Effect of Seed Priming with L-arginine and Sodium Nitroprusside on Some physiological Parameters and Antioxidant Enzymes of Sunflower Plants Exposed to Salt Stress.

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ABSTRACT
Salinity is one of the most important environmental stress factors limiting plant growth and productivity in different regions. Previously sodium nitroprusside (SNP) was applied as a nitric oxide (NO) donor to counteract the effect of salt stress while there is little information about exogenous arginine application as a precursor of NO in the possible anti-oxidative responses of plants against salinity. In this research the effects of seed priming with SNP and L-arginine (Arg) on alleviation of oxidative damages caused by salinity (150 mM NaCl) were investigated in sunflower plants. The experiments were performed in a completely randomized design with 3 replicates. Our findings showed that salinity stress has negative effects on growth parameters, and application of Arg and SNP could alleviate the harmful effects of salinity. Results showed that in salt stressed plants, activity of lipoxygenase in root and leave tissues increased while, antioxidative enzymes activity decreased when compared with control. Application of SNP increased the activity of catalase (CAT), guaiacol peroxidase (GPX) and ascorbate peroxidase (APX) enzymes in root and leaves of plants while Arg pretreatment could only increase the activity of CAT and APX in root and leaves of plant, when compared with untreated plants. In this research, the effect of SNP and Arg on most studied parameters, were the same so we assumed that the effects of Arg may related to the NO releasing from Arg directly or indirectly and we propose the application of Arg as a new compound which can be used instead of SNP.

Keywords: Ascorbate peroxidase, catalase, Helianthus annuus, guaiacol peroxidase, nitric oxide, polyamines, salinity.

Abbreviations:
APX: ascorbate peroxidase; Arg: L-arginine; Car: carotenoids; CAT: catalase; Chl: Chlorophyll; GPX: guaiacol peroxidase; GSH: glutathione; LOX: lipoxygenase; NO: nitric oxide; Pas: polyamines; ROS: reactive oxygen species; SNP: sodium nitroprusside; SOD: super oxide dismutase.

INTRODUCTION
Salinity is one of the most important environmental stress factors limiting plant growth and productivity in different regions, particularly in arid and semi-arid regions. It is estimated that over 800 million hectare of land in the world are affected by both salinity and sodic soil (Munns, 2005). Various detrimental effects of salt stress in crop plants have been reported which are responsible for severe decrease in the growth and yield of plants. Inhibition of plant growth and even plant death by NaCl, is due to a reduction in water availability (secondary drought), sodium ions accumulation and mineral imbalances (Zhu, 2001; Munns, 2002; Ashraf and Harris, 2004). All of these factors manifest themselves by morphological, physiological and metabolic modifications in plants, such as decrease in seed germination, shoot and root length, alterations in the integrity of cell membranes, inhibition of different enzymatic activities and photosynthesis (Dash and Panda, 2001; Sairam and Tyagi, 2004). Many abiotic environmental stresses including salinity, drought, temperature extremes, and metal toxicity disrupt the redox homeostasis of cells and exert a wide range of adverse effects on plant growth and metabolism (Sharma and Dubey, 2007; Nasibi and Kalantari, 2009). These stressful conditions induce overproduction of reactive oxygen species (ROS).
These compounds are known to damage cellular membranes by inducing lipid peroxidation and can damage DNA, proteins and chlorophyll (Mittova et al., 2002). The lifetime of active oxygen species within the cellular environment is determined by the antioxidant system, which provides crucial protection against oxidative damage. The antioxidative system comprises numerous enzymes such as super oxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) and guaiacol peroxidase (GPX) and non-enzymatic antioxidants with low molecular weight including ascorbic acid and reduced glutathione (GSH) (Sharma and Dubey, 2007).

L-arginine (Arg) is one of the most functionally diverse amino acids and a precursor for biosynthesis of polyamines (PAs) and nitric oxide (NO) (Liu et al., 2006). PAs modulate several biological processes in plants, including cell division, differentiation, and senescence and it has been suggested that they participate in cellular defense against oxidative damage through the inhibition of lipid peroxidation and scavenging of free radicals (Velikova et al., 2000). Nitric oxide is a small, lipophilic gas and a bioactive molecule that play an important role in different physiological processes. There is increasing evidence showing that NO acts like a signal molecule in physiological processes such as growth and development, respiratory metabolism, cell death, and ion leakage (Kopyra and Gwozdz, 2004; Lamotte et al., 2005). On the other hand, NO can also mediate plant growth regulators and ROS metabolism and increasingly evident, which NO involves in signal transduction and responses to biotic and abiotic stresses such as drought, low and high temperatures, UV and ozone exposure, heavy metal, herbicides, cold, and salt stresses (Neill et al., 2003; Del Rio et al., 2004; Fan et al., 2007). It has been reported that tolerance to drought, salt and heat stresses was enhanced in tomato (Lycopersicum sculentum), wheat (Triticum aestivum) and rice (Oryza sativa) seedlings when the plants were treated with NO donor and sodium nitroprusside (SNP) (Nasibi and Kalantari, 2009; Mata and Lamattina, 2001; Uchida et al., 2002).

In previous studies, researchers applied SNP, as a NO donor and exogenous polyamines to counteract the effect of salt stress (Wang, 2007; Tewari et al., 2008). However there are few researches on the effect of exogenous arginine as a precursor of these compounds in the possible anti oxidative responses of plants against salinity stress. In addition among different strategies which were used to cope with salinity stress, seed priming (pre-sowing seed treatment) is an easy, low cost and low risk technique and this approach has recently been used to overcome the salinity problem in saline agricultural lands. The present study was conducted to evaluate and compare the effects of seed priming with arginine and SNP on growth and alleviation of oxidative damages in sunflower plant under salt stress.

Materials and Methods:

Plant Material:
The seed of sunflower plants (Helianthus annuus L. var. KF84) were supplied by the agriculture research center of Kerman. Seeds of uniform size were disinfected with 2% sodium hypochlorite solution, and were washed with distilled water. Solutions of 1 and 5 mM of arginine and 100 µM of sodium nitroprusside (SNP) were used for seed priming and distilled water was used as control solution for 24 hours. After pre-soaking, seeds were sown in plastic pots contains perlite. After germination, seedlings were supplied with half strength of Hogland nutrient solution for three times at a week. After four weeks, all plants were divided in two groups, one group were exposed to salt stress (150 mM NaCl) and another group were irrigated with tap water as control for 14 days, then the shoot and root of plants were harvested and immediately frozen in liquid nitrogen and stored at -80°C for future biochemical analysis.

Growth Parameters:
Shoot and root dry weights, shoot and root length of treated plants were measured as growth parameters.

Chlorophyll Content:
Chlorophyll (Chl) content was determined using the methods of Lichtenthaler (1987). In this method Chl was extracted in the 80% acetone. Extracts were centrifuged at 3000g and the absorbance of supernatant was measured at 663.2, 646.8 and 470 nm with UV-VIS spectrophotometer. Chl a, Chl b, total Chl and carotenoids (Car) contents were calculated by following formulas:

\[\text{Chl } a = (12.25A_{663.2} - 2.79A_{646.8}) \times \text{volume of supernatant (ml)} \times \text{dilution factor/sample mass (g)}\]

\[\text{Chl } b = (21.21A_{646.8} - 5.1A_{663.2}) \times \text{volume of supernatant (ml)} \times \text{dilution factor/sample mass (g)}\]

\[\text{Total Chl } = \text{Chl } a + \text{Chl } b \]

\[\text{Car } = [1000A_{470-0.18Cl h} - 85.02\text{Chl } b]/198] \times \text{volume of supernatant (ml)} \times \text{dilution factor/sample mass}\]

In which, A: Absorbance at specific wave length.

Enzyme Extraction:
Five hundred milligrams of leaves and root were homogenized in cool 50 mM potassium phosphate buffer (pH=7.0) containing 1% (w/v) soluble PVP, 1 mM EDTA and 1mM PMSF with the addition of 10 mM ascorbic acid for APX assay. All of the procedures were done at 4°C. The homogenate was centrifuged at 20000g for 20 min and the supernatant was used for assay of the activity of enzymes.

Lipoxygenase Activity:
Lipoxygenase (LOX) (EC1.13.11.12) activity was estimated according to the method of Doderer

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et al. (1992). For measurement of LOX activity, the substrate solution was prepared by adding 35 µl linoleic acid to 5 ml distilled water containing 50 µl Tween-20. The solution was kept at pH 9.0 by adding 0.2 M NaOH, all the linoleic acid was dissolved and the pH remained stable. After adjusting the pH to 6.5 by adding 0.2 M HCl, 0.1 M phosphate buffer (pH=6.5) was added to make a total volume of 100 ml. LOX activity was determined spectrophotometrically by adding 50 µl of enzyme to 2.95 ml substrate. Solution absorbance was recorded at 234 nm and the activity was expressed as unit per mg protein in the leaves (extinction coefficient of 25000 M$^{-1}$ cm$^{-1}$).

**Catalase Activity:**
Catalase (EC 1.11.1.6) activity was assayed by measuring the initial rate of H$_2$O$_2$ disappearance at 240 nm using the extinction coefficient of 40 M$^{-1}$ cm$^{-1}$ for H$_2$O$_2$ (Velikova et al., 2000). The 3 ml reaction solution consisted of 50 mM potassium phosphate buffer (pH=7.0), 0.1 mM H$_2$O$_2$ and 100 µl of enzyme extract. Addition of H$_2$O$_2$ started the reaction and the decrease in absorbance was recorded after 30s.

**Guaiacol Peroxidase:**
The guaiacol peroxidase (EC1.11.1.7) activity was determined using the method by using Bovine serum albumin as a standard.

**Ascorbate Peroxidase:**
Ascorbate peroxidase (EC 1.11.1.11) was determined spectrophotometrically according to the oxidation of ASA. The reaction solution contained 50 mM potassium phosphate buffer (pH=7.0), 0.5 mM ascorbate, 0.1 mM H$_2$O$_2$ and 150 µl enzyme extract. H$_2$O$_2$-dependent oxidation of ASA was followed by measuring the decrease in absorbance within 1 min at 290 nm (extinction coefficient of 2.8 M$^{-1}$ cm$^{-1}$) (Nakano and Asada, 1981).

**Total Soluble Proteins:**
Protein content of roots and leaves was determined according to Bradford (1976) method by using Bovine serum albumin as a standard.

**Statistical Analysis:**
All measurements were carried out in three replicates and data were subjected to one way analysis of variance. Analysis of variance was performed using the ANOVA procedure. Statistical analyses were performed according to the MSTATC software. Significant differences between means were determined by Duncan’s multiple range tests. $P$ values less than 0.05 were considered statistically significant.

**RESULTS**

**Growth Parameters:**
Results showed that the length of shoots decreased significantly in salt condition and all of the pretreatment (Arg and SNP) increased the length of the salinity stressed plants. The highest plant height was obtained in control plants that were pretreated with Arginine (1 mM) (Table 1). In the case of root length, salinity decreased the root length; however Arg (5 mM) and especially SNP pretreatment alleviated the salt effect. Total biomass of plants decreased significantly in plants which were under salt stress when compared with control. Arg and SNP pretreatment significantly increased the total biomass of plants under control and salinity condition (Table 1).

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**Table 1.** Interaction of Arg and SNP with NaCl on growth parameters and photosynthetic pigments of sunflower plant (*Helianthus annuus* L.) under control and salinity stress (150 mM NaCl).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>Arg (1mM)</th>
<th>Arg (5mM)</th>
<th>SNP (100µM)</th>
<th>NaCl (150mM)</th>
<th>NaCl+Arg (1mM)</th>
<th>NaCl+Arg (5mM)</th>
<th>NaCl+SNP (100µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shoot length (cm)</td>
<td>17.25b</td>
<td>17.9a</td>
<td>15.25c</td>
<td>15c</td>
<td>11.33f</td>
<td>15c</td>
<td>12.25e</td>
<td>13.25d</td>
</tr>
<tr>
<td>Root length (cm)</td>
<td>25.00b</td>
<td>28.5a</td>
<td>24.2b</td>
<td>23.9b</td>
<td>17.25d</td>
<td>17.85d</td>
<td>21.25c</td>
<td>26.5 ab</td>
</tr>
<tr>
<td>Total biomass (mg/plant)</td>
<td>0.20c</td>
<td>0.23a</td>
<td>0.22b</td>
<td>0.22b</td>
<td>0.17 e</td>
<td>0.22 b</td>
<td>0.19 d</td>
<td>0.19 d</td>
</tr>
<tr>
<td>Chl a (mg g$^{-1}$f.m)</td>
<td>1.50d</td>
<td>2.66b</td>
<td>1.94c</td>
<td>1.43d</td>
<td>1.1e</td>
<td>1.89c</td>
<td>3.14a</td>
<td>2.16c</td>
</tr>
<tr>
<td>Chl b (mg g$^{-1}$f.m)</td>
<td>1.89b</td>
<td>2.65a</td>
<td>1.63b</td>
<td>1.78b</td>
<td>1.74b</td>
<td>1.91b</td>
<td>2.41a</td>
<td>1.70b</td>
</tr>
<tr>
<td>Total Chl (mg g$^{-1}$f.m)</td>
<td>3.39bc</td>
<td>5.31a</td>
<td>3.57bc</td>
<td>3.11c</td>
<td>2.84c</td>
<td>3.80b</td>
<td>5.55a</td>
<td>3.86b</td>
</tr>
<tr>
<td>Car (mg g$^{-1}$f.m)</td>
<td>2.6b</td>
<td>2.39b</td>
<td>2.63b</td>
<td>2.1b</td>
<td>3.7a</td>
<td>2.4b</td>
<td>2.7b</td>
<td>3.6a</td>
</tr>
</tbody>
</table>

*The mean comparisons of treatments were done using Duncan test at p<0.05 significant level. Means followed by the same letter(s) in each column are not significantly different at the 5% level.*
**Photosynthetic Compounds:**

Photosynthetic compounds include Chl \(a\), Chl \(b\) and carotenoids were measured in this experiment. Result showed that Chl \(a\) decreased under saline condition while Chl \(b\) and total Chl did not change in comparison with control plants. Carotenoids content increased in plants, which were under salt stress (Table 1). Pretreatment of plants with Arg and SNP increased chlorophyll content in plants under salinity stress while decreased the carotenoids content, the highest carotenoids content was observed in that plants which were not pretreated but were under salt stress.

**Lipoxygenase Activity:**

Lipoxygenase is an oxidative enzyme that contributes in oxidation of polyunsaturated fatty acids and lipid peroxidation of membrane. Assay of LOX activity in this study showed that, activity of this enzyme increased about 2 fold in leaves and 3 fold in roots of plants, which were under salt stress when compared with control (Fig. 1). Pretreatment of plants with Arg and SNP decreased the activity of this enzyme in stressed plants in comparison with non-pretreatment plants.

**Antioxidant Enzyme Activity:**

Changes in the activity of antioxidant enzymes is the consequence of oxidative stress. The effect of salt stress on CAT, GPX and APX-in leaves and roots of sunflower plant either with or without pretreatment was assayed. As it is shown in (Fig. 2) the activity of CAT, GPX and APX of leaf and root tissues of stressed plants were lower when compared with those of the control groups which may be a reflection of the oxidative burst under salt stress. Arg pretreatment increased the CAT activity in leaf of stressed plant but had no significant effects on CAT activity in root tissue (Fig. 2). SNP increased the activity of this enzyme in both root and leaf of plants, which were under salt stress. GPX is another antioxidant enzyme, which was measured in this study. Activity of this enzyme decreased in roots of stressed plants but in leaf tissue, the activity of this enzyme did not change significantly (Fig. 2).

Application of Arg as pretreatment had no significant effect on activity of this enzyme in root tissue. However, Arg (1 mM) decreased the activity of GPX in leaf tissue of plant under salt stress. SNP pretreatment increased the GPX activity in both leaf and root of plants under stress condition when compared with non-pretreatment plants. APX activity also decreased in leaf and root of plants, which were under salt stress. Arg pretreatment increased the activity of APX in leaf of plants under salinity condition but SNP pretreatment had no significant effects on APX activity in leaf tissue. In the roots of these plants which were under salt stress, application of both Arg and SNP increased the activity of APX (Fig. 2).

**DISCUSSION**

Salt stress disturbs intracellular ion homeostasis of plants, which leads to membrane dysfunction, attenuation of metabolic activity, and causes growth inhibition and ultimately leads to cell death (Sheokand et al., 2010). Our finding showed that salinity stress has the negative effects on shoot and root length and pre-treatment with Arg and SNP could alleviate these harmful effects. The reduction in vegetative growth due to high salinity effect, which was observed in this study, is in agreement with previous investigation on sunflower (Jabeen and Ahmad, 2012) and cowpea plants (Taffouo et al., 2009). The inhibition effects of salinity on growth parameters in sunflower plants might be due to the reduction of water absorption and metabolic activities, Na\(^+\) and Cl\(^-\) toxicity as well as nutrient deficiency that caused by ionic interference (Ghoulam et al., 2002; De Lacerda et al., 2003).
In this experiment, Arg (1mM) had the most effects on the increase of shoot length while SNP was more effective in the increase of root length (Table 1). These results are in agreement with those obtained by Zeid (2009) which observed that arginine pretreatment promoted growth parameters of bean plant under salt stress. Effect of NO on growth parameters of plants under drought, heavy metal and salinity stress has been reported (Nasibi and Kalantari, 2009; Zhao et al., 2001; Singh et al., 2008). However, it has been reported that the effects of arginine in alleviation of stress and increasing growth parameter may be related to the polyamine production, which have been implicated in a wide range of biological processes including growth, development and abiotic stress responses (Nasibi et al., 2011; Zeid, 2009).

Measurement of photosynthetic pigments showed that salinity stress decreased the amounts of chlorophyll content and pretreatment of plants with Arg and SNP decreased the NaCl damages on these pigments (Table 1). It has been reported that NO can scavenge the ROS and thus decreased the oxidative damage in photosynthetic apparatus and increased the chlorophyll content (Hsu and Kao, 2004; Lei et al., 2007). Leshem and Haramaty (1996) showed that nitric oxide could increase the chlorophyll content in leaves of pisum sativum. In this research both SNP and Arg increased the chlorophyll content in leaves of both control and stressed plants. Carotenoids have antioxidant role
in plants so in this experiment it seems that these compounds increased under salinity stress as non-enzymatic antioxidant. Pretreatment of plants with Arg or SNP alleviated the stress condition and decreased the amounts of these pigments.

One of the described damages, which provoked by stress such as salinity is the membrane injury and liberation of ions from the cell to extra cellular space (Halliwel and Gutteridge, 1984). This is a consequence of an oxidative burst leading to lipid peroxidation, membrane permeability and cell injury (Scandalios, 1993). The Reactive Oxygen Species has been reported to increase as a response to most abiotic stresses including salinity (Tsai et al., 2004). Lipoxygenase is an oxidative enzyme, which can contribute to lipid peroxidation. It is a non-heme enzyme that contains a single iron atom which is thought to oscillate between ferrous (inactive) and ferric (active) forms during each cycle of catalysis. A protective effect of NO on membrane injury has been previously reported under salt stress (Zhao et al., 2001), drought (Nasibi and Kalantari, 2009) and heavy metal stress (Singh et al., 2008).

In this experiment the activity of this enzyme increased in root and leaf of plants which were under salinity stress (Fig 1). Pretreatment of plants with Arg and SNP decreased the activity of this enzyme in both root and leaf tissues of plants, which were under stress. It has been reported that nitric oxide could inhibit enzyme activity by reducing the iron of the active site through the converted of active Fe$^{+3}$ to an inactive Fe$^{+2}$ form and trapping the iron in a reduced inactive form (Zhu et al., 2006). In this research we observed that Arg pretreatment also decreased the LOX activity in salt stressed plants. Our previous finding on tomato plants under drought stress indicated that Arg pretreatment could decrease the LOX activity in plants which were under stress condition—and the molecular analysis of research showed that in Arg pretreated plants, the expression of arginase I and II genes were higher than the non-Arg pretreated plants (Nasibi et al., 2013). Therefore, authors taught that under these situations, protective effect of Arg is related to polyamines or indirect synthesis of NO from polyamines, which were previously discussed (Tun et al., 2006; Nasibi et al., 2011).

The protective effects of Arg in salt stressed plants in this experiment may also be related to NO releasing from Arg, either directly or indirectly. Under normal conditions, the total amount of ROS formed in the plants is determined by the balance between the multiple ROS producing pathways and the ability of the enzymatic and non-enzymatic mechanism to deal with them. Under stress conditions, ROS formation is higher than ability of plants to remove them and this could result in oxidative damages (Lasipina et al., 2005). In sunflower plants the activity of APX, GPX and CAT decreased under salt stress condition (Fig 2), therefore, we can assume that the plants were damaged because the antioxidant machinery was not effectively struggling against stressful condition. It has been reported that the antioxidant enzymes have a significant role in imparting salt tolerance in plants (Ashraf and Harris, 2004).

In the present investigation it has been found that, in plants that were under salt stress, SNP pretreatment could increase the activity of CAT, GPX and APX enzymes in root and leaves of plants. While Arg pretreatment increased the activity of CAT and APX in roots and leaves of plants and had no significant effects on GPX activity when compared with non-pretreated plants. It has been reported that application of exogenous NO, induces the antioxidant enzyme activity in response to high salinity conditions (Molassiotis et al., 2010). Khalil et al. (2009) found that treatment of wheat plants with arginine or putrecin, activated the antioxidant defense system (SOD and CAT) and reduced the lipid damage when plants were exposed to high temperature. Nasibi et al., (2011) found that exogenous arginine treatment decreased the activity of catalase and guaiacol peroxidase while increased the activity of SOD, APX, and glutathione reductase in tomato plants under drought stress. Zhang et al. (2013) also demonstrated that exogenous arginine treatment alleviated chilling injury in cold-stored tomato fruit. They concluded that the effect of exogenous arginine treatment on alleviating chilling injury of tomato fruit might be attributed to its ability to enhance the accumulation of endogenous putrecin, proline and NO concentrations in fruit, which was primarily due to the increment of arginase, arginine decarboxylase, ornithine decarboxylase and nitric oxide synthase activities.

In this research, we observed that in many parameters, which were measured, the effect of SNP and Arg are the same so we assumed that the effects of Arg may relate to the NO releasing from Arg directly or indirectly and we propose the application of Arg as a new compound which can be used instead of SNP. However, further studies are necessary to find the mechanism of different pathways of arginine catabolism in stressful conditions.


Jabbar, N. and R. Ahmad. 2013. The activity of antioxidant enzymes in response to salt stress in sunflower (Heliannthus annuus L) and sunflower (Helianthus annuus L) seedlings rose from seed treated with chitosan. Journal of Agricultural and Food Science. 93: 1699-1705.


Molassiotis, A., G. Tanou and G. Diamantidis. 2010. NO says more than “yes” to salt tolerance—salt priming and systemic nitric oxide signaling in plants. Journal of Plant Signaling and Behavior. 5: 209-212.


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**REFERENCES**


Jabbar, N. and R. Ahmad. 2013. The activity of antioxidant enzymes in response to salt stress in sunflower (Heliannthus annuus L) and sunflower (Helianthus annuus L) seedlings rose from seed treated with chitosan. Journal of Agricultural and Food Science. 93: 1699-1705.


