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THE EFFECTS OF NITRIC OXIDE ON SOME ANTIOXIDANT ENZYME ACTIVITIES UNDER SALT STRESS IN SUNFLOWER PLANTS

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ABSTRACT

The effects of externally applied sodium nitroprusside on superoxide dismutase (SOD), glutathione peroxidase (GPx), ascorbate peroxidase (APX), and catalase (CAT) antioxidant enzyme activities, nitric oxide (NO) levels have been investigated in salt stress resistant and sensitive sunflower plants. NaCl treatments and SNP treatments simultaneously with salt application (NaCl+SNP) were performed. SOD, GPx, APX and CAT antioxidant enzyme activities and NO levels showed differences in leaf tissues treated with 100 µM SNP, different concentrations of NaCl, and NaCl+SNP. SOD, GPx and APX enzyme activities were generally increased in sensitive variety, but decreased in tolerant variety. However, while general increase in CAT enzyme activity was determined in tolerant type, a reduction was established in sensitive type. An increase was determined in both types in NO levels. It is evident from these results that administration of NO donor SNP can cope with reactive oxygen species in both varieties. This study indicates that negative effects of salt stress on different sun flower varieties can be recovered by nitric oxide.

Key words: salt stress, *Helianthus annuus* L., antioxidant enzymes, nitric oxide, SNP

INTRODUCTION

Salinity is an environmental stress agent that causes various problems such as osmotic stress in plants, ion toxicity, imbalance in mineral intake, resulting in reduction of growth phenomenon to the death of plant. Approximately 6% of the land area is affected by salinity [Flowers et al. 1977]. Considering that the agricultural land in the world is limited and the need for nutrients has increased exponentially, it is of the utmost importance that the existing land should be used more efficiently, at least for the saline soils to be treated and evaluated economically [Miller and Woods 1996]. Nitric oxide is a very important signaling molecule in plants. NO is considered responsible for regulating the various growth and development processes of plants. Nitric oxide is important in plant growth and development, which is involved in seed germination, primer and side root growth, flowering, regulation of pollen tube growth, fruit ripening, aging, defense response and abiotic stress, as well as being an important signaling molecule in intracellular different processes in high plants [Corpas et al. 2011]. Initial studies on nitric oxide have been performed in plants in park and factory areas, plant species in selected forests and toxic effects observed in photosynthetic apparatus and chlorophyll.

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However, beneficial effects of NO observed and researchers have reported that exogenously administered NO stimulates salt tolerance, accelerating photosynthesis and carbohydrate metabolism, leading to breakdown of dormancy or increased germination.

It involves a large reaction step [Liu et al. 2014] involving NO protein and non-protein thiols and superoxide anion (O_2^-), which are synthesized endogenously or taken up from an external source into the cell. In order to control the level of reactive oxygen species, the enzymes such as superoxide dismutase, catalase, glutathione peroxidase, ascorbate peroxidase, dehydroascorbate reductase, glutathione reductase and antioxidant defense systems with nonenzymatic components such as ascorbate, carotenoids, oxidized glutathione (GSH) and reduced glutathion (GSSG). Accumulation of the ROS resulting from various environmental stresses is an important cause of loss of product yield in the world. ROS affect a large number of cellular functions leading to damaged nucleic acids, oxidized protein and lipid products. Whether or not reactive oxygen species are harmful, protective or signaling factors depends on the precise balance between the production and the removing. Stress-induced ROS accumulation has been reported to be eliminated by enzymatic antioxidant systems such as the ROS scavenger SOD, APX, GPX, GST and CAT [Gill and Tuteja 2013].

Accordingly, the regulation of these antioxidant components by exogenous substance application can mediate the formation of salt stress tolerance [Boogar et al. 2014]. The increase of the salinity causes the irrigable agricultural areas to decrease gradually. For this reason, it is important to work on the determination of agricultural varieties which can grow under abiotic stress conditions that, less irrigated and relatively saline areas. In the present study, it was aimed to determine the changes of some biochemical parameters in sunflower plants under salt stress and compare the findings among the tolerant and resistant varieties. To gain insight into the mechanism underlying salt stress on agicultural important plants, changes in antioxidant enzyme activities of SOD, APX, GPx and CAT, and endogenous NO levels in sensitive and tolerant leaves of sunflower varieties in response to NaCl treatment were investigated in the present study. Also, this work designated to investigate whether exogenous sodium nitroprusside (SNP), -nitric oxide donor- may alleviate oxidative damage induced by salt stress in salt tolerant and sensitive sun flower plants and investigate the effects of SNP on some antioxidant enzyme activities in sunflower cultivars in abiotic stress tolerance.

MATERIAL AND METHOD

Seeds used as experimental material in this study were obtained from Aegean Agricultural Research Institute and Trakya Agricultural Research Institute. The 2517-A and 6535A seeds were selected from 22 varieties (Armada, Sanay, Sanbro, Confeta, Oliva, Palanci, Aegean, Tanay, Turay, Reyna, Pactol, TM-4, DKF2525, PR64H34, L65400H0, A-6626, A-6388, A-2453, A-9178, A-9661, A-2517, A-6535) of sunflower seeds according to the percentage of germination against various salt concentrations in the germination pots. The A-6535 variety is tolerant and the A-2517 variety is sensitive. Seeds were sterilized by washing in 0.5% sodium hypochlorite solution for 5 minutes and then 4–5 times in sterile dH_2O. After sunflower seeds are left overnight in distilled water, 20 seeds are planted in plastic pots containing perlite. The seeds were grown in a climate chamber with 12 hours dark/light, 25°C temperature and 60% humidity, and watered for 30 days at a 3 day interval with a ½ diluted Hoagland solution. On the 25th day of the growth; 200 mM NaCl, 400 mM NaCl and 100 µM, sodium nitroprusside (SNP), 100 µM SNP + 100 mM NaCl, 100 µM SNP + 200 mM NaCl and 100 µM SNP + 400 mM NaCl were applied in the Hoagland solution of both sunflower varieties. After application, leaf samples were taken in three replicates, two days intervals, on day 2 and day 4. Control group leaf samples were taken from plants that were not treated with SNP and/or NaCl.

Determination for the superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT) activities in leaves, Cayman Chem. kits (Catalog No 706002, 703102, 707002 respectively) were used and analysis for ascorbate peroxidase (APX) activity was used by MyBiosource APX elisa kit (Catalog No MBS2602897). Leaf tissue samples freshly weighed and 0.5 g sample were homogenized in pH 7.2, 50 mM Tris-HCl buffer and centrifuged at 10.000 g, for 15 minutes at 4°C (OLE DICH Instrument Makers APS Microcentrifuge 157 MP). Spectrophotomet-

ric measurement of enzyme activities were performed according to the kit protocol. The absorbance values of the standard and samples in the prepared microplate were measured in microplate reader (Bio-Tek, Epoch Microplate Spectrophotometer, US). Nitric oxide level was determined by Cayman Chem. NO measurement kit (Catalog No. 780001). Experimental steps were regulated according to the kit protocol and nitrite and nitrate standards were determined for each plant separately. Freshly weighed 0.5 g plant leaves were homogenized in pH 7.2, 200 mM phosphate buffer and centrifuged at 10,000 g for 10 minutes at 4°C.

Standard and extract of leaf tissue absorbances were determined spectrophotometrically with 540 absorbance spectra in a microplate reader (Bio-Tek, Epoch Microplate Spectrophotometer, USA) by adding the reagents identified in the protocol to the separate microplates for nitrite and nitrate. The amount of nitric oxide was determined by the sum of nitrite and nitrate concentrations determined for each sample. All experimental analyses were made in three biological replications under same conditions. A comparative analysis of variance was performed between the control group and the experimental group. Stastical analyses of the data were performed by using SPSS 15.0 software program. All measurements were subjected to analysis of variance (ANOVA) to discriminate significant differences (set as $P \leq 0.05$). Significant differences among treatments were calculated by Mann Whitney U test ($P = 0.05$). The data were shown as mean ±SD. Each group was compared with its own control group.

RESULTS

SOD enzyme activities. The activities of SOD enzyme had increased in NaCl (100, 200, and 400 mM) treated in leaf tissue of A-2517 cultivar compared to control group. Under stress conditions SOD enzyme activity increased from 100 mM to 400 mM and the highest enzyme activity was 18.38 ±0.96 U/ml (Tab. 1). Also, SOD enzyme activities had increased in all NaCl + SNP treatments in leaf tissue of A-2517 cultivar compared to control group. SOD enzyme activities observed in NaCl groups were higher on both 2nd and 4th day than NaCl+SNP application groups (Tab. 1). The highest SOD enzyme activity was 15.10 ±0.25 U/ml and 13.40 ±0.39 U/ml on the 2nd and 4th days of 400 mM NaCl + SNP treatment, respectively.

In leaf tissue of A-6535 cultivar, there was a decrease in SOD enzyme activities in 100 mM and 200 mM NaCl treatment groups on the 2nd day compared to control; whereas, an increase was determined in 400 mM NaCl treatment. As is seen in Table 1, the highest SOD enzyme activity was 14.18 ±0.99 U/ml in 400 mM NaCl treatment.

SOD activities in the leaves of A-6335 cultivar showed a decrease, at all NaCl+SNP treatment as compared with control. The highest SOD enzyme activity was 12.52 ±0.52 U/ml in 400 mM NaCl + SNP treatment on the 4th day. The most significant decrease occurred between SOD enzyme activities of 400 mM NaCl and 200 mM NaCl + SNP treatments on the 2nd day and these activities were 14.18 ±0.99 U/ml and 9.56 ±0.69 U/ml, respectively. SOD enzyme activity decreased on the 2nd and 4th days of SNP treatment compared to control group.

GPx enzyme activities. Glutathione peroxidase (GPx) activities in leaves of A-2517 cultivar were determined to increase on the 4th day of NaCl, NaCl + SNP and SNP treatments compared to control group (Tab. 2). The highest GPx enzyme activities (19.59 ±0.58 U/ml, 22.69 ±0.56 U/ml, 23.54 ±2.52 U/ml) in NaCl, NaCl + SNP and SNP treatment were determined on the 4th day of 100 mM NaCl, 400 mM NaCl+SNP and 100 µM SNP concentration respectively. GPx activity showed significant change at 400 mM NaCl + SNP in the leaf compared to control. Approximately 3 times of increase was determined in GPx enzyme activities at all concentrations on the 4th day of NaCl+SNP treatment compared to control group. GPx enzyme activities of SNP treatment on the 2nd and 4th days were found to be higher compared to all treatment groups. 100 mM NaCl treatment increased GPx activity in the leaves on day 2, while there was a increase in GPx activity at 100 mM+SNP in A-6535 cultivar on day 4 (Tab. 2). The highest GPx enzyme activity was determined on the 2nd day of 100 mM NaCl treatment (24.20 ±0.58 U/ml). While GPx activity increased on the 2nd day of 200 mM NaCl + SNP treatment compared to control group. The highest GPx enzyme activity was observed on the 4th day of 100 mM NaCl+SNP treatment (24.48 ±0.51 U/ml). GPx enzyme activities decreased in SNP treatments on the 2nd and 4th days compared to control group (Tab. 2).

Table 1. SOD activity (U/ml) on 2nd and 4th day of leaf tissues in sunflower varieties. Data are expressed as mean ±SE of three replicates

Groups	2nd day	4th day	2nd day	4th day
	A-2517	A-2517	A-6535	A-6535
Control	9.83 ±0.70[a]	8.82 ±0.89[a]	12.24 ±1.12[a]	13.45 ±0.73[a]
100 mM NaCl	12.19 ±0.70[b]	11.06 ±1.18[b]	11.54 ±1.06[a]	11.35 ±0.49[b]
200 mM NaCl	12.99 ±1.15[b]	12.75 ±0.61[c]	11.12 ±0.61[a]	12.91 ±0.90[a]
400 mM NaCl	18.38 ±0.96[d]	13.55 ±0.67[c]	14.18 ±0.99[b]	13.45 ±0.93[a]
100 mM NaCl + SNP	10.81 ±0.63[a]	11.01 ±0.58[b]	11.38 ±0.54[a]	10.84 ±0.22[b]
200 mM NaCl + SNP	12.56 ±1.17[b]	11.11 ±0.59[b]	9.56 ±0.69[b]	9.35 ±0.27[c]
400 mM NaCl + SNP	15.10 ±0.25[c]	13.40 ±0.39[c]	11.98 ±0.57[a]	12.52 ±0.52[a]
100 µM SNP	15.25 ±0.60[c]	16.02 ±0.20[d]	11.41 ±0.08[a]	13.29 ±1.15[a]

Different letters in each column show a statistical difference

Table 2. GPx activity (U/ml) on 2nd and 4th day of leaf tissues in sunflower varieties. Data are expressed as mean ±SE of three replicates

Groups	2nd day	4th day	2nd day	4th day
	A-2517	A-2517	A-6535	A-6535
Control	7.43 ±0.21[a]	7.60 ±0.08[a]	22.07 ±0.43[a]	23.13 ±0.61[a]
100 mM NaCl	7.55 ±0.18[a]	19.59 ±0.58[b]	24.20 ±0.58[b]	22.39 ±0.55[a]
200 mM NaCl	7.60 ±0.04[a]	18.83 ±0.78[b]	21.29 ±0.76[a]	22.50 ±1.17[a]
400 mM NaCl	7.97 ±0.18[a]	18.53 ±0.38[b]	21.71 ±1.11[a]	21.01 ±0.38[b]
100 mM NaCl + SNP	7.00 ±0.37[a]	21.43 ±0.77[c]	21.60 ±0.56[a]	24.48 ±0.51[a]
200 mM NaCl + SNP	7.72 ±0.38[a]	21.49 ±0.58[c]	23.04 ±0.27[a]	22.26 ±1.18[a]
400 mM NaCl + SNP	7.20 ±0.22[a]	22.69 ±0.56[c]	21.76 ±0.09[a]	21.97 ±0.18[a]
100 µM SNP	25.31 ±0.75[b]	23.54 ±0.06[c]	21.01 ±1.16[a]	22.34 ±2.52[a]

Different letters in each column show a statistical difference

Table 3. APX activity (U/ml) on 2nd and 4th day of leaf tissues of sunflower varieties. Data are expressed as mean ±SE of three replicates

Groups	2nd day	4th day	2nd day	4th day
	A-2517	A-2517	A-6535	A-6535
Control	4.70 ±0.05[a]	4.90 ±0.04[a]	7.24 ±0.06[a]	7.23 ±0.05[a]
100 mM NaCl	9.08 ±0.02[d]	7.24 ±0.19[d]	4.08 ±0.01[d]	6.88 ±0.07[a]
200 mM NaCl	8.25 ±0.12[c]	6.48 ±0.23[c]	4.45 ±0.08[d]	6.63 ±0.11[b]
400 mM NaCl	7.54 ±0.10[b]	6.28 ±0.11[c]	6.80 ±0.58[b]	7.25 ±0.12[a]
100 mM NaCl + SNP	9.12 ±0.03[d]	5.19 ±0.18[b]	4.14 ±0.06[d]	6.42 ±0.23[b]
200 mM NaCl + SNP	9.94 ±0.04[d]	6.61 ±0.15[c]	4.81 ±0.46[d]	6.75 ±0.12[b]
400 mM NaCl + SNP	8.97 ±0.57[c]	5.96 ±0.17[b]	6.10 ±0.1[b]	7.94 ±0.02[a]
100 µM SNP	8.11 ±1.09[c]	6.21 ±0.01[c]	5.97 ±0.2[c]	5.76 ±0.03[c]

Different letters in each column show a statistical difference

APX enzyme activities. APX enzyme activity increased on the 2nd and 4th days at all three concentrations (100, 200, and 400 mM) of NaCl and NaCl + SNP treatment compared to control group in sensitive A-2517 variety. The highest APX enzyme activity was detected on the 2nd day of 200 mM NaCl + SNP treatment (9.94 ±0.04 U/ml). APX enzyme activity increased in all NaCl+SNP treatments compared to control group (Table 3). Also APX enzyme activities increased on the 2nd and 4th days of SNP treatments compared to control group. APX enzyme activities determined in leaf tissue of A-6535 cultivar at all three concentrations of NaCl + SNP treatments decreased compared to control group (except for the 4th day of 400mM NaCl + SNP treatment). The highest APX enzyme activity was determined on the 4th day of 400mM NaCl + SNP treatment (7.94 ±0.02 U/ml). APX enzyme activities decreased on the 2nd day of 400 mM NaCl treatment compared to 100 mM and 200 mM NaCl + SNP treatment (4.14 ±0.06 U/ml and 4.81 ±0.46 U/ml respectively) (Tab. 3).

CAT enzyme activities. As seen in Table 4, CAT enzyme activities in leaf tissue of A-2517 cultivar was observed to decrease on the 2nd day of 100 mM, 200 mM, and 400 mM NaCl treatments compared to control group. The highest CAT enzyme activity was found on the 4th day of 400 mM NaCl treatment (15.75 ±0.62 U/ml). CAT enzyme activities in NaCl + SNP treatments decreased compared to control group. In leaf tissue of A-2517 cultivar, CAT enzyme activities decreased for SNP treatment compared to control group. Decrease in CAT enzyme activity on the

Table 4. CAT activity (U/ml) on 2nd and 4th day of leaf tissues of sunflower varieties. Data are expressed as mean ± SE of three replicates (Different letters in each column show a statistical difference)

Groups	2nd Day	4th Day	2nd Day	4th Day
	A-2517	A-2517	A-6535	A-6535
Control	13,92 ±0,46^{a}	14,00 ±0,15^{a}	5,46 ±0,57^{a}	6,26 ±0,40^{a}
100 mM NaCl	9,21 ±0,29^{c}	13,44 ±1,49^{a}	15,65 ±1,21^{e}	15,78 ±0,61^{b}
200 mM NaCl	8,68 ±0,18^{d}	14,25 ±2,23^{a}	11,96 ±0,22^{c}	18,51 ±0,18^{c}
400 mM NaCl	8,95 ±0,43^{d}	15,75 ±0,62^{b}	12,51 ±0,72^{d}	15,34 ±1,70^{b}
100 mM NaCl+SNP	8,50 ±0,04^{d}	8,51 ±0,75^{d}	11,23 ±1,21^{c}	18,60 ±2,17^{c}
200 mM NaCl+SNP	9,80 ±0,35^{c}	9,62 ±0,44^{c}	10,19 ±1,76^{b}	19,16 ±0,16^{d}
400 mM NaCl+SNP	8,60 ±0,29^{d}	8,91 ±0,27^{d}	12,67 ±0,53^{d}	20,35 ±2,04^{d}
100 µM SNP	12,22 ±0,25^{b}	6,20 ±0,20^{e}	10,71 ±1,07^{b}	24,20 ±0,68^{e}

Table 5. NO Levels (µM) on leaf tissues of sunflower varieties. Data are expressed as mean ± SE of three replicates (Different letters in each column show a statistical difference)

Groups	2nd Day	4th Day	2nd Day	4th Day
	A-2517	A-2517	A-6535	A-6535
Control	2216,21 ±109,66^{a}	2224,23 ±4,58^{a}	1630,42 ±47,21^{a}	1628,13 ±43,79^{a}
100 mM NaCl	2687,60 ±152,53^{c}	2804,85 ±35,12	2738,92 ±49,48^{d}	1839,75 ±3,71^{c}
200 mM NaCl	2299,47 ±0,69^{a}	2748,75 ±21,45	2735,00 ±7,64^{d}	1738,71 ±9,93^{b}
400 mM NaCl	2688,17 ±40,95	3012,58 ±9,57	2758,08 ±16,98^{d}	1891,00 ±14,85^{c}
100 mM NaCl+SNP	2278,84 ±109,03^{a}	2412,88 ±23,75^{b}	2282,88 ±0,90^{b}	2272,79 ±62,63^{d}
200 mM NaCl+SNP	2431,46 ±57,83^{b}	2894,54 ±4,53	2259,88 ±22,49^{b}	2256,75 ±86,87^{d}
400 mM NaCl+SNP	2865,80 ±6,43^{d}	2985,92 ±4,50	2264,75 ±51,84^{b}	1759,67 ±18,67^{b}
100 µM SNP	2791,29 ±37,98^{c}	2373,75 ±7,11^{a}	2360,42 ±78,24^{c}	1940,50 ±14,80^{c}

4th day in SNP treatment was approximately as much as half compared to control group (Tab. 4). CAT enzyme activities determined for NaCl treatment groups on the 2nd day decreased compared to SNP treatments. CAT enzyme activities determined on the 2nd day of 100 mM and 400 mM NaCl + SNP treatments in leaf tissue of A-2517 cultivar decreased compared to 100 mM and 400 mM NaCl treatments. When we look at the data in Table 4, it was observed that CAT enzyme activity in leaf tissue of A-6535 cultivar increased approximately 3 times in NaCl treatments (100, 200, and 400 mM) compared to control group. The highest CAT enzyme activity was found to be 24.20 ±0.68 U/ml on the 4th day of 100 mM SNP treatment. All NaCl + SNP treatments led CAT enzyme activities to increase in comparison with control group. The highest CAT enzyme activity in NaCl + SNP treatments in leaf tissue of A-6535 cultivar was determined on the 2nd and 4th days of 400 mM NaCl + SNP treatment (12.67 ±0.53 U/ml and 20.35 ±2.04 U/ml, respectively). On the other hand, in SNP treatments, CAT enzyme activities increased approximately 2-4 times compared to control group (Tab. 4).

NO Levels. Nitric oxide levels in all NaCl treatments in leaf tissue of A-2517 cultivar increased compared to control group (Tab. 5). The highest NO level was determined on the 4th day of 400 mM NaCl treatment (3012.58 ±9.57 µM). In NaCl+SNP treatments, NO levels increased at all three concentrations compare to control. The highest NO level in NaCl + SNP treatments was observed on the 4th day of 400 mM NaCl+SNP treatment (2985.92 ±4.50 µM) (Tab. 5). In SNP treated groups, on the other hand, NO levels increased compare to control. In leaf tissue of A-6535 cultivar, NO levels increased on the 2nd and 4th days of NaCl treatments (100 mM, 200 mM, and 400 mM) compared to control group. NO levels increased approximately 1.5 times on the 2nd day of NaCl treatment compared to control group and the highest NO level occurred on the 2nd day of 400 mM NaCl treatment (2758.08 ±16.98 µM) in the leaves of A-6535 cultivar (Tab. 5). In NaCl + SNP treatments, on the other hand, NO levels increased compared to control group. The highest NO level was observed on the 2nd day of 100 mM NaCl + SNP treatment (2282.88 ±0.90 µM). NO levels in leaf tissue of A-6535 cultivar increased in SNP treatment compared to control group. NO levels on the 2nd day of NaCl + SNP treatments decreased compared to NaCl treatments.

DISCUSSION

Under abiotic stress conditions, many metabolic functions are restricted, such as closure of stomas, formation of reactive oxygen species in mitochondria and peroxisomes. Oxidative stress resulting from the increase in ROS amount destroys macromolecules such as DNA, protein carbo hydrate, lipid. As a result, antioxidant enzyme activities may vary depending on whether the plant is resistant to stress conditions. Salt stress causes ROS accumulation in high amounts in plant cells. Plants increase the levels of antioxidant metabolites under stress conditions. These metabolites include enzymes such as SOD, CAT, APX and GR [Schutzendubell et al. 2002, Ahmad et al. 2010, Hayat et al. 2012].

As mentioned before in the literature, SOD enzyme activity increased in the sensitive cultivar with boron treated to barley and there was no significant change in SOD enzyme activity in the tolerant cultivar. This reveals that antioxidant enzyme activities of two plants to stress conditions were different. *Cleome spinosa* (C3) and *Cleome gynandra* (C4) plants were subjected to drought stress for 10 days after growth in order to reveal the difference between antioxidant enzyme activities of C3 and C4 plants in drought stress. Study revealed that higher SOD enzyme activity was determined in *C. gynandra* in drought treatment [Uzilday et al. 2012]. These results indicate that SOD enzyme activities which have a major role in antioxidant system of plants, may vary in different cultivars of the plant. Benefeciary effect of NO on antiantioxidant enzyme actitivites has been reported under drought and salt stress [Garcia-Mata eta l. 2001, Laspina et al. 2005, Zhang et al. 2006, Caverzan et al. 2016, Kaya et al. 2015]. Results of studies for this purpose revealed that SNP treatment activated SOD enzyme activity and NO played an active role in antioxidant enzyme system of plant against stress [Laspina et al. 2005, Gao et al. 2008, Unal et al. 2014]. In our study, SOD enzyme activities decreased in NaCl + SNP treatments in both cultivars compared to NaCl treatments. These results are similar with the study by Laspina et al. [2005], Chawla et al. [2013], Guo et. al. [2009], Kaya

et al. [2015]. They concluded that nitric oxide (NO) inhibited the increase of superoxide dismutase (SOD) activity induced by stress. NO plays a role as a signal molecule that has effect against environmental stress and it has been reported that external NO applications can reduce the damage caused by stresses [Gill et al. 2013]. SOD enzyme activity increased in SNP treated A-2517 cultivar compared to control group, this increase was approximately 2 times on the 4th day and these results are similar the study performed by Uchida et al. [2002] and they reported that NO levels in plants increased with stress treatment and pretreatment of SNP. In A-6535 cultivar, on the other hand, SNP treatment decreased SOD enzyme activity compared to control group. Results suggested that SNP decreased enzyme activity helps to its regenerative effect.

In the present study, GPX enzyme activities increased with NaCl treatment compared to control group in sensitive to salt sress (A-2517) cultivar and particularly increases on the 4th day were 2.5 times greater more than control group. GPx enzyme activities generally increased in both cultivars, under salt stress and this is accordance concordate with the studies by Laspina et al. [2005]. In the present study, it was found that while 200 mM NaCl + SNP treatment increased GPx enzyme activity in sensitive A-2517 cultivar compared to 200 mM NaCl treatment, the other two NaCl + SNP treatments decreased compared to NaCl treatments. GPx enzyme activity decreased in 200 mM NaCl + SNP treatment on the 4th day compared to 200 mM NaCl treatment but increased in 100 mM and 400 mM NaCl + SNP treatments. Only SNP treatments increased approximately 3 times in GPx enzyme activities in sensitive A-2517 cultivar compared to control group but decreased in tolerant A-6535 cultivar compared to control group. This results indicated that NO had a different effect on enzyme activities of plants with different susceptibility, which is compatible with the study reported by Kirecci an Yurekli [2017].

According to results of the present study, APX enzyme activity increased approximately 2 times in NaCl treatment compared to control group in A-2517 cultivar which is sensitive to salt stress and this result is compatible with the study by Praxedes et al. [2014], Caverzan et al. [2016], APX enzyme activities decreased with NaCl treatment in A-6535 cultivar. This difference between tolerant and sensitive cultivars is compatible with the APX enzyme activity determined by Karabal et al. [2003] in roots of wheat tolerant and sensitive to boron toxicity. While APX enzyme activities increased in SNP treatment under salt stress plants compared to control group in A-2517 cultivar, and it decreased in A-6535 cultivar. APX enzyme activity increased approximately 2 times in exogeneous NO treatment compared to control group in A-2517 cultivar, it decreased in A-6535 cultivar. It has been reported that APX enzyme activity in sunflower plant increased 2 times with NO treatment compared to control group [Laspina et al 2005].

In the present study, CAT enzyme activities decreased in NaCl treatment on the 2nd day compared to control group in A-2517 cultivar; whereas, they increased in A-6535 cultivar. Different studies mention similar results [Caverzan et al. 2016]. While CAT enzyme activities in A-2517 cultivar decreased in SNP treatments in salt stress relative to the control group, considerable increases were determined on the 2nd and 4th days in A-6535 cultivar and similar results were obtained in the study by Laspina et al. [2005], Parvaiz et al. [2016]. These increases are the stress response of antioxidant defense system against ROS production increasing under stress conditions. In a previous study, researchers reported that nitric oxide (NO) decreased Cd stress-induced catalase (CAT) activity at the rate of 44% in sunflower plants subjected to Cd [Laspina et al. 2005, Kaya et al. 2015]. Also SNP provided increase CAT activity 4-fold in the fourth day compared to control group. It reveals that NO has an effect on enzyme activities and is an important signal molecule for abiotic stress and its mechanism may vary from plant to plant.

This study supports the idea that NO is an effective molecule against salt stress because the NO content increased due to salt application in both plant. The findings we obtained in this study consistent with the findings of other researchers [Li et al. 2008, Arasimowicz-Jelonek et al. 2009, Yurekli and Kirecci 2016]. Also in the present study, NO levels increased in both cultivars compared to control group in NaCl + SNP treatments and this is compatible with the study indicating that NO levels increased with temperature stress and SNP treatment Song et al. [2006]. Significant changes in antioxidant enzyme activities in re-

sponse to salt stress have also been shown in our study due to generally positive and healing effects.

CONCLUSIONS

Sunflower is an economically important plant in Anatolia as well as in the world. In this study, we analyzed SOD, GPX, APX and CAT antioxidant enzyme activities and NO levels in different concentrations of NaCl and NaCl+SNP in leaf tissues of different sunflower plant. When we consider the fact that plants have a system of scavenging and recovering features, the findings we have obtained showed that tolerant variety possessed more effective catalase/superoxide dismutase/ascorbate-glutathione cycle under high salinity. Our study shows that exogenous NO application in sunflower plants is effective in reducing salt stress. The investigation of salt tolerance of cultivated plants is very important in terms of utilization of saline soils. In recent years there has been intensive research to explain on the effects of salt tolerance genes on the development of crops against over expression of salt, the effects of salinity on plants, and the complex responses of plants to these effects. As known that plants are develop tolerance to salinity and other abiotic stress factors, it is important to identify biochemical, physiological and molecular mechanisms that allow plants tolerance to salt stress. The results of this study provide some basic information and system for further study of the genetic and biochemical implications of salt tolerance. It can be concluded that pretreatment with SNP attenuated salt stress induced injuries in sunflower plants via up-regulation of the activities of antioxidant enzymes and prevention of lipid peroxidation.

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