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Morphological traits of lentil (*Lens culinaris* Medik.) affected by ascorbic acid application and salinity stress

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Abstract

In order to investigate the effects of different salinity levels and ascorbic acid application on morphological traits of lentil (*Lens culinaris* Medik.), an experiment with randomized complete block design in three replications was conducted in 2013 in the greenhouse. Two ascorbic acid levels (Control and application of 50 ppm ascorbic acid) were tested in pot experiment under different salinity levels (0 (control), 4, 8 and 12 $dS.m^{-1}$ NaCl). Results indicated that salt stress severely reduced morphological traits and grain yield of lentil as compared with control, particularly at high NaCl level (12 $dS.m^{-1}$). Morphological traits of lentil were decreased as a result of salt stress, leading to reductions in plant growth and final grain yield. Ascorbic acid application enhanced the morphological traits and grain yield, but it did not change the number of branches per plant of the salt stressed plants and control treatment. In general, it was become clear that application of seed priming with ascorbic acid in lentil ameliorated the adverse effects of salt stress and improved morphological traits and grain yield of this plant.

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Introduction

Salinity is a common abiotic stress factor seriously affecting crop production 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 salinity (Munns, 2005). In most of the cases, the negative effects of salinity have been attributed to increase in Na^+ and Cl^- ions in different plants hence these ions produce the critical conditions for plant survival by intercepting different plant mechanisms. Although both Na^+ and Cl^- are the major ions which produce many physiological disorders in plants, Cl^- is the most dangerous (Tavakkoli *et al.,* 2010). Salinity at higher levels causes both hyper-ionic and hyperosmotic stress and can lead to plant demise. The outcome of these effects may cause membrane damage, nutrient imbalance, altered levels of growth regulators, enzymatic inhibition and metabolic dysfunction, including photosynthesis which ultimately leads to plant death (Hasanuzzaman *et al.,* 2012). Most of crop species *i.e.* bean, corn, sugarcane, potato and cabbage are sensitive to salinity (EC 1.0 - 1.8 $dS.m^{-1}$), which reduce crop productivity about 6-19 percent. In general, biochemical, physiological, morphological and anatomical characteristics of crop species directly affected by soil salinity are well established (Parida and Das, 2005).

Salinity can limit growth and plant yield in three ways including reducing osmotic potential, creating ion toxicity, causing disarrangement and imbalance of ion uptake causing disorders in enzyme activities and membrane and metabolic activities in the plant (Marschner, 1986). These processes could affect morphological parameters and plant growth and will result in reduced vegetative growth, consequently reducing plant dry weight and ultimately crop yield (Pesqueira *et al.,* 2003).

Plants use two systems to defend against and repair damage caused by oxidizing agents. First, the enzymatic antioxidant system which is mainly represented by superoxide dismutase (SOD), peroxidase (PRX), catalase (CAT) and ascorbate peroxidase (ASPX) (Harinasut *et al.,* 2000), then, the non-enzymatic antioxidant system, consisting of molecules involved in ROS (Reactive Oxygen Species) scavenging, such as ascorbic acid (vitamin C), alpha-tocopherol (vitamin B), β-carotene, glutathione (tri-peptide) (Piotr and Klobus, 2005). It has also been highlighted that improving abiotic stresses tolerance in crops is possible through genetic improvement of its antioxidant systems or following exogenous addition of antioxidants (Hung *et al.,* 2005). Ascorbic acid is involved in the regulation of many critical biological processes such as photo-inhibition and cell elongation (Noctor *et al.,* 1998). Furthermore, such positive effects of ascorbic acid in overcoming the adverse effects of salt stress were attributed to the stabilization and protection of photosynthetic pigments and the photosynthetic apparatus from oxidative damage (Hamada, 1998).

Lentil (*Lens culinaris* Medick.) is a lens-shaped grain legume well known as a high nutritious food. It grows as an annual bushy leguminous plant typically 20-45 cm tall. Lentil seed is a rich source of protein, minerals (K, P, Fe and Zn) and vitamins (Bhatty, 1988).

However, information on how ascorbic acid regulates physiological and biochemical processes in lentil plants subjected to salt stress is not much available in the literature. Thus, the main objective of the present study was to study the effects of ascorbic acid on morphological traits and grain yield of lentil under salinity stress.

Material and methods

Site description and experimental design

An experiment (using RCB design) with three replications was carried out in 2013 at the greenhouse to evaluate the effects of ascorbic acid on morphological traits and grain yield of lentil under saline conditions. Salinity stress treatment comprised non-saline (control) and three saline (4, 8 and 12 $dS.m^{-1}$NaCl) conditions and the second treatment comprised of ascorbic acid application in two levels (control and application of 50 ppm ascorbic acid).

Seeds of lentil were divided into two subsamples, one of which was kept as control (unprimed) and other sub-sample was prepared for priming. A sub-sample was soaked in freshly prepared ascorbic acid (50 ppm ≈ 0.3 mM; El-Tayeb, 1995) for 4 hours at 20^{oC}. After priming, seeds dried back to primary moisture. Twenty seeds after treating with 2 g/kg *Benomyl* were sown 2 cm deep in each pot filled with 1200 g perlite, using 24 pots in general. After emergence, seedlings were thinned to keep six plants in each pot. Salinity treatments (0, 4, 8 and 12 $dS.m^{-1}$) were applied immediately after sowing. During the growth period, the pots were weighed and the losses were made up with Hoagland solution (EC= 1.3 dS.m-1). Perlites within the pots were washed every 30 days and non-saline and salinity treatments were reapplied in order to prevent further increase in electrical conductivity (EC) due to adding Hoagland solution. Tap water (EC= 0.6 dS.m-1) and saline solutions were added to the pots in accordance with the treatments to achieve 100% FC.

Measurement of traits

At maturity, plants of each pot were separately harvested and plant height, number of branches, leaf number, stem dry weight and grain yield per plant for each treatment at each replicate was determined. The grain yield was harvested and adjusted to 12 percent moisture content.

Statistical analysis

Statistical analysis of the data based on factorial design was performed using MSTAT-C software. Duncan multiple range test was applied to compare means of each trait at the 5% probability level.

Results and discussion

Plant height of lentil was significantly affected by salinity and ascorbic acid treatments, but interaction between salinity and ascorbic acid application was not significant (Table 1). The highest plant height (31.33 cm) was obtained in control treatment (Fig. 1). Ascorbic acid treatment to salinized plants considerably increased the plant height (Table 2). Increase in salinity levels from C_1 to C_3 (4 and 12 $dS.m^{-1}$NaCl respectively), resulted in significant reduction of plant height. Salinity stress can cause osmotic stress and salt toxicity in plants leading to a reduction in growth and ultimately in yield (Ebrahim, 2005). Hussein *et al.* (2011) found that salt stress decreased all growth and yield parameters of wheat plant. Zeid *et al.* (2009) reported that exogenous ascorbic acid enhanced the productivity of wheat plants under salinity stress conditions. These effects may be attributed to the protective role of ascorbic acid in plant cells from the oxidative stress induced by salinity.

Table 1. Analysis of variance of morphological traits and grain yield of lentil affected by salinity and ascorbic acid application treatments.

S.O.V	df	Plant height	Number of branches	Leaf number	Stem dry weight	Grain yield
Block	2	3.691	1.704	56.336	0.132 *	0.007
Salinity	3	42.651 **	72.663 **	553.919 **	2.806 **	0.107 **
Ascorbic acid	1	23.949 *	9.391	322.441 **	0.176 *	0.064 *
Interaction	3	7.212	2.502	78.621	0.044	0.032
Error	14	4.353	2.561	31.442	0.029	0.012

* and ** , Significant at 5% and 1% probability level, respectively.

On the basis of our results, number of branches per plant was influenced as salinity levels increased, but ascorbic acid application had no significant effect on this trait (Table 1). Maximum and minimum number of branches per plant were achieved in control (3.48) and C_3 (0.56) treatments respectively, but there was not significant different between control with C_1 treatments (Fig. 2). Similarly Mahmoodi-Yengabad (2012) observed that salinity stress significantly decreased total biomass, plant height and leaf number. Amri *et al.* (2011) showed that the use of different degrees of exogenous can reduce the effects of salt stress on growth of *Punica granatum*. Treated seedlings under salt stressed condition did not show clear differences in plant growth, however it significantly reduced the Na^+ and Cl^- content and increased protein content. However, El-Hifny and El-Sayed, (2011) reported that foliar application of ascorbic acid significantly increased yield and its components of pepper plants.

Table 2. Mean comparisons for different traits of lentil under ascorbic acid application.

Treatment	Plant height (cm)	Leaf number	Stem dry weight (gr)	Grain yield (gr/plant)
Control	22.13 ^{b}	71.46 ^{b}	0.383 ^{b}	0.53 ^{b}
Ascorbic acid	26.54 ^{a}	78.25 ^{a}	0.442 ^{a}	0.62 ^{a}

The means with same letters in each column are not significantly different at $p \leq 0.05$.

Salinity stress and ascorbic acid application had significant effect on leaf number of lentil (Table 1). Leaf number was reduced as salinity levels increased. The highest leaf number (105.61) was observed under control treatment (Fig. 3). Plants that treated with ascorbic acid had greater leaf number when compared with control (Table 2). Munns (1993) indicated that salt in plants reduces growth by causing premature senescence of old leaves and hence reduced supply of assimilates to growing regions. Iyengar *et al.* (1977) observed reduction of leaf number per plant of barley under the influence of root zone salinity. El-Tohamy *et al.* (2008) in research on eggplant indicated that spraying with ascorbic acid had favorable effects on yield particularly with the higher concentration. This might be due to increasing of endogenous promoting hormones in plant (Abd El-Halim, 1995) in addition to stimulating of plant growth and CO_2 as well as microbial activity.

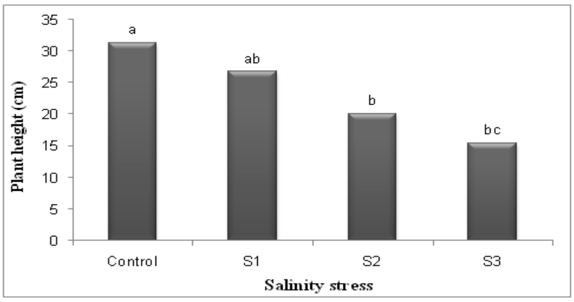

Fig. 1. Effect of different salinity stress treatment levels (non-saline (control) and three saline levels (S1, S2 and S3: 4, 8 and 12 $dS.m^{-1}$NaCl) on plant height of lentil (Different letters indicate significant differences at $p \leq 0.05$).

Analysis of variance indicated that salt stress and ascorbic acid application significantly affected stem dry weight, but their interaction had no effect on this trait (Table 1). Stem dry weight was reduced as salinity stress increased. Maximum and minimum stem dry weight were achieved in control (0.71 gr) and C_3 (0.25 gr) treatments respectively (Fig. 4). A 15% increase in stem dry weight occurred in plants treated with ascorbic acid compared to untreated plants (Table 2). Under high saline conditions water uptake by plants was reduced due to soil osmotic potential (Jamil *et al.,* 2007). Reduction in plant shoot and root dry matter is due to combined effects of osmotic and Cl^- and Na^+ ions (Hajer *et al.,* 2006). Ascorbic acid also beneficially influenced damage reduction caused by salt. This may be due to salinity resulting in increased activity of reactive oxygen species (ROS) which may cause severe cellular damage. One proposed biochemical mode of ascorbate is to act as an antioxidant scavenging hydrogen peroxide (Beltagi, 2008).

Fig. 2. Effect of different salinity stress treatment levels (non-saline (control) and three saline levels (S1, S2 and S3: 4, 8 and 12 $dS.m^{-1}$NaCl) on number of branches per plant (Different letters indicate significant differences at $p \leq 0.05$).

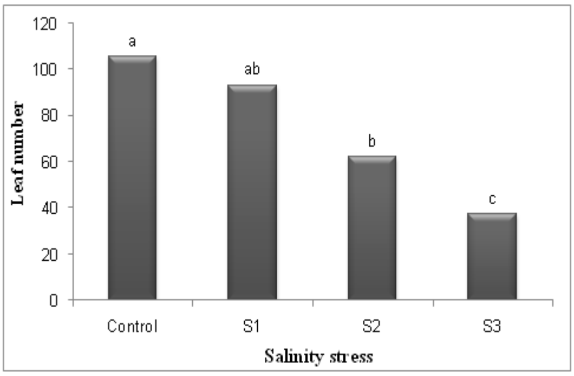

Fig. 3. Effect of different salinity stress treatment levels (non-saline (control) and three saline levels (S1, S2 and S3: 4, 8 and 12 $dS.m^{-1}$NaCl) on leaf number of lentil (Different letters indicate significant differences at p≤ 0.05).

Fig. 4. Effect of different salinity stress treatment levels (non-saline (control) and three saline levels (S1, S2 and S3: 4, 8 and 12 $dS.m^{-1}$NaCl) on stem dry weight of lentil (Different letters indicate significant differences at p≤ 0.05).

Results indicated that, grain yield of lentil was significantly affected by salt stress and ascorbic acid application, but interaction of salinity and ascorbic acid was not significance for this trait (Table 1). Maximum grain (0.71 gr/plant) was obtained from control; Irrigation with tap water (EC= 0.6 dS.m-1), and the minimum grain yield (0.31 gr/plant) obtained from C_3 salinity stress treatment, respectively (Fig. 5). In general, grain yield was considerably reduced, as the intensity of salt stress increased. Priming of seeds with ascorbic acid mitigated effects of salinity. Priming seed with 50 ppm ascorbic acid produced more grain yield than control (Table 2). Grain soaking in ascorbic acid had an inhibitory effect on the accumulation of sodium in different organs under various concentrations of NaCl (Al-Hakimi and Hamada, 2001). Helsper *et al.* (1982) observed that application of ascorbic acid at 1000 mg/L increased wheat grain yield by 15.35 and 15.64 % when compared with control plants. The positive response of wheat plants may be due to the effect of ascorbic acid on some enzymes which are important in regulation of photosynthetic carbon reduction. Hussein *et al.* (2011) reported that increasing the rate of spraying of ascorbic acid from 0 to 200 ppm and under different levels of salinity water led to increase in wheat spikes yield in most cases. Ascorbic acid could be used as a potential growth regulator to improve salinity stress resistance in several plant species (Sheteawi, 2007). Also, ascorbic acid would inhibit stress-induced increases in the leakage of essential electrolytes following per-oxidative damage to plasma membranes (McKersie *et al.,* 1999).

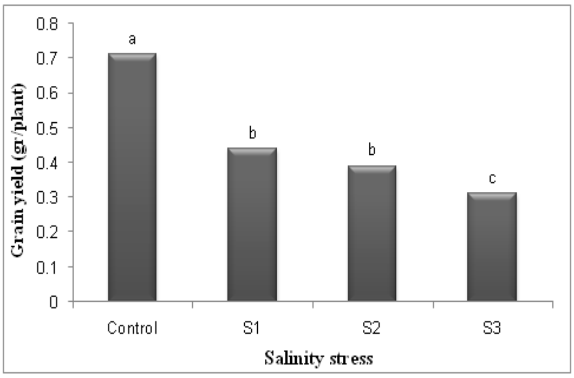

Fig. 5. Effect of different salinity stress treatment levels (non-saline (control) and three saline levels (S1, S2 and S3: 4, 8 and 12 $dS.m^{-1}$NaCl) on grain yield per plant of lentil (Different letters indicate significant differences at p≤ 0.05).

Conclusion

Results of this study indicated that, morphological traits and grain yield of lentil considerably reduced under moderate and severe salinities, but the extent of this reduction varied among salinity levels. Therefore, lentil is a sensitive crop to salinity and acceptable yield of this crop can be obtained under non-saline conditions. Priming seed with ascorbic acid reduced adverse effects of salt stress and increased yield and morphological traits of lentil. Therefore, negative effects of salinity on lentil morphological traits, plant growth and grain yield can be reduced by use of 50 ppm ascorbic acid.

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