TÜBİTAK

Research Article

Turk J Bot
36 (2012) 697-706

doi:10.3906/bot-1201-5

The response of the xerophytic plant *Gypsophila aucheri* to salt and drought stresses: the role of the antioxidant defence system

Aşkım Hediye SEKMEN ESEN*, Rengin ÖZGÜR, Barış UZİLDAY, Zehra Özgecan TANYOLAÇ, Ahmet DİNÇ

Department of Biology, Faculty of Science, Ege University, Bornova, 35100, İzmir - TURKEY

Received: 03.01.2012 ● Accepted: 05.06.2012

Abstract: The aim of the present study was to determine the antioxidant system responses of a xerophytic plant to salinity and drought, which were not elucidated before. Physiological and antioxidant responses of the xerophytic plant *Gypsophila aucheri* Boiss. were investigated under salinity (100 and 300 mM NaCl) and drought (withholding watering) treatment for 2 weeks in a controlled growth chamber. Besides growth responses (relative growth rate (RGR)), superoxide dismutase (SOD), catalase (CAT), peroxidase (POX), ascorbate peroxidase (APX), and glutathione reductase (GR) activities, and SOD and CAT isoenzymes activities were determined under stress conditions. Moreover, the level of lipid peroxidation was determined as thiobarbutiric acid reactive substances (TBARS) content. Tolerance of *G. aucheri* to salinity and drought was evaluated based on lipid peroxidation (oxidative damage) and RGR. According to the experiments, *G. aucheri* was found to be tolerant to drought and 100 mM NaCl and its antioxidant system was able to cope with oxidative stress under these conditions. However, 300 mM NaCl treatment had detrimental effects on plant growth and membrane integrity. Under this concentration, oxidative stress cannot be precluded in spite of increased SOD, POX, and APX activities. These data indicate that xerophytic *G. aucheri* is a moderate halophytic plant that uses an efficient antioxidant defence mechanism under salinity.

Key words: *Gypsophila aucheri*, antioxidant enzymes, salt stress, drought stress, lipid peroxidation

Introduction

Salt and drought conditions affect a wide variety of physiological and metabolic processes in plants, leading to growth reduction (Di Baccio et al., 2008; Uzilday et al., 2012). In the first phase, the initial growth reduction is due to the osmotic effect of salt outside the roots. Following this, salinity has other effects such as accumulation of ions, which causes a second phase of growth reduction (Munns, 2005). Plants growing naturally on saline soils have evolved various mechanisms to cope with the negative effects of ionic stress (Flowers et al., 1977; Munns, 2002; Parida et al., 2004; Turkan & Demiral, 2009; Çekiç et al., 2012). These mechanisms rely on controlled uptake, exclusion, compartmentalisation, and increased extrusion of salts (Flowers & Colmer, 2008).

Absence of water (drought) or inability to uptake water from medium (i.e. salinity) decreases stomatal conductance under drought and/or saline conditions. This decrease in gas exchange lowers the intrinsic CO_2 concentrations. Because of this water-conserving response, photosynthesis and many aspects of the plant metabolism are affected. Loss of coordination between different metabolic pathways causes electrons with high energy state to be accepted by molecular O_2 (Asada, 2006; Noctor et al., 2007). As a result, reactive oxygen species like singlet oxygen ($^1O_2$), hydrogen peroxide (H_2O_2),

* E-mail: hediye.sekmen@ege.edu.tr

super oxide (O_2^-), and hydroxyl ($OH^\bullet$) are produced in different compartments of the plant cell such as the chloroplast, mitochondria, and peroxisomes.

These effects caused by drought and salinity retard the yield of crops up to 50% (FAO, 2008). To overcome this issue, an understanding of the physiological, biochemical, and molecular responses of plants naturally tolerant to drought and salinity is important in order to engineer stress tolerant crops. One of the most important tolerance mechanisms to these stresses is known to be ROS scavenging capacity of the plant antioxidant system. This system includes an array of enzymatic and non-enzymatic antioxidants to scavenge ROS. Enzymatic antioxidants include superoxide dismutase (SOD), peroxidase (POX), catalase (CAT), ascorbate peroxidase (APX), and glutathione reductase (GR), whereas ascorbic acid, α-tocopherols, carotenoids, and flavonoids act as non-enzymatic antioxidants (Schafer et al., 2002). $O_2^{\bullet -}$ anion radical produced under normal conditions or under stress is scavenged by SOD and this reaction results in the formation of H_2O_2 and O_2. Following this, H_2O_2 is mainly detoxified by CAT (Shigeoka et al., 2002). In addition, APX and GR, members of the ascorbate-glutathione cycle, work together to eliminate H_2O_2 (Foyer & Noctor, 2005).

In our previous study, we investigated the antioxidant defence system responses of *Gypshophila oblanceolata* Bark., an endemic halophytic plant that occurs very abundantly in salt marshes of Salt Lake in the Central Anatolian part of Turkey (Hamzaoğlu & Aksoy, 2009). One of the other endemic species of the genus Gypsophila, *Gypsophila aucheri* Boiss., is a drought-tolerant xerophytic plant that is distributed in calcium rich-soils on arid rocky hillsides that are dry in summer. The aim of the present study was to *(i)* see how a xerophytic plant responds to salinity stress, *(ii)* investigate the possibility of *G. aucheri* being a xerohalophytic plant, and *(iii)* determine the differences in antioxidant defence system responses under drought and salinity. For this aim, SOD, CAT, POX, APX, and GR enzyme activities, isoenzyme activities of SOD and CAT, and lipid peroxidation were determined in *G. aucheri* under salinity (100 and 300 mM NaCl) and drought stresses. To the best of our knowledge, this is the first study investigating the antioxidant system responses of a xerophytic plant under salinity.

Material and methods

Plant material

Seeds of *G. aucheri* were provided by Haşim Altınözlü from Hacettepe University, Ankara, Turkey. Seeds were collected from the central-eastern part of Turkey. *G. aucheri* seeds were sterilised with 5% sodium hypochlorite for 10 min and were washed thoroughly with deionised water. They were germinated in petri dishes covered with 3 layers of filter paper at 24 °C. After germination, seedlings were transferred into pots containing perlite and grown under a constant temperature regime of 25 ± 1 °C for 16 h photoperiod at 70 ± 5% relative air humidity and at a photosynthetic photon flux density (PPFD) of 100 μmol m^{-2} s^{-1} light intensity in a controlled growth chamber. Plants were watered every other day with half-strength Hoagland's solution (Hoagland & Arnon, 1950).

Stress treatments

Sixty-day-old plants were used for stress treatments. Preliminary studies using various concentrations of NaCl (100, 200, 300, and 500 mM NaCl) showed that 100 and 300 mM NaCl were appropriate for determining the effects of mild and severe stresses. Salinity stress was applied to pots filled with perlite by addition of 100 and 300 mM NaCl to Hoagland's solution. The plants were subjected to drought stress by withholding water. Both types of stresses were applied for 2 weeks and then the plants were harvested. Samples were stored at –80 °C until further analysis.

Growth analysis

Six random plants for each group were used for the growth analyses. They were separated into shoot and root fractions on days 0 and 14 of stress treatment. Dry weights (DW)—after the samples were dried at 70 °C for 72 h—of shoots were measured to calculate the relative growth rate (RGR) of seedlings according to Hunt et al. (2002), as follows:

$$RGR = (\ln (DW_2) - \ln (DW_1)/(t_2 - t_1)$$

where DW_1 = dry mass (g) at time 1; DW_2 = dry mass (g) at time 2; and t_1 and t_2 = initial harvest time 1 and final harvest time 2 in days.

Enzyme extractions and assays

Extraction and electrophoretic separation procedures were performed at 4 °C. For protein and enzyme extractions, 0.5-g samples were ground to fine powder with liquid nitrogen and then homogenised in 1.25 mL of 50 mM Tris-HCl, pH 7.8, containing 0.1 mM ethylenediaminetetraacetic acid (EDTA), 0.2% (v/v) Triton-X100, 1 mM phenylmethylsulfonyl fluoride (PMSF), and 2 mM dithiothreitol (DTT). For APX activity determination, 5 mM ascorbate was added to the homogenisation buffer and PVPP (2% w/v) was used instead of DTT to prevent APX inhibition. Samples were centrifuged at 14,000 × *g* for 30 min, and supernatants were used for the determination of protein content and enzyme activities. Total soluble protein contents of the enzyme extracts were determined according to Bradford (1976) using bovine serum albumin as a standard. All spectrophotometric analyses were conducted on a Shimadzu (UV 1600) spectrophotometer.

SOD (EC 1.15.1.1) activity was assayed based on ability to inhibit photochemical reduction of nitrotetrazolium blue chloride (NBT) at 560 nm (Beauchamp & Fridovich, 1971). The assays were carried out at 25 °C and the reaction mixture (3 mL) contained 0.033 mM NBT, 10 mM L-methionine, 0.66 mM EDTA Na_2, and 0.0033 mM riboflavin in 50 mM Na-phosphate buffer (pH 7.8). Riboflavin was added last and the test tubes containing the reaction mixture were incubated for 10 min under 300 μmol m^{-2} s^{-1} irradiance. The reaction mixture with no enzyme developed maximum colour because of the maximum rate of reduction of NBT. A non-irradiated reaction mixture was used as the control as it did not develop colour. One unit of SOD was defined as the amount of enzyme that inhibits 50% NBT photoreduction.

CAT (EC 1.11.1.6) activity was estimated according to Bergmeyer (1970), measuring the initial rate of disappearance of H_2O_2 at 240 nm. The reaction mixture contained 50 mM Na-phosphate buffer (pH 7.0) with 0.1 mM EDTA and 3% H_2O_2. The decrease in the absorption was followed for 3 min and 1 mmol H_2O_2 mL^{-1} min^{-1} was defined as 1 unit of CAT.

POX (EC1.11.1.7) activity was based on the method described by Herzog and Fahimi (1973). The reaction mixture contained 3,3′-diaminobenzidine-tetra hydrochloride dihydrate solution (DAB) containing 0.1% (w/v) gelatine and 150 mM Na-phosphate-citrate buffer (pH 4.4) and 0.6% H_2O_2. The increase in absorbance at 465 nm was followed for 3 min. A unit of POX activity was defined as mmol H_2O_2 decomposed mL^{-1} min^{-1}.

APX (EC 1.11.1.11) activity was measured according to Nakano and Asada (1981). The assay depends on the decrease in absorbance at 290 nm as ascorbate is oxidised. The reaction mixture contained 50 mM K-phosphate buffer (pH 7.0), 0.5 mM ascorbate, 0.1 mM EDTA Na_2, 0.1 mM H_2O_2, and 0.1 mL of enzyme extract in a final assay volume of 1 mL. The concentration of oxidised ascorbate was calculated by using an extinction coefficient of 2.8 mM^{-1} cm^{-1}. One unit of APX was defined as 1 mmol mL^{-1} ascorbate oxidised min^{-1}.

GR (EC 1.6.4.2) activity was measured according to Foyer and Halliwell (1976). The assay medium contained 25 mM Na-phosphate buffer (pH 7.8), 0.5 mM GSSG, and 0.12 mM NADPH Na_4, and 0.1 mL enzyme extract in a final assay volume of 1 mL. NADPH oxidation was followed at 340 nm. Activity was calculated using the extinction coefficient of NADPH (6.2 mM^{-1} cm^{-1}). One unit of GR was defined as 1 mmol mL^{-1} GSSG reduced min^{-1}.

The specific enzyme activity for all enzymes was expressed as in unit mg^{-1} protein.

Identification of isoenzymes

Samples containing equal amounts of protein were subjected to non-denaturing polyacrylamide gel electrophoresis (PAGE) as described by Laemmli (1970) except that sodium dodecyl sulphate was omitted.

For the separation of SOD isoenzymes, 4.5% stacking and 12.5% separating gels under constant current (30 mA) were used. SOD activity was detected by photochemical staining with riboflavin and NBT as described by Beauchamp and Fridovich (1973). The different types of SOD were determined by incubating gels in inhibitors of SOD before staining, such as 2 mM KCN to inhibit CuZn SOD activity and 3 mM H_2O_2 to inhibit CuZn SOD and Fe SOD activities as described by Vitória et al. (2001) (Mn SOD activity is resistant to both inhibitor treatments).

CAT isoforms were detected according to Woodbury et al. (1971). The electrophoretic separation was performed on non-denaturating polyacrylamide mini gels using 10% separating gel under constant current (30 mA). The gels were incubated in 0.01% H_2O_2 for 5 min. After incubation the gels were washed with distilled water twice and incubated for 20 min in staining solution containing 1% $FeCl_3$ and 1% $K_3Fe(CN_6)$.

Gels stained for SOD and CAT activities were photographed with a Vilber Lourmat gel imaging system and then analysed with a Bio-Profil Bio-1D (Vilber Lourmat, Marne la Vallée, France). In densitometric analyses of SOD and CAT activities, activities of control plants were taken as 100% and % of control values for each treatment are shown. The values are the average of data from 3 independent gels. Within each isoenzyme, means with the same letter are not significantly different at $P < 0.05$ according to Tukey's post-test.

Lipid peroxidation

The level of lipid peroxidation was determined in terms of thiobarbutiric acid reactive substances (TBARS) content according to the method described by Madhava Rao and Sresty (2000). TBARS concentration was calculated from the absorbance at 532 nm and measurements were corrected for non-specific turbidity by subtracting the absorbance at 600 nm. The concentration of TBARS was calculated using an extinction coefficient of 155 mM^{-1} cm^{-1}.

Statistical analyses

The experiment was conducted in a completely randomised design and measurements were performed with 6 replicates (n = 6). Statistical variance analysis of the data was performed using ANOVA and differences among treatments were compared using Tukey's post-test with least significant differences at the 5% level.

Results

Effects of salt and drought stress on relative growth rate (RGR)

RGR of 100 mM NaCl- and drought-treated plants was not affected by stress as compared to the control groups, while 300 mM NaCl decreased the RGR by 16.11% (Figure 1).

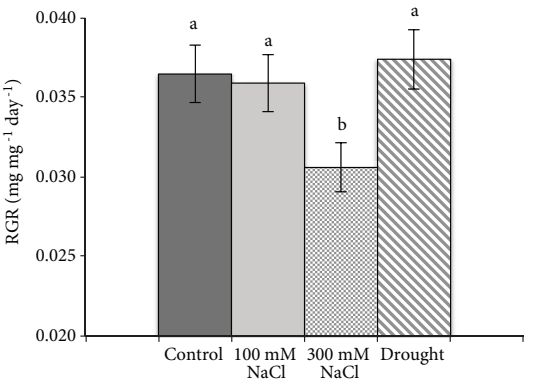


Figure 1. Effects of salinity and drought stress on relative growth rate of *G. aucheri*. Vertical bars indicate ±SE and different letters represent significantly different ($P < 0.05$) values.

Effects of salt and drought stress on the total activities of antioxidant enzymes

SOD activity was increased under salt and drought stress (Figure 2A). At 100 and 300 mM NaCl, SOD activity was increased by 42% and 67.73%, respectively. Moreover, similar to the induction of SOD under 300 mM NaCl stress, drought stress also enhanced SOD activity, by 69.34%.

Catalase activity of 100 mM NaCl-treated plants was increased by 75.56% as compared to the control group, while no significant change was observed in 300 mM NaCl-treated plants. Drought enhanced the catalase activity by 61.1%, as compared to control plants (Figure 2B).

POX activity of *Gypshophila aucheri* was induced significantly by both stress treatments (Figure 2C). Treatment with 100 mM and 300 mM NaCl increased the activity of POX by 25.26% and 26.31%, respectively, whereas activity of POX increased by 16.84% under drought stress.

Salt and drought stress enhanced APX activity of *G. aucheri* (Figure 2D). The highest APX activity (3.34-fold) was observed under drought stress, as compared to the control. Moreover, its activity was increased by 104% and 74.46% in 100 mM and 300 mM NaCl-treated plants, respectively.

GR activity was increased 2.1-fold under drought stress, as compared to the control. Moreover, 100 mM NaCl stress also enhanced (2.50-fold) its activity,

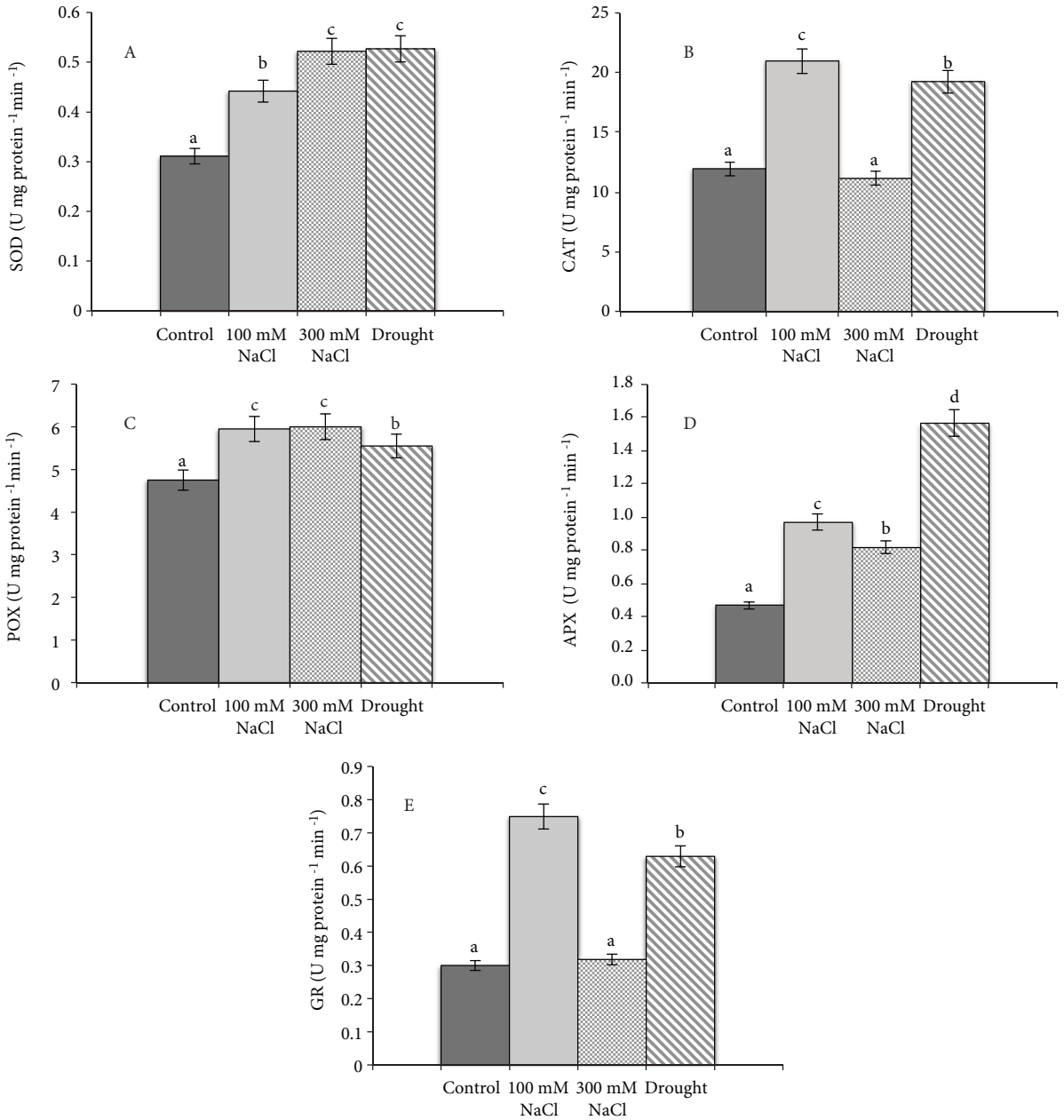
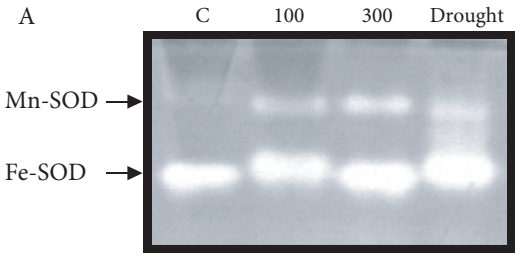


Figure 2. The effects of salinity and drought stress on SOD (a), CAT (b), POX (c), APX (d), GR (e) in seedlings of *Gypshophila aucheri*. Vertical bars indicate ±SE and different letters represent significantly different ($P < 0.05$) values.

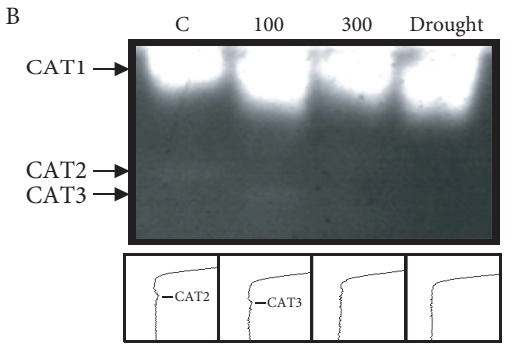
while no change was observed in GR activity of 300 mM NaCl treated-plants (Figure 2E).

Effects of salt and drought stress on the activities of SOD and CAT isoenzymes

As shown in Figure 3A, SOD isoenzymes were identified as 1 Mn SOD (not inhibited by KCN or H_2O_2) and 1 Fe SOD (not inhibited by KCN, but inhibited by H_2O_2). Only 1 band (Fe SOD) was determined in control plants. However, in stress-treated plants, both Fe SOD and Mn SOD isoenzymes were determined. Fe SOD had the highest SOD isoenzyme activity under both conditions. Moreover, intensities of Mn SOD were increased by 42.69% and 48.13% under 300 mM NaCl and drought stress, respectively.



	C	100	300	Drought
Mn-SOD	ND	100	142.69 ± 8.4	148.13 ±10.2
Fe-SOD	100	113.9 ± 4.34	127.8± 4.3	127.8 ± 4.1



	C	100	300	Drought
CAT1	100	142.23 ± 1.2	127.6 ± 1.07	159.84 ± 1.26
CAT2	100	ND	ND	ND
CAT3	ND	100	ND	ND

Figure 3. Activity staining and % induction of SOD and CAT isoenzymes in the crude extract of seedlings of *Gypshophila aucheri*. Samples applied to the gels contained 20 µg of protein. In densitometric analyses of SOD and CAT activities, activities of control plants were taken as 100% and % of control values for each treatment are shown. The values are the average of data from 3 independent gels.

As shown in Figure 3B, 3 different catalase isoenzymes (CAT1, CAT2, CAT3) were determined in *G. aucheri*. CAT1 was seen in all treatment groups. However, CAT2 isoenzyme was only determined in control plants, while CAT3 activity was only detected in 100 mM NaCl-treated plants. CAT1 activity was increased following stress treatments. Moreover, 100 mM and 300 mM NaCl enhanced CAT1 activity by 42.23% and 27.6%, respectively. Drought treatment increased CAT1 by 59.8% as compared to control plants.

Effects of salt and drought stress on lipid peroxidation

To determine the level of damage caused by oxidative stress to cellular membranes, lipid peroxidation was measured by determining the thiobarbituric acid reactive substances (TBARS). The TBARS levels of 100 and 300 mM NaCl treated-plants increased by 16.3% and 54.04%, respectively, as compared to control plants. However, a slight, but significant, increase (9.6%) in drought-treated plants was observed as compared to control plants (Figure 4).

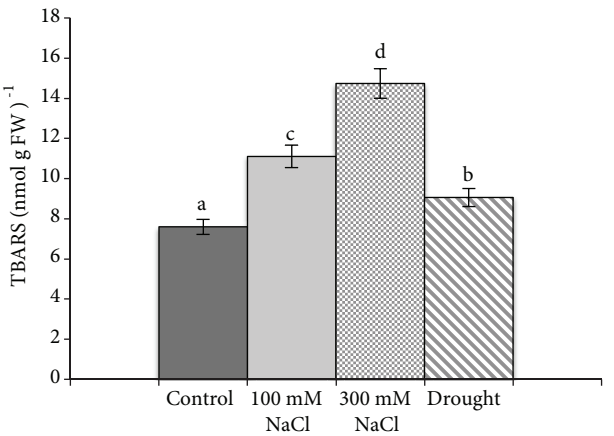


Figure 4. The effects of salinity and drought stress on lipid peroxidation (TBARS content) in seedlings of *Gypshophila aucheri*. Vertical bars indicate ±SE and different letters represent significantly different ($P < 0.05$) values.

Discussion

Similar to some halophytes such as *Gypsophila oblanceolata* (Sekmen et al., 2012), *Crithmum maritimum* (Hamed et al., 2007), and *Plantago maritima* (Sekmen et al., 2007), the RGR of *Gypsophila aucheri* was not stimulated or inhibited by 100 mM NaCl. However, 300 mM NaCl caused a significant decrease in the RGR of *G. aucheri* (Figure 1). This reduction in growth might be due to osmotic effects or ionic imbalances resulting from nutritional deficiency or excess ions as reported by Greenway and Munns (1980). On the other hand, like 100 mM NaCl, drought also did not affect growth of *G. aucheri* as was evident from the unchanging RGR of seedlings.

Parallel to our results, RGR of *Cleome gynandra* was not affected by 10 days of water withholding (Uzilday et al., 2012). In contrast, Hassine et al. (2008) and Ozkur et al. (2009) found inhibition in growth of drought-tolerant *Atriplex halimus* and *Capparis ovata* under stress. However, growth of *Phaseolus acutifolius* was stimulated under 10% polyethylene glycol-induced drought stress (Turkan et al., 2005).

SOD scavenges $O_2^{\cdot-}$ to H_2O_2 and plays a role in the antioxidant system as the first line of defence (Mittler, 2002). This detoxification mechanism is especially important in chloroplasts and mitochondria, where $O_2^{\cdot-}$ is produced by metabolic imbalance under stress conditions (Asada, 2006; Noctor et al., 2007). Three types of SOD can be found in plants according to their metal cofactors: Mn SOD, CuZn SOD, and Fe SOD. Changes in the pattern, composition, and regulation of these isoenzymes are well-known adaptation mechanisms to oxidative stress, as presented by previous studies (Perez-Lopez et al., 2009; Tan et al., 2010; Sekmen et al., 2012). In the present study, SOD activities of all treatment groups were significantly increased by salt and drought stress (Figure 2A). Similarly, Ozkur et al. (2009), Tan et al. (2010), and Tounekti et al. (2011) also observed significant increases in SOD activities of drought-tolerant caper and maize and moderate-halophyte rosemary plants.

CAT is indispensable for the plant's antioxidant defence system to detoxify photorespiratory H_2O_2. If considered in terms of mass flow photorespiration, it is thought to be the second most important process after photosynthesis itself (Bauwe et al., 2010). Therefore, scavenging of this H_2O_2 is vital for maintenance of cellular redox state. In previous studies, CAT activity under stress was found to be higher in salt- and drought-tolerant plant species such as drought-tolerant *Capparis ovata* (Ozkur et al., 2009) and salt-tolerant *Centaurea tuzgoluensis* (Yıldıztugay et al., 2011), providing protection against the oxidative stress. In our study, CAT activity was increased in *G. aucheri* plants under 100 mM NaCl and drought stress (Figure 2B). Similarly, in our previous study, we observed that salt stress (100 mM NaCl) induced CAT activity in the moderate-halophyte *G. oblanceolata* (Sekmen et al., 2012). These results indicated that H_2O_2 is scavenged effectively by induced levels of CAT in *G. aucheri* plants under the same conditions. On the other hand, at 300 mM NaCl, CAT activity was neither increased nor decreased, as compared to control plants. Moreover, unchanged CAT activity was not compensated for by induction of other H_2O_2 scavenging enzymes (POX and APX) at 300 mM NaCl (Figure 2C and 2D).

POXs are important scavengers of H_2O_2. Under stress conditions, with increased production of ROS, induction of POX activity is reported in stress-tolerant plants like *Capparis ovata* (Ozkur et al., 2009) and *Centaurea tuzgoluensis* (Yıldıztugay et al., 2011). In the present study, salt and drought treatments significantly induced POX activities of *G. aucheri*. While there is no information about responses of xerophytic plants to salt stress, it enhanced the POX activity of xerophytic *G. aucheri* as compared to drought stress (Figure 2C).

APX works in coordination with GR to scavenge H_2O_2 in the Asada-Halliwell pathway (Mittler, 2002). In contrast to CAT, affinity of APX towards H_2O_2 is very high and this attribute makes it an important regulator of H_2O_2 in plant cells. Many studies report increases in APX activity under salinity like in *Sueda salsa*, *Cakile maritima*, and *Centaurea tuzgoluensis* (Cai-Hong et al., 2005; Amor et al., 2006; Yıldıztugay et al., 2011). In addition, APX activity is also induced by drought in *Capparis ovata*, a xerohalophyte (Ozkur et al., 2009). In accordance with these results, in the present study, APX activity of stress-treated groups was distinctively induced (Figure 2D).

Oxidised glutathione is reduced by GR via a NADPH-dependent process (Foyer & Halliwell, 1976). Moreover, GR activity is induced by salinity stress in halophytes (Amor et al., 2006; Yıldıztugay et al., 2011). In the present study, 100 mM NaCl and drought significantly increased the GR activity in *G. aucheri*, similar to the halophyte *Cakila maritima* (Amor et al., 2006) and xerophyte *Capparis ovata* (Ozkur et al., 2009), whereas 300 mM NaCl had no impact on GR activity (Figure 2E). Therefore, the activity of the ascorbate-glutathione cycle cannot prevent 300 mM NaCl-induced oxidative stress in *G. aucheri* due to insufficient induction of APX and unaltered activity of GR.

In the present study, activities of Mn SOD and Fe SOD isoenzymes showed concomitant increases with total SOD activity under stressed conditions

(Figure 3A). These changes in isoenzyme patterns might indicate increased production of $O_2^{\cdot-}$ in the mitochondria and chloroplast of *G. aucheri* under drought and salinity stress. Therefore, efficient scavenging of $O_2^{\cdot-}$ produced in these organelles might be important for tolerating drought and salinity. On the other hand, this study showed that SOD isoenzyme patterns of *G. aucheri* are different from those of the moderate-halophyte *G. oblanceolata* under control conditions. In *G. oblanceolata*, 2 Mn SOD, 2 Fe SOD, and one CuZn SOD isoenzyme were detected (Sekmen et al., 2012), while only Fe SOD was determined in *G. aucheri* under the same condition. However, under salt stress, similar to *G. oblanceolata*, we found that Mn SOD and Fe SOD play a major role against salt-induced oxidative stress in *G. aucheri.*

Differences in the isoenzymatic pattern of CAT were detected in *G. aucheri* under salt and drought stress. While CAT1 was determined in all treatment groups and was enhanced by both stresses, CAT2 was determined only in the control group and was inhibited by drought and salinity (Figure 3B). Similar to our findings, salt stress inhibited CAT1 isoenzyme in *Bruguiera parviflora* (Parida et al., 2004). On the other hand, a novel isoenzyme, CAT3, was observed under 100 mM NaCl stress, suggesting that this isoenzyme is important under salt stress. In our previous study, in contrast to *G. aucheri*, no differentiation in CAT isoenzyme pattern was observed in *G. oblanceolata* (Sekmen et al., 2012)

Lipid peroxidation is a marker of stress-induced damage (Hernandez & Almansa, 2002). Salt treatments increased the TBARS levels consistently with enhanced salinity concentrations, while drought stress slightly affected the TBARS levels of xerophytic *G. aucheri* as compared to salt treatments. The highest TBARS levels were seen in 300 mM salt-treated plants, which can be explained by unchanged activities of CAT and GR activities. The tolerance of *G. aucheri* to drought and 100 mM NaCl stress correlate with a limited extent of oxidative damage as a result of increased activities of antioxidants (SOD, CAT, POX, APX and GR), preventing toxic accumulation of ROS (Figure 4).

Conclusion

(i) The present study shows an antioxidant response of *G. aucheri* to moderate (100 mM), severe NaCl (300 mM), and drought stress. Similar trends in the activities of antioxidant enzymes indicated that *G. aucheri* reacted similarly to moderate NaCl and drought stress. (ii) As compared to 100 mM NaCl and drought stress, 300 mM NaCl led to higher oxidative stress as indicated by increased lipid peroxidation. In addition, 300 mM NaCl-induced oxidative damage cannot be precluded in spite of increased SOD, POX, and APX activities. (iii) These results suggested that the xerophyte *G. aucheri* is a moderately salt tolerant species that presents an efficient antioxidant defence mechanism like *Rosmarinus officinalis* (Tounekti et al., 2011), *G. oblanceolata* (Sekmen et al., 2012), and *C. tuzgoluensis* (Yıldıztugay et al., 2011).

Acknowledgements

This work was supported by Ege University Research Foundation [2010-FEN 067]. The authors wish to thank Haşim Altınözlü for providing the *G. aucheri* seeds.

References

Amor NB, Jimenez A, Megdiche W, Lundqvist M, Sevilla F & Abdelly C (2006). Response of antioxidant systems to NaCl stress in the halophyte *Cakile maritima*. *Physiologia Plantarum* 126: 446-457.

Asada K (2006). Production and scavenging of reactive oxygen species in chloroplasts and their functions. *Plant Physiology* 141: 391-396.

Bauwe H, Hagemann M & Fernie AR (2010). Photorespiration: players, partners and origin. *Trends in Plant Science* 15: 330-336.

Bergmeyer N (1970). Methoden der enzymatischen Analyse, Vol. 1. Akademie Verlag, Berlin.

Beauchamp C & Fridovich I (1971). Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. *Analytical Biochemistry* 44: 276-287.

Beauchamp C & Fridovich I (1973). Isoenzymes of superoxide dismutase from wheat germ. *Biochimica et Biophysica Acta* 317: 50-64.

Bradford MM (1976). A rapid and sensitive method for the quantization of microgram quantities of protein utilizing the principle of the protein-dye binding. *Analytical Biochemistry* 72: 248-254.

Cai-Hong P, Su-Jun Z, Zhi-Zhong G & Bao-Shan W (2005). NaCl treatment markedly enhances H_2O_2-scavenging system in leaves of halophyte *Suaeda salsa. Physiologia Plantarum* 125: 490–499.

Çekiç FÖ, Ünyayar F & Ortaş İ (2012). Effects of arbuscular mycorrhizal inoculation on biochemical parameters in *Capsicum annuum* grown under long term salt stress. *Turkish Journal of Botany* 36: 63-72.

Di Baccio D, Navari-Izzo F & Izzo R (2004). Seawater irrigation: antioxidant defence responses in leaves and roots of a sunflower (*Helianthus annuus* L.) ecotype. *Journal of Plant Physiology* 161: 1359-1366.

Flowers TJ & Colmer TD (2008). Salinity tolerance in halophytes. *New Phytologist* 179: 945-963.

Flowers TJ, Troke PF & Yeo AR (1977). The mechanism of salt tolerance in halophytes. *Annual Review of Plant Physiology* 28: 89-121.

Food and Agriculture Organization (FAO) (2008). Land and Plant Nutrition Management Service, Available at http://www.fao.org/ag/ag/agll/spush.

Foyer CH & Halliwell B (1976). The presence of glutathione and glutathione reductase in chloroplasts: a proposed role in ascorbic acid metabolism. *Planta* 133: 21-25.

Foyer CH & Noctor G (2005). Oxidant and antioxidant signalling in plants: a re-evaluation of the concept of oxidative stress in a physiological context. *Plant Cell and Environment* 28: 1056-1071.

Greenway H & Munns R (1980). Mechanisms of salt tolerance in nonhalophytes. *Annual Review of Plant Biology* 31: 149-190.

Hamed KB, Castagna A, Salem E, Ranieri A & Abdelly C (2007). Sea fennel (*Crithmum maritimum* L.) under salinity conditions: a comparison of leaf and root antioxidant responses. *Plant Growth Regulation* 53: 185-194.

Hamzaoğlu E & Aksoy A (2009). Phytosociological studies on the halophytic communities of Central Anatolia. *Ekoloji* 18: 1-14.

Hassine AB, Ghanem ME, Bouzid S & Lutts S (2008). An inland and a coastal population of the Mediterranean xero-halophyte species *Atriplex halimus* L. differ in their ability to accumulate proline and glycinebetaine in response to salinity and water stress. *Journal of Experimental Botany* 59: 1315-1326.

Hernández JA & Almansa MS (2002). Short term effects of salt stress on antioxidant systems and leaf water relations of pea leaves. *Physiologia Plantarum* 115: 251-257.

Herzog V & Fahimi H (1973). Determination of the activity of peroxidase. *Analytical Biochemistry* 55: 554-562.

Hoagland DR & Arnon DI (1950). The water culture method for growing plants without soil. *California Agricultural Experiment Station Circular* 347: 1-32.

Hunt R, Causton DR, Shipley B & Askew AP (2002). A modern tool for classical plant frowth analysis. *Annals of Botany* 90: 485-488.

Laemmli UK (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 227: 680-685.

Madhava Rao KV & Sresty TVS (2000). Antioxidative parameters in the seedlings of pigeon pea (*Cajanus cajan* L. Millspaugh) in response to Zn and Ni stresses. *Plant Science* 157: 113-128.

Mittler R (2002). Oxidative stress, antioxidants and stress tolerance. *Trends in Plant Science* 7: 405-10.

Munns R (2002). Comparative physiology of salt and water stress. *Plant Cell and Environment* 25: 239-250.

Munns R (2005). Genes and salt tolerance: bringing them together. *New Phytologist* 167: 645-663.

Nakano Y & Asada K (1981). Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. *Plant and Cell Physiology* 22: 867-880.

Noctor G, Paepe RD & Foyer CH (2007). Mitochondrial redox biology and homeostasis in plants. *Trends in Plant Science* 12: 125-134.

Ozkur O, Ozdemir F, Bor M & Turkan I (2009). Physiochemical and antioxidant responses of the perennial xerophyte *Capparis ovata* Desf. to drought. *Environmental and Experimental Botany* 66: 487-492.

Parida AK, Das AB & Mohanty P (2004). Defense potentials to NaCl in a mangrove *Brugieara parviflora*: Differential changes of isoforms of some antioxidant enzymes. *Journal of Plant Physiology* 161: 531-542.

Pérez-López U, Robredo A, Lacuesta M, Sgherri C, Muñoz-Rueda A, Navari-Izzo F & Mena-Petite A (2009). The oxidative stress caused by salinity in two barley cultivars is mitigated by elevated CO2. *Physiologia Plantarum* 135: 29-42.

Schafer FQ, Wang HP, Kelley EE, Cueno KL, Martin SM & Buettner GR (2002). Comparing β-carotene, vitamin E and nitric oxide as membrane antioxidants. *Biological Chemistry* 383: 671-681.

Sekmen AH, Turkan I & Takio S (2007). Differential responses of antioxidative enzymes and lipid peroxidation to salt stress in salt-tolerant *Plantogo maritima* and salt-sensitive *Plantago media. Physiologia Plantarum* 131: 399-411.

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. *Environmental and Experimental Botany* 77: 63-76.

Shigeoka S, Ishikawa T, Tamoi M, Miyagawa Y, Takeda T, Yabuta Y & Yoshimura K (2002). Regulation and function of ascorbate peroxidase isoenzymes. *Journal of Experimental Botany* 53: 1305-1319.

Tan YF, O'Toole N, Taylor NL & Millar AH (2010). Divalent metal ions in plant mitochondria and their role in interactions with proteins and oxidative stress-induced damage to respiratory function. *Plant Physiology* 152: 747-761.

Tounekti T, Vadel AM, Ennajeh M, Khemira H & Munné-Bosch S (2011). Ionic interactions and salinity affect monoterpene and phenolic diterpene composition in rosemary (*Rosmarinus officinalis*). *Journal of Plant Nutrition and Soil Science* 174: 504-514.

Turkan I, Bor M, Ozdemir F & Koca H (2005). Differential responses of lipid peroxidation and antioxidants in the leaves of drought-tolerant *P. acutifolius* Gray and drought-sensitive *P. vulgaris* L. subjected to polyethylene glycol mediated water stress. *Plant Science* 168: 223-231.

Turkan I & Demiral T (2009). Recent developments in understanding salinity tolerance. *Environmental and Experimental Botany* 67: 2-9.

Uzilday B, Turkan I, Sekmen AH, Ozgur R & Karakaya HC (2012). Comparison of ROS formation and antioxidant enzymes in *Cleome gynandra* (C4) and *Cleome spinosa* (C3) under drought stress. *Plant Science* 182: 59-70.

Vitória AP, Lea PJ, Azevedo RA (2001). Antioxidant enzymes responses to cadmium in radish tissues. *Phytochemistry* 57: 701-710.

Woodbury W, Spencer AK & Stahman MA (1971). An improved procedure for using ferricyanide for detecting catalase isozymes. *Analytical Biochemistry* 44: 301-305.

Yıldıztugay E, Sekmen AH, Turkan I & Kucukoduk M (2011). Elucidation of physiological and biochemical mechanisms of an endemic halophyte *Centaurea tuzgoluensis* under salt stress. *Plant Physiology and Biochemistry* 49: 816-824.