



Effect of seed pre-treatment with L-arginine on improvement of seedling growth and alleviation of oxidative damage in canola plants subjected to salt stress

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Abstract

Soil salinity is one of the major abiotic stresses that adversely affect plant productivity and quality. Therefore, an experiment was conducted to investigate the effects of seed treatment with L-arginine on some morphological and physiological parameters of *Brassica napus* under salinity stress. The seeds of canola were pre-treated with three arginine concentrations (0, 5, and 10 μ M Arg) for 24 hours. Then they were subjected to three levels of salt treatments (0, 50, and 100 mMNaCl) for 7 days. Results of this experiment indicate that salinity stress caused a number of morphological and physiological changes in the canola plant, including decrease in root and shoot length. Hydrogen peroxide and malondialdehyde (MDA) content increased in leaves of canola plant under salt stress. Salt stress also induced changes in antioxidant enzymes activities such as catalase (CAT), ascorbate peroxidase, (APX), and Guaiacol peroxidase (GPX). In conclusion, the adverse effects of salt stress on canola can be alleviated by the arginine pre-treatment through modulating activities of antioxidant enzymes.

Keywords: antioxidant enzymes; arginine; hydrogen peroxide; lipid peroxidation; salt stress

Abbreviations:

Arg: arginine; APX: ascorbate peroxidase; CAT: catalase; GPX: guaiacol peroxidase; MDA: malondialdehyde; NO: nitric oxide; ROS: reactive oxygen species; SOD: super oxide dismutase

Nasibi, F., Kh. Manouchehri Kalantari and A. Barand. 2014. 'Effects of seed pre-treatment with L-arginine on improvement of seedling growth and alleviation of oxidative damage in canola plants subjected to salt stress'. *Iranian Journal of Plant Physiology* 5 (1), 1217-1224.

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 hectares of land in the world are affected by both salinity and sodicity (Munns 2005). There are various detrimental effects of salt stress on crop plants, 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

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Received: July, 2014

Accepted: October, 2014

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, decrease in shoot and root length, alterations in the integrity of cell membranes, inhibition of different enzymatic activities, and photosynthesis (Munns, 2002; Sairamet al., 2002). Many abiotic environmental stresses including salinity, drought stress, 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 anti-oxidative system comprises numerous enzymes such as SOD, CAT, APX, and GPX as well as non-enzymatic antioxidants with low molecular weight including ascorbic acid and reduced glutathione (GSH) (Sharma and Dubey, 2007).

Amino acids have a specific role in plant responses to stresses. L-arginine is one of the most functionally diverse amino acids and a precursor for biosynthesis of polyamines and nitric oxide (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 free radicals (Velikova et al., 2000). Research has shown that PAs applied exogenously to plants confer some protective effects against heavy metal stress such as Copper (Wang, 2007) and Cd stress (Hsu and Kao, 2004). The involvement of NO in salinity tolerance has drawn much attention in the past few years (Molassiotis et al., 2010). For instance, under salinity condition, the exogenous NO can enhance salt tolerance by alleviating oxidative damage (Shi

et al., 2007; Zheng et al., 2009). Although salt stress affects all growth stages of a plant, seed germination and seedling growth stages are known to be more sensitive in most plant species (Ashraf, 1994; Munns, 2002). Furthermore, germination and seedling stage is predictive of plant growth responses to stress. In addition, among different strategies 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 agriculture lands. In previous studies, researchers applied SNP as a NO donor and/or exogenous polyamines to counteract the effect of salt stress (Wang, 2007; Hsu and Kao, 2004; Tewari et al., 2008). However, there is little research on the effect of exogenous arginine as a precursor of these compounds in the possible anti-oxidative responses of plants against salinity stress. The present study was conducted to evaluate the effect of arginine on seedling growth and alleviation of oxidative damages in canola plant under salt stress.

Materials and Methods

Plant material

The seed of canola 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 5 and 10 μM of arginine were used for seed priming and distilled water was used as control solution for 2 days. After pre-soaking, the seeds were surface dried and then allowed to germinate on filter paper in a Petri dish. Filter papers in the Petri dishes were moistened with 50 or 100mMNaCl for salinity treatment and with distilled water as control condition. The Petri dishes were kept in dark for 24h and then transferred to light for further growth for 7 days.

Growth parameters

Shoot and root fresh weigh, shoot and root lengths of seedlings were measured as growth parameters.

Lipid peroxidation

One hundred mg of the leaf tissue of plants were homogenized in 10 ml of 0.1% trichloroacetic acid (TCA), and then centrifuged at 10000×g for 15min. One ml of supernatant was then added to 4 ml of 20% TCA containing 0.5% 2-thiobarbituric acid (TBA) and the solution was heated for 30 min at 90°C. Samples were cooled immediately and then re-centrifuged for 10min at 10000×g. The absorbance was measured at 532 nm. The value for the non-specific absorption at 600 nm was subtracted. The level of lipid peroxidation was expressed as μM of MDA formed using an extinction coefficient of $155 \text{ mM}^{-1} \text{ cm}^{-1}$ (Heath and Packer 1968).

Hydrogen peroxide content

Fresh leaf (0.5 g) was homogenized in ice bath with 5 ml 0.1% (w/v) (TCA). The homogenate was centrifuged at $12,000 \times g$ for 15 min and 0.5 ml of the supernatant was added to 0.5 ml of 10 mM potassium phosphate buffer (pH 7.0) and 1 ml of 1 M KI. The reaction was carried out for 1h in darkness and absorbance was measured at 390 nm. The amount of hydrogen peroxide was calculated using a standard curve prepared with known concentration of H_2O_2 (Alexieva et al., 2001).

Enzyme extraction and activity determination

Five hundred mg leaves were homogenized in an ice cold mortar using 50mM potassium phosphate buffer pH 7.0 containing 1mM EDTA and 1% (w/v) soluble PVP. After centrifugation (20,000 g, 20 min) the supernatant was used for determination of CAT, GPX, and APX activities.

Catalase activity (EC 1.11.1.6)

Catalase activity was assayed by measuring the initial rate of H_2O_2 disappearance at 240nm using the extinction coefficient $40 \text{ mM}^{-1} \text{ cm}^{-1}$ for H_2O_2 (Velikova et al., 2000).

Guaiacol peroxidase (GPX) (EC1.11.1.7)

The GPX activity was determined using the method of Plewa et al. (1991) following the formation of tetraguaiacol by measuring the absorbance at 470nm and using an extinction coefficient $25.5 \text{ mM}^{-1} \text{ cm}^{-1}$.

Ascorbate peroxidase (APX) (EC 1.11.1.11)

Ascorbate peroxidase was determined spectrophotometrically according to the oxidation of ASA. The reaction solution contained 50mM potassium phosphate buffer (pH 7.0), 0.5mM ascorbate, 0.1 mM H_2O_2 and 150 μl enzyme extract. H_2O_2 -dependent oxidation of ASA was followed by measuring the decrease in absorbance within 1min at 290 (extinction coefficient of $2.8 \text{ mM}^{-1} \text{ cm}^{-1}$) (Nakano and Asada, 1981).

Total soluble proteins

Protein content was determined according to the method of Bradford (1976) using Bovine serum albumin as standard.

Statistical analysis

All the experiments were performed in triplicate (in this experiment each replicate was one Petri dish with 10 seedlings). Values indicated mean values \pm standard errors of the mean. Duncan's test was used to analyze the difference between treatments taking $p < 0.05$ as significance level.

Results

Growth parameters

As shown in Table 1, treatment of plants with 50mM NaCl had no significant effect on shoot length while it reduced the length of root. 100mM NaCl however, reduced both shoot and root length of plants. Pre-treatment of plants with 5 or 10 μM Arg improved the shoot and root growth under salinity and no-salinity conditions.

Table 1

Effects of arginine pre-treatment on shoot and root length and weight of canola plants in control and salinity stress condition. Data are means of three replicates. The mean comparisons of treatments were done using Duncan's test at $p < 0.05$ significant level. Means followed by the same letter in the same column are not significantly different.

	Control	5 μ M Arg	10 μ M Arg	50 mM NaCl	50 mM NaCl +5 μ M Arg	100 mM NaCl	100 mM NaCl +5 μ M Arg	100 mM NaCl +10 μ M Arg	
Shoot Length (cm)	3.3cd	4.83b	5.86a	2.7d	3.73c	4.66b	1.86e	2.8d	3.83c
Root Length (cm)	3.2d	4.6b	6.03a	2.26e	3.03d	3.5c	1.2f	1.9f	2.4e

Lipid peroxidation and MDA content

Results showed that salt stress at both 50 and 100 mM NaCl significantly increased the amount of MDA as an indicator of lipid peroxidation (Fig. I). Under salt stress lipid peroxidation decreased in plants which were pre-treated with 5 and 10 μ M Arg when compared with non-pre-treated plants.

Hydrogen peroxide content

As shown in Fig. II, salinity stress caused an increase in hydrogen peroxide content while Arg pre-treatment significantly decreased the amounts of H_2O_2 in leaf of canola plants. In plants which were under salt stress, priming of plants with 10 μ M Arg was more effective than 5 μ M in decrease of H_2O_2 content. Pre-treatment of plant with Arg had no significant effect on H_2O_2 content in control plants.

Antioxidant enzyme activity

The effect of salt stress on CAT, GPX, and APX in canola plant leaves, either with or without Arg pre-treatment was assayed. As is shown in Fig.(III), in plants which were under 50mMNaCl treatment the activity of GPX was higher in stressed plants in comparison with those of the control groups. Arg pre-treatment (in 5 μ M concentration) increased the activity of CAT and APX while it had no significant effect on GPX activity in comparison with non-pre-treated plants. Treatment of plants with 100mMNaCl increased the activity of all enzymes (CAT, GPX, and APX) when compared with control plants which may be a reflection of the key role

of these enzymes in ROS detoxification under these situation. As shown in Fig. (III) application of 5 μ M Arg pre-treatment decreased the activity of CAT and GPX compared with non-pre-treated plants while 10 μ M arginine pre-treatment increased the activity of CAT and APX.

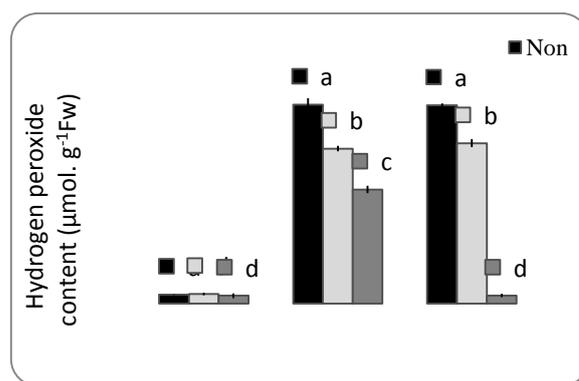


Fig. I. Effect of Arg pre-treatment on MDA content in canola plant leaves under control and salinity stress condition; Data are means \pm SE of three replicates. The mean comparisons of treatments were done using Duncan's test at $p < 0.05$.

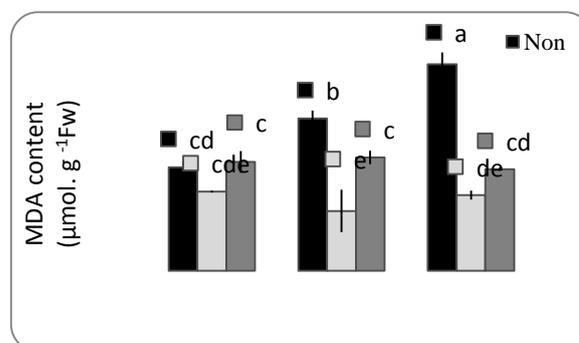


Fig. II. Effect of Arg pre-treatment on H_2O_2 content in leaves of canola plants under control and salinity stress condition; Data are means \pm SE of three replicates. The mean comparisons of treatments were done using Duncan's test at $p < 0.05$.

Discussion

Data in Table 1 make it clear that salt stress significantly decreased growth parameters as compared with untreated plants. The reduction in vegetative growth due to high salinity effect is in agreement with previous investigations on sunflower (Jabeen and Ahmad, 2012) and cowpea plants (Taffouo et al., 2009). The inhibition effects of salinity on growth parameters in canola plants might be due to the reduced water absorption, reduced metabolic activities as a result of Na^+ and Cl^- toxicity, and nutrient deficiency caused by ionic interference (Ghoulam et al., 2002 and De Lacerda et al., 2003). The results showed that pre-treatment with arginine could alleviate the harmful effect of salinity on plant height and fresh weight. These results are in agreement with those obtained by Zied (2009) who observed that arginine pre-treatment promoted growth parameters and germination percentage of bean plant under salt stress. These effects of arginine may be related to the polyamine production which has been shown to be engaged in a wide range of biological processes including growth, development, and abiotic stress responses (Kuchenbuch and Phillips, 2005).

The reactive oxygen species (ROS) increases as a response to most abiotic stresses including salinity (Sudhakar et al., 2001; Tsai et al., 2004). Excessive ROS induce overproduction of Malondialdehyde (MDA) which is one of the final products of stress-induced lipid peroxidation (Zhang et al., 2010; Joseph and Jini, 2010, 2011). Therefore, an enhanced level of MDA indicates oxidative damage in plants tissues. There are studies showing that salt stress and level of lipid peroxidation are correlated (Davenport, 2003). As shown in Fig. (I) MDA increased in canola plant under salt stress. These results correspond well with the results of H_2O_2 content measurement (Fig. II). It has been reported that salinity leads to oxidative stress through the increase of ROS such as H_2O_2 (Mudgal et al., 2010). In this experiment, when plants were pre-treated with Arg, the amounts of MDA and H_2O_2 decreased and this effect is very important for salt stress tolerance (Figs. I and II). In a previous study, our findings showed that application of exogenous arginine

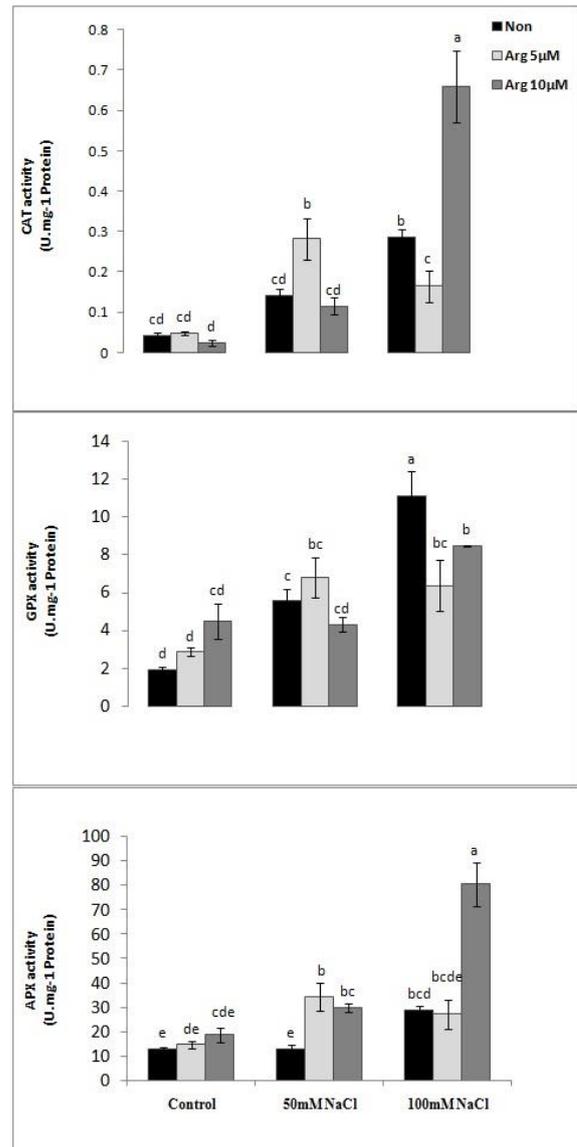


Fig. III. Effect of Arg pre-treatment on CAT, GPX, and APX activity in canola plant leaves under control and salinity stress condition; Data are means \pm SE of three replicates. The mean comparisons of treatments were done using Duncan's test at $p < 0.05$.

reduced the lipid peroxidation and hydrogen peroxide content in tomato seedling under drought stress (Nasibi et al., 2011). Zeid (2009) also reported that application of arginine could alleviate the adverse effects of salinity stress on bean seedlings. 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 it and this could result in oxidative damages (Laspina et al., 2005). In canola plants under salt stress (especially in 100 μ M NaCl), APX, GPX, and CAT activities were elevated over the controls (Fig. III); therefore, we can assume that the plant antioxidant machinery was 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). Also, the increased activity of antioxidant enzymes during increased salt stress has been reported in wheat (Sairam et al., 2002), tomato (Mittova et al., 2002), rice (Vaidyanathan et al., 2003), sugar beet (Bor et al., 2003), and maize (Azevedo-Neto et al., 2006). It has been found in the present investigation that Arg pre-treatment at 5 μ M concentration could increase the activity of CAT and APX enzymes in plants under moderate salinity (50mM NaCl) while 10 μ M Arg could increase the activity of CAT and APX in plants under 100 mM NaCl (Fig. III). Therefore, it seems that under moderate level of salinity, low concentration of Arg is more effective than high concentration while at high level of salinity, high concentration of Arg is effective. Khalil et al. (2009) found that treatment of wheat plants with arginine or putrescine activated the antioxidant defense system (SOD, CAT) and reduced the lipid damage when plants were exposed to high temperature. Nasibiet al. (2011) found that exogenous arginine treatment decreased the activity of catalase and guaiacol peroxidase while it decreased the activity of superoxide dismutase (SOD), ascorbate peroxidase, and GR 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 may be attributed to its ability to enhance accumulation of endogenous Put, proline, and NO concentrations in fruit which was primarily due to the increased arginase, ADC, ODC, OAT, and NOS activities.

The effects of Arg in this study may be attributed to the NO or polyamines as these compounds have been reported to alleviate salt stress in previous studies. For example,

treatment with NO donors is known to improve salt stress tolerance in several plant species (Siddiqui and et al., 2011). In addition, exogenously applied NO is known to induce antioxidant enzyme activity in response to high salinity conditions (Molassiotis et al., 2010). Importance of PAs in salt stress tolerance has been reported in several plant species (Alcazar et al., 2006; Liu et al., 2007). It is argued that polyamines counteract oxidative damage in plants by acting as direct free radical scavengers or through induction of antioxidant enzyme activity to scavenge free radicals (Bors et al., 1989). However, further studies are necessary to find mechanism of different pathways of arginine catabolism in stressful conditions.

Acknowledgements

We would like to acknowledge the support of Ecology Department, Institute of Science, High Technology and Environmental Science in Graduate University of Advance Technology of Kerman.

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