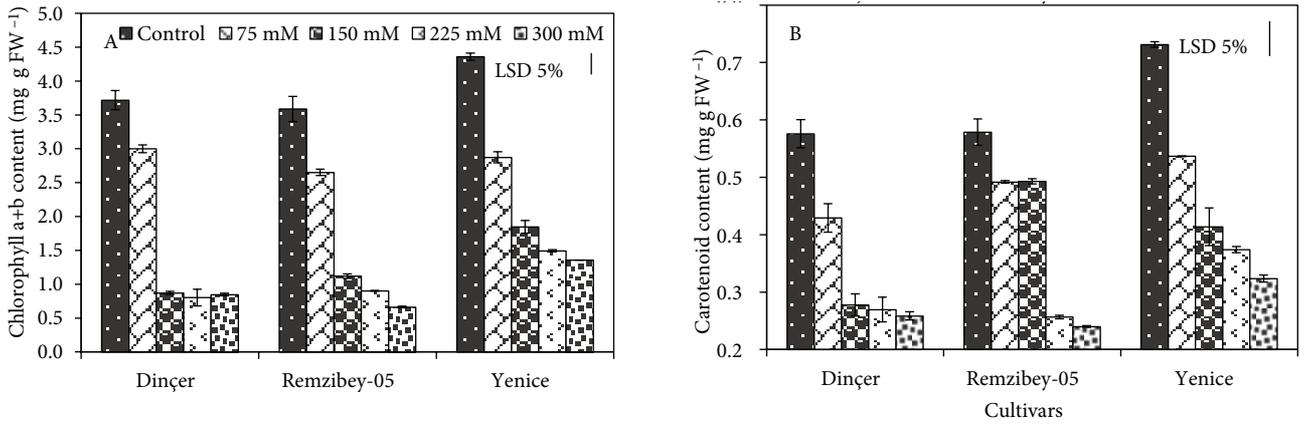
Figure 3. Effect of salt stress on total chlorophyll (A) and carotenoid (B) contents of safflower leaves (n = 3).

Table 2. Effects of salt stress on K^+ content, Na^+ content, and K^+/Na^+ ratio of safflower cultivars. Each value represents the mean ± SE (n = 3).

Cultivars	Salt level (mM)	K^+ content (mg g DW^{-1})	Na^+ content (mg g DW^{-1})	K^+/Na^+ ratio
Dinçer	Control	30.3 ± 0.9	12.0 ± 0.4	2.52 ± 0.06
	75	27.0 ± 1.7	23.1 ± 0.9	1.17 ± 0.05
	150	21.7 ± 1.9	25.7 ± 2.4	0.87 ± 0.15
	225	21.7 ± 3.0	49.1 ± 3.1	0.45 ± 0.09
	300	14.3 ± 0.9	63.1 ± 1.0	0.23 ± 0.02
Remzibey-05	Control	23.7 ± 2.9	12.5 ± 0.3	1.88 ± 0.21
	75	23.3 ± 1.2	34.4 ± 2.2	0.68 ± 0.02
	150	23.7 ± 1.8	36.8 ± 2.4	0.65 ± 0.06
	225	18.7 ± 1.7	47.5 ± 0.8	0.39 ± 0.04
	300	14.3 ± 1.2	69.3 ± 1.2	0.21 ± 0.02
Yenice	Control	36.7 ± 1.7	13.2 ± 0.7	2.80 ± 0.23
	75	24.0 ± 2.9	33.8 ± 0.7	0.71 ± 0.10
	150	21.0 ± 0.6	35.4 ± 2.9	0.60 ± 0.07
	225	20.3 ± 0.3	57.1 ± 1.6	0.36 ± 0.02
	300	13.0 ± 1.0	70.8 ± 6.3	0.18 ± 0.01
LSD 5%		6.9	9.2	0.39

lowest Na^+ content was determined in Dinçer according to its related control for all NaCl applications, except for 225 mM NaCl. Depending on alterations in K^+ and Na^+ contents, K^+/Na^+ ratios were reduced in the 3 safflower cultivars at all NaCl concentrations. With the lowest NaCl treatment, K^+/Na^+ ratios of cultivars declined by 54%, 64%, and 75% for Dinçer, Remzibey-05, and Yenice, respectively, compared to the controls.

3.5. Lipid peroxidation

The lipid peroxidation level in the leaves of safflower cultivars, measured as the content of MDA, is shown in Table 3. The MDA content increased progressively due to salt treatments in the 3 safflower cultivars, but this increase was not significant with the 75 mM NaCl treatment for Remzibey-05 and Yenice or at 150 mM NaCl for Remzibey-05. At the highest salt concentration (300 mM

Table 3. Changes in leaf MDA (nmol g FW^{-1}) content of safflower cultivars exposed to different salt concentrations. Each value represents the mean ± SE (n = 3).

Cultivars	Salt level (mM)				
	Control	75	150	225	300
Dinçer	26.8 ± 1.8	41.3 ± 3.3	42.7 ± 3.0	45.8 ± 2.7	46.1 ± 2.8
Remzibey-05	36.8 ± 2.5	40.6 ± 1.2	41.3 ± 1.2	50.2 ± 3.0	51.6 ± 3.0
Yenice	27.2 ± 2.4	30.3 ± 0.7	39.6 ± 2.1	46.1 ± 2.5	47.8 ± 4.5
LSD 5%	10.3				

NaCl), the MDA content was 1.72- and 1.76-fold higher in Dinçer and Yenice, respectively, and 1.4-fold higher in Remzibey-05 compared to their controls.

3.6. Proline content

The free proline content of safflower cultivars increased markedly in response to all salt treatments, except for Yenice at 75 mM NaCl treatment (Table 4). Among all salt treatments, the increase of proline content was the highest in Remzibey-05 (more than 195-fold), although the highest proline content was determined in Dinçer.

3.7. Activities of antioxidant enzymes

Antioxidant enzyme activities in the leaves of safflower cultivars showed differences under the various salt concentrations (Figure 4). SOD activities increased significantly in safflower cultivars, except in Yenice at 75 mM NaCl, compared to the controls. Salinity at 75 mM resulted in the highest SOD activity in Dinçer, whereas the SOD activity at higher salt concentrations declined (only significant at 300 mM) compared to 75 mM NaCl treatment for this cultivar. Among all salt treatments, the lowest increase in SOD activity was determined in Remzibey-05 compared to the controls (Figure 4A). With increasing salt levels, the APX activities of Dinçer increased while the activities of other cultivars decreased (Figure 4B). The GR activities of the safflower cultivars showed similarities among APX activities under salt stress (Figure 4C). GR activity increased only in Dinçer, whereas in Remzibey-05 and Yenice the GR activity was significantly reduced. The minimum APX and GR activities were determined in Remzibey-05 at the highest NaCl concentration and these reductions were 66% and 71% of the controls, respectively. NaCl treatments of the safflower cultivars caused significant increase in POD activity, especially in Dinçer with the highest activity (Figure 4D). However, among the cultivars, Remzibey-05 had the lowest increase in POD activity for all salt concentrations when compared to the controls.

4. Discussion

Increasing concentrations of NaCl in the growth medium caused a marked reduction in vegetative growth of the safflower cultivars. All growth parameters of Remzibey-05 and Yenice were affected more severely than those of Dinçer (Table 1). Kaya et al. (2003) reported that Remzibey-05 (spiny) was more resistant to high salt concentrations than Dinçer and Yenice (spineless) based on some parameters (emergence rate, root and shoot length, root and shoot dry weight, etc.). Contrary to these results, in the present study, Dinçer showed better performance than the others in terms of growth and chlorophyll *a* fluorescence parameters with antioxidant enzyme activities. The highest NaCl treatment (300 mM) led to the highest decrease of shoot length of

Table 4. Changes in leaf proline (μmol g FW^{-1}) content of safflower cultivars exposed to different salt concentrations. Each value represents the mean ± SE (n = 3).

Cultivars	Salt level (mM)				
	Control	75	150	225	300
Dinçer	0.014 ± 0.002	0.294 ± 0.007	0.822 ± 0.041	1.032 ± 0.033	1.132 ± 0.031
Remzibey-05	0.001 ± 0.000	0.199 ± 0.004	0.645 ± 0.034	0.730 ± 0.051	0.989 ± 0.045
Yenice	0.004 ± 0.001	0.074 ± 0.001	0.410 ± 0.005	0.669 ± 0.037	0.921 ± 0.039
LSD 5%	0.11				
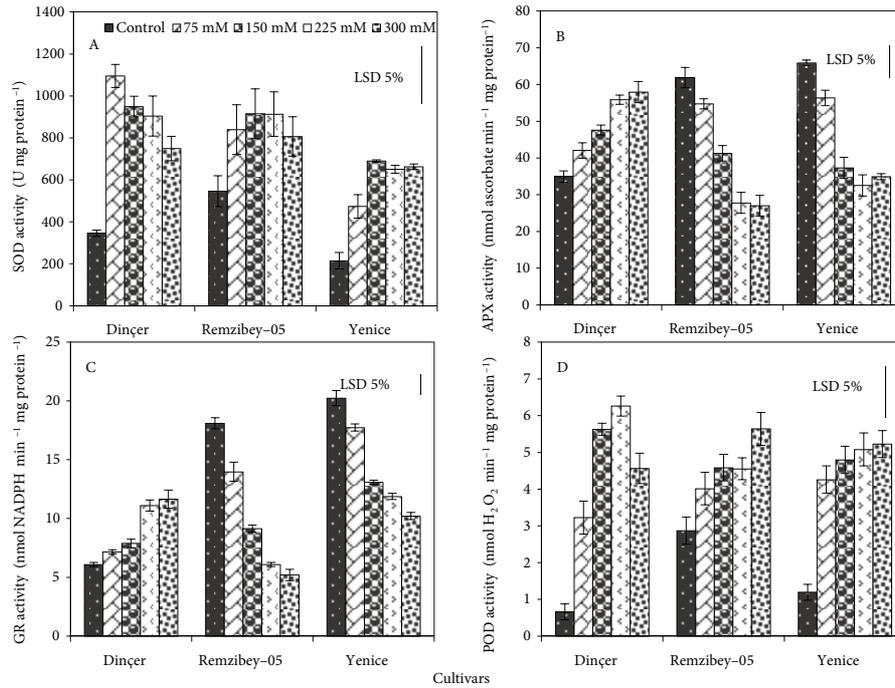
Figure 4. Changes in antioxidant enzyme activities of safflower cultivars exposed to different salt concentration: SOD activity (A), APX activity (B), GR activity (C), POD activity (D) (n = 3).

cultivar Yenice (decrease of 72.5% compared to control), while the reduction in shoot fresh weight was the most remarkable for Remzibey-05 (decrease of 85.5% compared to control). As with the length and fresh weight of shoots, water contents of the cultivars were also markedly reduced, which was most obvious in Yenice. The decrease of water contents in safflower cultivars might be explained by the decrease of water-flow from the root to the shoot due to the impaired hydraulic conductivity in roots. Similar results were reported by Siddiqi and Ashraf (2008), who found reduction in shoot fresh biomass and all water parameters, such as RWC, water potential (Ψw), and osmotic potential (Ψs), of safflower cultivars. In the present study, the changes in photosynthetic efficiency, pigment and ion contents, and antioxidant enzyme activities might be the reason for reduced growth parameters of the safflower cultivars under saline conditions.

Along with plant growth, photosynthesis is the other essential physiological process affected by salt stress. The effect of stress on quantum efficiency of electron transport through PSII can be evaluated by using the chlorophyll *a* fluorescence technique, which estimates effects rapidly and noninvasively (Genty et al., 1989; Baker and Rosenqvist, 2004). The chlorophyll *a* fluorescence parameters showed that lower stress levels (75 and 150 mM NaCl) did not induce photoinhibition of PSII of the cultivars (Figures 1 and 2), except for F_M values. F_O is considered to be the evaluated amount of oxidized primary quinone electron

acceptors of PSII (Q_A) when all reaction centers are open (Yusuf et al., 2010). In this study, high salt concentrations induced significant decreases in F_O parameters in Dinçer and Yenice (Figure 1A). Mehta et al. (2010) reported that an increase in salt concentration cause a marked decrease in F_O in wheat leaves. This reduction may reflect the impairment of the integrity of thylakoid membranes and the decrease in the photosynthetic pigment content. The F_M parameter, which represents a reduction in the amount of the primary electron acceptor Q_A (Lutts et al., 1996), declined under salinity due to severity of stress in safflower cultivars, except for the 75 mM NaCl treatment (Figure 1B). The decreasing F_M parameter was indicated by the inactive PSII reaction centers as well as the degradation of D1 protein (Kalaji et al., 2011). Changes in the F_O and F_M parameters led to a reduced F_V/F_M ratio (Figure 1C), but this decline was only significantly important under the highest salt concentration in Dinçer and Remzibey-05 leaves. For the other salt applications, the alterations of F_V/F_M parameters were not found significant, and these results indicated no damage to the donor side or acceptor side of PSII (Chen et al., 2004) and optimal functionality of PSII (Sekmen et al., 2012). Our results showed that decreased values of F_V/F_M exhibited a correlation with ΦPSII, F'_V/F'_M, and ETR (Figures 1D, 2A, and 2B). With the 225 mM NaCl treatment, these parameters declined distinctly only in Yenice. Zribi et al. (2009) reported that decreased ΦPSII was determined by qP and F'_V/F'_M, and their results

showed a high correlation between either ΦPSII and qP or ΦPSII and F'_V/F'_M. In this study our data showed that decreased ΦPSII was more related to F'_V/F'_M than qP because F'_V/F'_M, which expressed the level of quenching in PSII reaction centers and antenna, was affected more than qP under salt stress. However, it was seen that a decrease in F_V/F_M was related to the inhibition of circulation and use of photosynthetic electrons (M'rah et al., 2006) and also reduced ETR. Except at 225 mM NaCl for Yenice, ΦPSII, F'_V/F'_M, and ETR were significantly reduced in safflower cultivars only at the highest salt treatment compared to controls (almost 40% for Dinçer, 60% for Remzibey-05, and 50% for Yenice), and this might reflect that salt stress did not induce susceptibility of PSII to inhibition. qP, which explains the trapping photon energy that derives photosynthesis, was not affected significantly in Dinçer at any salt concentrations (Figure 2C). Likewise, the values of NPQ of Dinçer exhibited no significant change for all NaCl treatments, and this result indicates that the cultivar used different mechanisms to dissipate excitation energy from PSII and its antenna (Figure 2D). The NPQ declined in Remzibey-05 at 300 mM and in Yenice at 225 and 300 mM salt treatments, and the reason for this might be the decrease in pigment content (Figure 3). The results for chlorophyll *a* fluorescence indicated that Dinçer was more successful than other safflower cultivars in adapting to salinity.

Salt stress influenced the pigment content of the safflower leaves, which led to decrease in their chlorophyll contents (Figure 3A). The decrease in chlorophyll contents might be related to an increase of chlorophyll degradation or a decrease of chlorophyll synthesis (Santos, 2004). It was also reported that, under saline conditions, chlorophyll content declined in soy bean (Çiçek and Çakırlar, 2008), sugarcane (Cha-um and Kirdmanee, 2009), and cotton (Kawakami et al., 2013). As with chlorophyll content, carotenoid amounts reduced in relation to salt treatments (Figure 3B). Previous studies have shown that carotenoid content of leaves decreased due to salt stress (Çiçek and Çakırlar, 2008; Cha-um and Kirdmanee, 2009; Cambrollé et al., 2011). The reason for the decrease in pigment contents could be the increasing levels of ROS and the loss of membrane integrity.

Besides osmotic effects, salt stress affects plant growth and development by causing an accumulation of ions in detrimental concentrations in tissues (e.g., Na$^+$ and Cl$^-$) and alterations to the nutritional content of essential ions (e.g., Ca^{+2} and K$^+$) (Rejili et al., 2007). Our results showed that Na$^+$ concentration increased while K$^+$ content reduced, especially at 225 and 300 mM salt treatments for all cultivars (Table 2). The alterations of Na$^+$ and K$^+$ contents were more excessive at all salt levels for Yenice. Changes in Na$^+$ and K$^+$ concentrations were reflected in the K$^+$/Na$^+$

ratio for safflower cultivars (Table 2). It has been reported that the K$^+$/Na$^+$ ratio is an important selection criterion for salt tolerance (Morant-Manceau et al., 2004; Ashraf and Orooj, 2006; Çiçek and Çakırlar, 2008). Although the K$^+$/Na$^+$ ratio was decreased by increasing salt concentration in safflower cultivars, the highest K$^+$/Na$^+$ ratio was determined in Dinçer.

Peroxidation of membrane lipids, known as MDA, caused by salt stress was reported in many previous studies on various species such as *Arabidopsis thaliana* (M'rah et al., 2006), chickpea (Eyidogan and Öz, 2007), and *Vigna radiata* (Hayat et al., 2010). Under salt stress, Dinçer had the highest increase in MDA content at 75 and 150 mM salt treatments compared to the control (Table 3), while the photosynthetic activities remained unchanged for this cultivar at these NaCl concentrations (Figure 1).

Salt stress caused an increase in proline contents and these contents of the cultivars rose progressively depending on increasing NaCl concentrations (Table 4). Although proline accumulation resulting from high salt stress is known as the earliest response in higher plants, its role as an adaptive process is still a matter of debate. On one hand, positive relations between salt tolerance and proline accumulation were reported by some researchers (Ashraf and Orooj, 2006; Hajlaoui et al., 2010), but, on the other hand higher accumulations of proline in sensitive cultivars than in those with tolerance were also reported (Heidari, 2010; Çelik and Atak, 2012). Dinçer had the highest free proline contents, while Yenice had the lowest for all treatments. As a result, we may suggest that there is a positive relation between proline accumulation and salt tolerance.

In many cases, salt tolerance is related to a higher activity of antioxidant enzymes in plants (Türkan and Demiral, 2009). Salt stress caused a significant increase in all the examined antioxidant enzymes activities (SOD, POD, APX, and GR) in Dinçer for all salt treatments (Figure 4). Many previous studies on various species demonstrated the increase of some antioxidant enzyme activities because of environmental fluctuations, such as SOD, POD, and GR in *Vigna radiata* (Hayat et al., 2010); APX in canola (Heidari, 2010); and POD in safflower (Hosseini et al., 2010). In Dinçer, the increases in activities of SOD, APX, GR, and POD were not sufficient to prevent lipid peroxidation as verified by the MDA formation. Similarly, it was reported that lipid peroxidation increased in *Catharanthus roseus* in spite of the induction of antioxidant enzymes in response to salinity (Elkahoui et al., 2005). Salt stress increased SOD activities while it reduced APX and GR activities in Remzibey-05 and Yenice (Figures 4A–4C). This shows that these cultivars may not use the AsA-GSH cycle to overcome the accumulation of H$_2$O$_2$. While APX and GR activities were reduced, POD

activities were significantly increased in Remzibey-05 and Yenice, except with 75 mM NaCl in Remzibey-05 (Figure 4D). Increases in POD activities may indicate that both cultivars make use of this mechanism for protecting the deleterious effects of H_2O_2 instead of APX and GR.

In conclusion, determination of the cultivars' responses to salt stress and the development of more tolerant species/cultivars are important. The results of this study showed that salt stress negatively affected the safflower cultivars' growth, water and ion contents, pigment amounts, and photosynthesis at the vegetative stage. However, the safflower cultivars tried to withstand severe salinity conditions by upregulating protective mechanisms. Although Dinçer showed similar results to those of Remzibey-05 and Yenice

for accumulation of MDA and reduced pigment contents, it exhibited much better responses in terms of growth parameters, chlorophyll *a* fluorescence measurements, and antioxidant enzyme activities. It may be suggested that the best-performing safflower cultivar under these salt conditions is Dinçer. Consequently, Dinçer may be used as a gene source to develop more tolerant cultivars and to increase the salt stress tolerance capacity of oil seed plants in further studies.

References

Ashraf M, Ali Q (2008). Relative membrane permeability and activities of some antioxidant enzymes as the key determinants of salt tolerance in canola (*Brassica napus* L.). Environ Exp Bot 63: 266–273.

Ashraf M, Orooj A (2006). Salt stress effects on growth, ion accumulation and seed oil concentration in an arid zone traditional medicinal plant ajwain (*Trachyspermum ammi* [L.] Sprague). J Arid Environ 64: 209–220.

Aymen EM, Kaouther Z, Fredj MB, Cherif H (2012). Seed priming for better growth and yield of safflower (*Carthamus tinctorius*) under saline condition. J Stress Physiol Biochem 8: 135–143.

Baker NR, Rosenqvist E (2004). Applications of chlorophyll fluorescence can improve crop production strategies: an examination of future possibilities. J Exp Bot 55: 1607–1621.

Bates LS, Waldren RP, Teare ID (1973). Rapid determination of free proline for water stress studies. Plant Soil 39: 205–207.

Baydar H, Gökmen OY (2003). Hybrid seed production in safflower (*Carthamus tinctorius* L.) following the induction of male sterility by gibberellic acid. Plant Breeding 122: 459–461.

Bergmeyer HU (1974). Methods of Enzymatic Analysis, Vol. 2. 2nd ed. New York, NY, USA: Academic Press.

Beyer WF, Fridovich I (1987). Assaying for superoxide dismutase activity: some large consequences of minor changes in conditions. Anal Biochem 161: 559–566.

Bradford MM (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72: 248–254.

Cambrollé J, Redondo-Gómez S, Mateos-Naranjo E, Luque T, Figueroa ME (2011). Physiological responses to salinity in the yellow-horned poppy *Glaucium flavum*. Plant Physiol Bioch 49: 186–194.

Çelik Ö, Atak Ç (2012). The effect of salt stress on antioxidative enzymes and proline content of two Turkish tobacco varieties. Turk J Biol 36: 339–356.

Cha-um S, Kirdmanee C (2009). Proline accumulation, photosynthetic abilities and growth characters of sugarcane (*Saccharum officinarum* L.) plantlets in response to iso-osmotic salt and water-deficit stress. Agr Sci China 8: 51–58.

Chen HX, Li WJ, An SZ, Gao HY (2004). Characterization of PSII photochemistry and thermostability in salt-treated *Rumex* leaves. J Plant Physiol 161: 257–264.

Çiçek N, Çakırlar H (2008). Effects of salt stress on some physiological and photosynthetic parameters at three different temperatures in six soya bean (*Glycine max* L. Merr.) cultivars. J Agron Crop Sci 194: 34–46.

Coşge B, Gürbüz B, Kıralan M (2007). Oil content and fatty acid composition of some safflower (*Carthamus tinctorius* L.) varieties sown in spring and winter. Int J Nat Eng Sci 1: 11–15.

Degl'Innocenti E, Hafsi C, Guidi L, Navari-Izzo F (2009). The effect of salinity on photosynthetic activity in potassium-deficient barley species. J Plant Physiol 166: 1968–1981.

Ejaz B, Sajid ZA, Aftab F (2012). Effect of exogenous application of ascorbic acid on antioxidant enzyme activities, proline contents, and growth parameters of *Saccharum* spp. hybrid cv. HSF-240 under salt stress. Turk J Biol 36: 630–640.

Elkahoui S, Hernández JA, Abdelly C, Ghrir R, Limam F (2005). Effects of salt on lipid peroxidation and antioxidant enzyme activities of *Catharanthus roseus* suspension cells. Plant Sci 168: 607–613.

Esterbauer H, Cheeseman KH (1990). Determination of aldehydic lipid peroxidation products: malonaldehyde and 4-hydroxynonenal. Method Enzymol 186: 407–431.

Eyidogan F, Öz MT (2007). Effect of salinity on antioxidant responses of chickpea seedlings. Acta Physiol Plant 29: 485–493.

Farrant JM (2000). A comparison of mechanisms of desiccation tolerance among three angiosperm resurrection plant species. Plant Ecol 151: 29–39.

Fraj MB, Al-Dakheel AJ, McCann IR, Shabbir GM, Rumman GA, Al Gailani AQAM (2013). Selection of high yielding and stable safflower (*Carthamus tinctorius* L.) genotypes under salinity stress. Agr Sci Res J 3: 273–283.

Francois LE, Bernstein L (1964). Salt tolerance of safflower. Agron J 56: 38–40.

Genty B, Briantais JM, Baker NR (1989). The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. Biochim Biophys Acta 990: 87–92.

Gondim FA, Gomes-Filho E, Costa JH, Alencar NLM, Prisco JT (2012). Catalase plays a key role in salt stress acclimation induced by hydrogen peroxide pretreatment in maize. Plant Physiol Bioch 56: 62–71.

Hajlaoui H, El Ayeb N, Garrec JP, Denden M (2010). Differential effects of salt stress on osmotic adjustment and solutes allocation on the basis of root and leaf tissue senescence of two silage maize (Zea mays L.) varieties. Ind Crop Prod 31: 122–130.

Hayat S, Hasan SA, Yusuf M, Hayat Q, Ahmad A (2010). Effect of 28-homobrassinolide on photosynthesis, fluorescence and antioxidant system in the presence or absence of salinity and temperature in Vigna radiata. Environ Exp Bot 69: 105–112.

Heidari M (2010). Nucleic acid metabolism, proline concentration and antioxidants enzyme activity in canola (Brassica nupus L.) under salinity stress. Agric Sci China 9: 504–511.

Hoagland DR, Arnon DI (1950). The water culture method for growing plants without soil. Circ Calif Agr Exp Sta 347: 1–39.

Hosseini T, Shekari F, Ghorbanli M (2010). Effect of salt stress on ion content, proline and antioxidative enzymes of two safflower cultivars (Carthamus tinctorius L.). J Food Agric Environ 8: 1080–1086.

Hu Y, Schmidhalter U (2005). Drought and salinity: a comparison of their effects on mineral nutrition of plants. J Plant Nutr Soil Sc 168: 541–549.

Hussain TM, Chandrasekhar T, Hazara M, Sultan Z, Saleh BK, Gopal GR (2008). Recent advances in salt stress biology – a review. Biotechnol Mol Biol Rev 3: 8–13.

Kalaji HM, Govindjee Bosa K, Kościelniak J, Żuk-Golaszewska K (2011). Effects of salt stress on photosystem II efficiency and CO$_2$ assimilation of two Syrian barley landraces. Environ Exp Bot 73: 64–72.

Kawakami, EM, Oosterhuis DM, Snider JL (2013). Nitrogen assimilation and growth of cotton seedlings under NaCl salinity and in response to urea application with NBPT and DCD. J Agron Crop Sci 199: 106–117.

Kaya MD, İpek A, Öztürk A (2003). Effects of different soil salinity levels on germination and seedling growth of safflower (Carthamus tinctorius L.). Turk J Agric For 27: 221–227.

Lichtenthaler HK (1987). Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. Method Enzymol 148: 350–382.

Lutts S, Kinet JM, Bouharmont J (1996). NaCl-induced senescence in leaves of rice (Oryza sativa L.) cultivars differing in salinity resistance. Ann Bot 78: 389–398.

Mehta P, Jajoo A, Mathur S, Bharti S (2010). Chlorophyll a fluorescence study revealing effects of high salt stress on Photosystem II in wheat leaves. Plant Physiol Bioch 48: 16–20.

Morant-Manceau A, Pradier E, Tremblin G (2004). Osmotic adjustment, gas exchanges and chlorophyll fluorescence of a hexaploid triticale and its parental species under salt stress. J Plant Physiol 161: 25–33.

M'rah S, Ouerghi Z, Berthomieu C, Havaux M, Jungas C, Hajji M, Grignon C, Lachaâl M (2006). Effects of NaCl on the growth, ion accumulation and photosynthetic parameters of Thellungiella halophila. J Plant Physiol 163: 1022–1031.

Nagy Z, Galiba G (1995). Drought and salt tolerance are not necessarily linked: a study on wheat varieties differing in drought resistance under consecutive water and salinity stress. J Plant Physiol 145: 168–174.

Pessarakli M, Szabolcs I (1999). Soil Salinity and Sodicity as Particular Plant/Crop Stress Factors. In: Pessarakli M, editor. Handbook of Plant Crop Stress. Boca Raton, FL, USA: CRC Press, pp. 3–22.

Qados AMSA (2011). Effect of salt stress on plant growth and metabolism of bean plant Vicia faba (L.). J Saudi Soc Agric Sci 10: 7–15.

Rao VM, Hale BA, Omrod DP (1995). Amelioration of ozone induced oxidative damage in wheat plants grown under high carbon dioxide. Plant Physiol 109: 421–432.

Rejili M, Vadel AM, Guetet A, Neffatti M (2007). Effect of NaCl on the growth and the ionic balance K$^+$/Na$^+$ of two populations of Lotus creticus (L.) (Papilionaceae). S Afr J Bot 73: 623–631.

Santos CV (2004). Regulation of chlorophyll biosynthesis and degradation by salt stress in sunflower leaves. Sci Hortic 103: 93–99.

Sekmen AH, Turkan I, Tanyolac ZO, Ozfidan C, Dinc A (2012). Different antioxidant defense responses to salt stress during germination and vegetative stages of endemic halophyte Gypsophila oblanceolata Bark. Environ Exp Bot 77: 63–76.

Sekmen Esen AH, Özgür R, Uzilday B, Tanyolac ZO, Dinç A (2012). The response of the xerophytic plant Gypsophila aucheri to salt and drought stresses: the role of the antioxidant defence system. Turk J Bot 36: 697–706.

Siddiqi EH, Ashraf M (2008). Can leaf water relation parameters be used as selection criteria for salt tolerance in safflower (Carthamus tinctorius L.). Pak J Bot 40: 221–228.

Siddiqi EH, Ashraf M, Al-Qurainy F, Akram NA (2011). Salt-induced modulation in inorganic nutrients, antioxidant enzymes, proline content and seed oil composition in safflower (Carthamus tinctorius L.). J Sci Food Agric 91: 2785–2793.

Siddiqi EH, Ashraf M, Arkam NA (2007). Variation in seed germination and seedling growth in some diverse lines of safflower (Carthamus tinctorius L.) under salt stress. Pak J Bot 39: 1937–1944.

Shahzad M, Witzel K, Zörb C, Mühling KH (2012). Growth-related changes in subcellular ion patterns in maize leaves (Zea mays L.) under salt stress. J Agron Crop Sci 198: 46–56.

Sudhir P, Pogoryelov D, Kovács L, Garab G, Murthy SDS (2005). The effects of salt stress on photosynthetic electron transport and thylakoid membrane proteins in the cyanobacterium *Spirulina platensis*. J Biochem Mol Biol 38: 481–485.

Tayefi-Nasrabadi H, Dehghan G, Daeihassani B, Movafegi A, Samadi A (2011). Some biochemical properties of guaiacol peroxidases as modified by salt stress in leaves of salt-tolerant and salt-sensitive safflower (*Carthamus tinctorius* L.cv.) cultivars. Afr J Biotechnol 10: 751–763.

Türkan I, Demiral T (2009). Recent developments in understanding salinity tolerance. Environ Exp Bot 67: 2–9.

Tuteja N (2007). Mechanisms of high salinity tolerance in plants. Method Enzymol 428: 419–438.

Vijayan K (2009). Approaches for enhancing salt tolerance in mulberry (*Morus*L) - a review. Plant Omics J 2: 41–59.

Wang SY, Jiao H, Faust M (1991). Changes in ascorbate, glutathione and related enzyme activity, during thidiauron-induced bud break of apple. Plant Physiol 82: 231–236.

Weimberg R (1987). Solute adjustments in leaves of two species of wheat at two different stages of growth in response to salinity. Physiol Plant 70: 381–388.

Yusuf MA, Kumar D, Rajwanshi R, Strasser RJ, Tsimilli-Michael M, Govindjee Sarin NB (2010). Overexpression of γ-tocopherol methyl transferase gene in transgenic *Brassica juncea* plants alleviates abiotic stress: physiological and chlorophyll *a* fluorescence measurements. BBA-Bioenergetics 1797: 1428–1438.

Zareie S, Mohammadi-Nejad G, Sardouie-Nasab S, (2013). Screening of Iranian safflower genotypes under water deficit and normal conditions using tolerance indices. Aust J Crop Sci 7: 1032–1037.

Zribi L, Fatma G, Fatma R, Salwa R, Hassan N, Néjib RM (2009). Application of chlorophyll fluorescence for the diagnosis of salt stress in tomato "*Solanum lycopersicum* (variety Rio Grande)". Sci Hortic 120: 367–372.