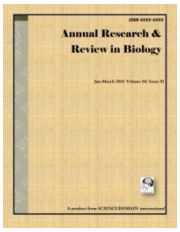

Annual Research & Review in Biology
4(24): 3656-3665, 2014

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Ascorbic Acid and It'S Effects on Alleviation of Salt Stress in Sunflower

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Author's contribution

This whole work was carried out by author SNF.

Original Research Article

Received 10th March 2014
Accepted 8th May 2014
Published 30th June 2014

ABSTRACT

Salinity, a severe environmental factor, has limited the growth and productivity of crops. Many compounds have been applied to minimize the harmful effects of salt stress on plant growth. A laboratory experiment was carried out to assess the effects of seed priming with ascorbic acid (ASA) on germination characteristics of sunflower (*Helianthus annuus* L.). The seeds were treated with different concentrations of ASA (0, 1 and 2 mM) before germination, then the primed seeds were germinated under salinity stress conditions using different concentrations of NaCl (0, 100 and 200 mM). Some germination characteristics including germination percentage, germination rate, seed stamina index, hypocotyl dry weight, radicle dry weight, relative water content and ion leakage were measured after imposing salinity stress for 8 days. Salinity stress caused a significant reduction in germination and seedling growth of sunflower. Seeds primed with various concentrations of ascorbic acid proved to be effective in salinity tolerance at the germination stage of sunflower. In general, priming with 2 mM ascorbic acid was more effective than the other concentrations.

Keywords: Ascorbic acid; germination; salinity stress; sunflower.

1. INTRODUCTION

Salt stress, similar to many abiotic stress factors, is known to induce oxidative damage toplant cells from reactive oxygen species that affect the physiology and biochemistry of

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plants and that can lead to a reduction in plant yield [1]. Seven percent of the land's surface and 5% of cultivated lands are affected by salinity, and it is an important factor which can limit the growth and productivity of plants [2]. The inhibitory effect of salinity stress is largely due to the ionic and osmotic stress [3].

Exogenous application of plant growth regulators, fertilizers, osmoprotectants and antioxidants have been reported to successfully alleviate the adverse effects of salt stress on plants [4,5]. Ascorbic acid (AsA) is an important antioxidant in plants which accumulates in plants as an adaptive mechanism to environmental stress such as salinity. AsA regulates stress response as a result of a complex sequence of biochemical reactions such as activation or suppression of key enzymatic reactions, induction of stress responsive proteins synthesis, and the production of various chemical defense compounds [6].

Seed priming, a controlled hydration technique that allows the pre-germination metabolisms without actual germination [7], is one of the most pragmatic and short-term approaches to combat the effects environmental stresses on seedling emergence and stand establishment [8,9]. Ascorbic acid significantly reduced the effect of salinity in sunflower [10]. The seed pretreatment with ascorbic acid could be applied by growers just before seeding and enable the plants to establish in saline soils [11]. In Fenugreek, Salt stress on seedlings could by significantly reduced by the use of an ascorbic acid pretreatment of seeds [11]. The reduction in harmful effects of salt on seedling growth should prove useful to growers. When the plants were exposed to the highest levels of NaCl, the lowest levels of AsA were not beneficial; the highest level of AsA worked better however AsA in different concentrations helped the plants under salt stress in *Lactuca sativa* [12]. Other works also demonstrated that seed priming with ascorbic acid before sowing ameliorated the negative changes of NaCl- stress on productivity and yield parameters [13]. The objective of this study was to investigate the effects of seed priming with AsA on the some germination parameters of sunflower (*Helianthus annus* L.) plants under saline conditions.

2. MATERIALS AND METHODS

This experiment was carried out to study the effect of seeds priming with different concentrations of ascorbic acid on germination and seedling growth of sunflower under different salinity levels. According to Saiednejad et al. [14] the seeds were surface sterilized by immersion in 0.5% sodium hypochlorite (NaOCl) solution for 5 min to prevent fungal infections and then washed three times with sterile, distilled water to remove any NaOCl residue. After washing, the seeds were previously treated with ascorbic acid by soaking for 24 h at room temperature in an ascorbic acid solution at 0, 1, and 2 mM. To determine the growth and effects of ascorbic acid seed treatment on sunflower seeds and seedlings under different levels of salt stress, the seeds pretreated with ascorbic acid were exposed to 0, 100 and 200 mM NaCl during germination and seedling development. Seed germination was recorded daily up 7 days after the start of the experiment. At the end of experiment, plants were harvested to measure the germination characteristics. To measure the dry weight, seedlings were dried in aerated oven at 65ºC for 48 h. Germination percentage, Germination rate and seed stamina index were calculated with the following formula:

$G = (n \div N) \times 100$ [15]
$RG = \Sigma (Ni \div Di)$ [15]
$SSI = [G \times (HL + RL)] \div 100$ [16]
$RWC = [(FW-DW) \div (TW-DW)] \times 100$ [17]

G: germination percentage, n: number of seeds germinated, N: total number of seeds in each Petri dish, RG: rate of germination (seed/day), Ni: germinated seed in each numeration, Di: day of each numeration, SSI: seed stamina index, HL: hypocotyl length, RL: radicles length, RWC: relative water content, FW: fresh weight, DW: dry weight, TW: turgid weight.

All data were analyzed using SAS software [18]. Significant differences among the mean values of treatments were compared by the least significant difference (LSD) test method at 5% using the MSTAT-C computer program.

3. RESULTS

Compared with control germination percentage was reduced significantly by salinity stress, so that the highest level (200 mM) reduced it by 48.2% (Table 1). Pretreatment with ascorbic acid (ASA) markedly reduced the harmful effects of salinity stress and also improved all the measured parameters significantly. Results showed that only concentration of 2 mM ASA significantly affected germination percentage compared to control, and the highest germination percentage (84.04%) recorded for 2 mM of ASA (Table 2). The response of germination percentage to ASA concentrations × salinity levels were the same statistically (Fig. 1).

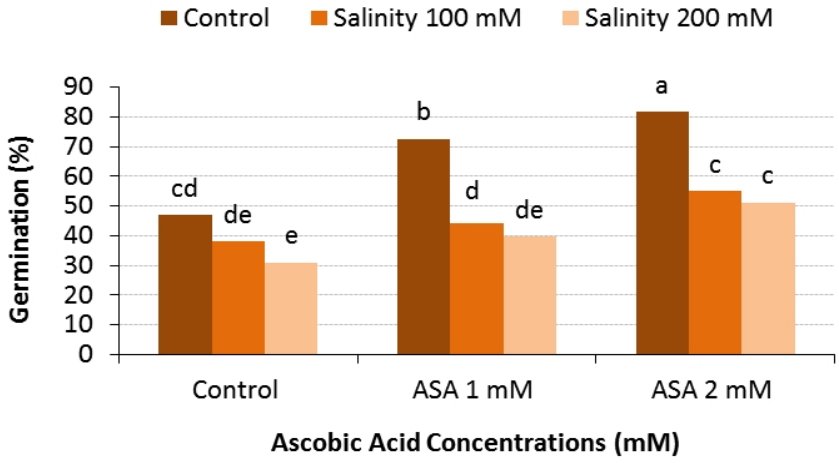

Fig. 1. The effect of ASA on germination percentage of Sunflower under salinity stress

The response of germination rate to salinity and ASA levels was the same with germination percentage (Tables 1 and 2). The germination rate of Sunflower at the level of control was 30 seed day^{-1}. Significant reduction in germination rate started with lower concentration of salinity (100 mM) and it decreased as salinity levels were increasing. Application of first level of ASA (1 mM) affected the germination rate significantly; also seeds pretreated with 2 mM of ASA indicated a better response in all salinity levels compared to control (Fig. 2). Based on these results, it seems that concentrations of 1 and 2 mM of ASA ameliorate germination rate.

Table 1. Mean Comparison of Sunflower germination characteristics under salinity stress

Relative water content (%)	Seedling dry weight (gr)	Seedling fresh weight (gr)	Radicle length (cm)	Hypocotyl length (cm)	Seed stamina index	Germination rate (seed/day)	Germination (%)	Salinity levels
80.68 a	0.0414 a	0.0684 a	5.1 a	5.23 a	5.27 a	30.79 a	96.5 a	Control
66.47 b	0.0316 b	0.0413 b	4.6 b	4.56 b	4.02 a	24.08 b	78.26 b	100mM
63.03 b	0.0189 c	0.0265 c	4.5 b	2.63 c	0.13 b	12.63 c	49.51 c	200mM

Variants possessing the same letters (a, b and c) are not statistically significant at P<0.05 level, according to LSD test

Table 2. Effect of Ascorbic acid on germination and seedling growth of Sunflower

Relative water content (%)	Seedling dry weight (gr)	Seedling fresh weight (gr)	Radicle length (cm)	Hypocotyl length (cm)	Seed stamina index	Germination rate (seed/day)	Germination (%	ASA levels
73..14 c	0.0117 b	0.0296 b	3.42 b	2.78 b	2.86 c	20.61 b	71.21 b	Control
75.72 b	0.0163 a	0.0406 a	5.56 a	5.12 a	3.26 b	21.03 b	70.73 b	1 mM
77.84 a	0.0189 a	0.0433 a	5.51 a	5.12 a	3.50 a	24.67 a	82.14 a	2 mM

Variants possessing the same letters (a, b and c) are not statistically significant at P<0.05 level, according to LSD test

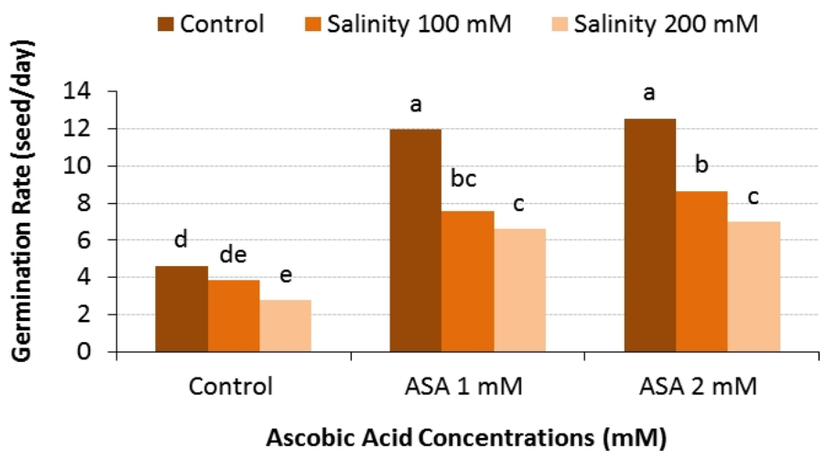

Fig. 2. The effect of ASA on germination rate of Sunflower under salinity stress

The effect of salinity levels on SSI was significant (Table 1). The highest value of SSI belonged to the salinity level of 0 mM (control), whereas the lowest value belonged to 200 mM treatment. The effect of ASA pretreatment on SSI was also statistically significant. Increasing concentration of ASA caused a remarkable increase in the mentioned trait. The greatest increase in SSI was observed in the concentration of 2 mM ASA as 47.6% compared to control (Table 2). Interaction between salinity stress and ASA pretreatment indicated that priming with 1 and 2 mM ASA had the same effect on SSI under normal and stress conditions (Fig. 3).

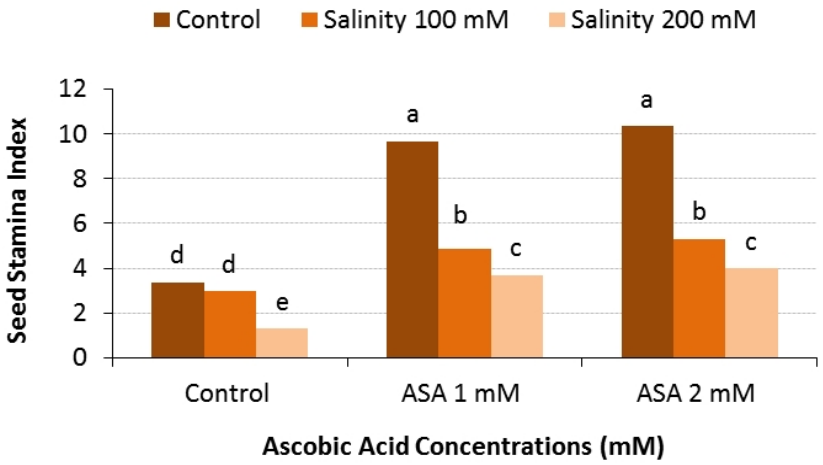

Fig. 3. The effect of ASA on SSI of Sunflower under salinity stress

The effect of salinity levels on hypocotyl length of Sunflower is shown in Table 1. Hypocotyl length of Sunflower decreased significantly ($p<0.0001$) with increasing in salinity concentration. The first applied level of salinity resulted in a 6.8% reduction in hypocotyl length, but the reduction value for the higher concentration was 98.3%.

The interaction effects of salinity and ASA on hypocotyl length presented in Fig. 4. The response of this trait varied at different levels of salinity with different concentration of ASA. It shows that the concentration of 1 and 2 mM of ASA were effective in the all salinity levels in compared to control.

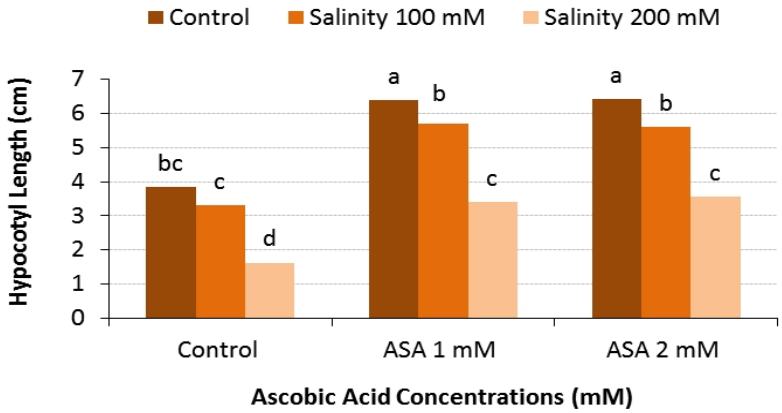

Fig. 4. The effect of ASA on hypocotyl length of Sunflower under salinity stress

Comparison between salinity levels showed that application of 100 and 200 mM of salinity decreased the mean of this trait (Table 1). As Table 2 shows the highest and lowest radicle length obtained from concentration of 0 and 2 mM of ASA respectively. Interaction between salinity stress and ASA pretreatment indicated that priming with 2 mM ASA had the highest radicle length under normal and stress condition (Fig. 5).

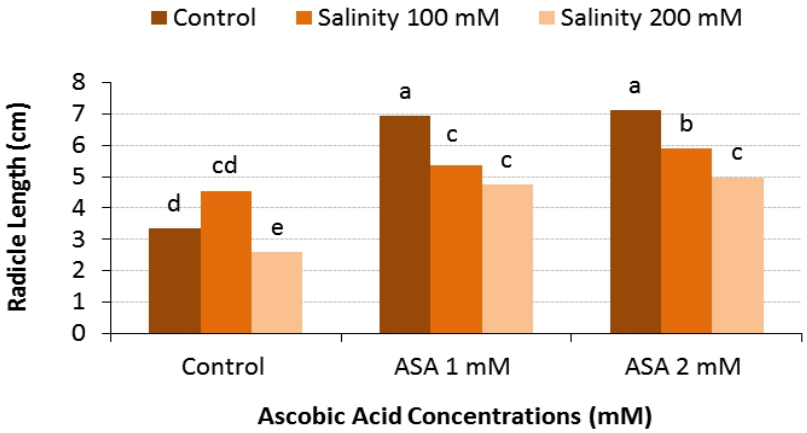

Fig. 5. The effect of ASA on radicle length of Sunflower under salinity stress

The effects of salinity stress on hypocotyl and radicle dry weight of Sunflower were shown in Table 1. On the basis of these results, increasing salinity levels caused remarkably decreases in hypocotyl and radicle dry weight (Table 1). All the levels of salinity stress were significantly different with each other. So that, the highest hypocotyl dry weight (0.0784 g) and radicle dry weight (0.0394 g) were recorded for control treatment, they were decreased by increasing salinity. ASA had a significant effect on hypocotyl and radicle dry weight of Sunflower (Table 2). The difference in these traits was statistically significant between control and all concentrations of ASA. The concentration of 1 mM ASA caused an increment of 57.7% and 43.3% in hypocotyl and radicle dry weight compared to control respectively. The interaction of salinity and ASA showed that treatment of sunflower seeds with ASA could prevent the decrease in hypocotyl and radicle dry weight caused by salinity stress. Moreover, seedling pretreatment with 2 mM ASA had the highest of these traits under normal and stress conditions (Figs. 6 and 7).

As shown in Table 1, relative water content was reduced significantly as salinity increasing. The highest value of relative water content belonged to control, but the lowest of this trait belonged to 200 mM treatment. The effect of ASA pretreatment on relative water content was statistically significant. Increasing concentration of ASA caused remarkably increase in the mentioned trait (Table 2). The most increase in relative water content was observed in the concentration of 2 mM ASA as 8.1% compared to control. Interaction between salinity stress and ASA pretreatment indicated that priming with 1 and 2 mM ASA was more effective than 0 mM ASA (control) under normal and salinity stress condition (Fig. 8).

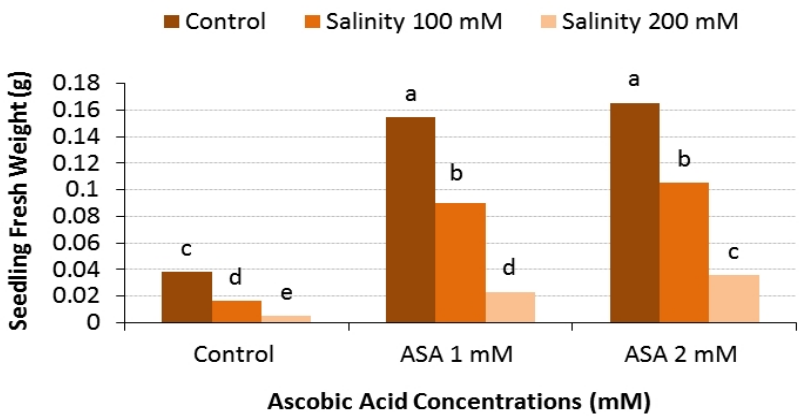

Fig. 6. The effect of ASA on hypocotyls dry weight of Sunflower under salinity stress

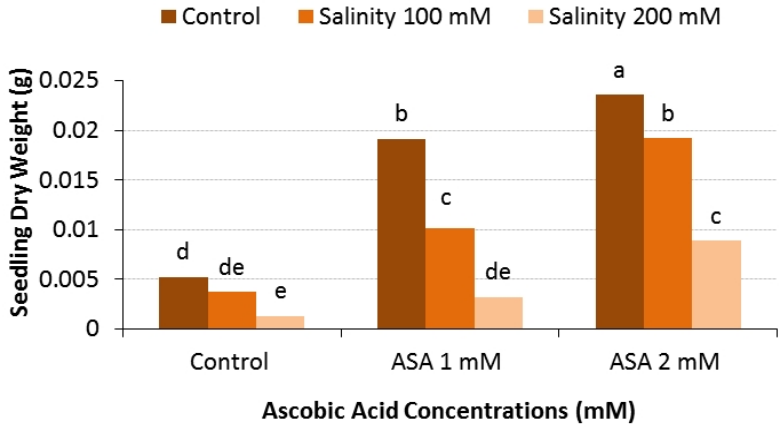

Fig. 7. The effect of ASA on radicle dry weight of Sunflower under salinity stress

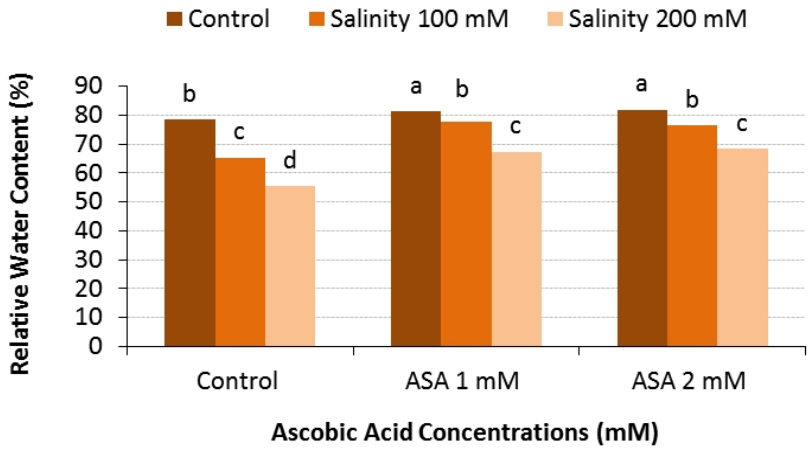

Fig. 8. The effect of ASA on relative water content of Sunflower under salinity stress

4. DISCUSSION

In the present study, salt stress caused significant inhibition in emergence and growth of sunflower seedlings. Similar results were reported by Tejera et al. [19,20], who indicated that growth of common bean plants considerably decreased by salt stress. Salinity can inhibit seedling growth by altering the water potential, increasing the ion toxicity, inhibiting the cell division and cell expansion, or causing an ion imbalance [21] .In this context, Younis et al. [22] reported that the growth reduction caused by salinity stress is due to inhibited apical growth in plants as well as endogenous hormonal imbalance. In both cases, reduction could have been caused by the toxic effects of ions on metabolism or from adverse water relations. In addition, a secondary aspect of salinity stress in plants is the stress-induced production of ROS [23]. The enhanced production of ROS during salinity stress lead to the progressive oxidative damage and ultimately cell death and growth suppression [24].

ASA is one of the most important antioxidants protecting plants from oxidative stress [25]. It is also involved in regulating photosynthetic capacity, flowering and senescence [26], also counteracting adverse effects of salt stress in tomato [4] and wheat [27]. It may have been due to increase in cell division and/or cell enlargement, although these phenomena were not examined in the present study. Furthermore, there are some reports which provide evidence that ASA accelerates cell division and cell enlargement as observed in different plants such as Pisum [28], and *Lupinus albus* [29]. These findings and the results of the present study suggest that growth promoting effect of ASA under salinity or control conditions may have been due to enhanced antioxidant capacity, and increase in cell division and cell enlargement. There is evidence that ASA has an important role in stomata regulation [30]. However, detailed information on how ASA can cause changes in photosynthesis remains to be elucidated. The ability of ascorbate to lose or donate electrons to produce MDHA is the basis of its biologically useful antioxidant capacity [31]. Endogenous ASA can be increased by exogenous application of ASA through the rooting medium [30], as a foliar spray or as seed priming.

From present investigation, it is quite clear that seeds primed with various concentration of ascorbic acid proved to be effective in including salt tolerance in the germination stage in Sunflower. The results showed that hormonal priming with 2 mM ascorbic acid in sum of measured traits was more effective than other concentrations. Hypocotyl and radicle's dry weight were increased in seedlings raised from seeds primed with ascorbic acid.

5. CONCLUSION

The results revealed that inhibition of germination in salinity stress condition probably resulted from osmotic effect. Seeds primed with ASA gave better performance than control (non-primed) under salinity stress condition. It seems priming increased the tolerance of seeds to salinity stress, therefore it can be concluded that priming is a simple, cheap and unsophisticated tool that has a practical importance and could be recommended to farmers to achieve higher germination and uniform emergence under field conditions.

COMPETING INTERESTS

Author has declared that no competing interests exist.

REFERENCES

1. Azevedo-Neto D, Prisco J, Eneas J, De Abreu C, Gomes E. Effect of salt stress on antioxidative enzymes and lipid peroxidation in leaves and roots of salt-tolerant and saltsensitive maize varieties. Environ. Exp. Bot. 2006;56:87-94.
2. Flowers TJ, Troke PF, Yeo AR. The mechanism of salt tolerance in halophytes. Ann. Rev. Plant Physiol. 1977;28:89–121.
3. Magome H, Yamaguchi S, Hanada A, Yuji Kamiya Y, Oda K. The DDF1 transcriptional activator upregulates expression of a gibberellin-deactivating gene, GA2ox7, under high-salinity stress in Arabidopsis. Plant J. 2008;56:613–626.
4. Shalata A, Neumann PM. Exogenous ascorbic acid (Vitamin C) increases resistance to salt tolerance and reduced lipid peroxidation. J. Exp. Bot. 2001;364:2207–2211.
5. Khadri M, Tejera NA, Lluch C. Sodium chloride interaction in two common bean (*Phaseolus vulgaris*) cultivars differing in salinity tolerance. Environ. Exp. Bot. 2007;60:211-218.
6. Khan TA, Mazid M, Mohammad F. A review of ascorbic acid potentialities against oxidative stress induced in plants. J. Agrobiology. 2011;28(2):97–111.
7. Bradford KJ. Manipulation of seed water relations via osmotic priming to improve germination under stress conditions. Hort. Sci. 1986;21:1105–1112.
8. Farooq M, Aziz TM, Hussain H, Rehman K, Jabran, Khan MB. Glycinebetaine improves chilling tolerance in hybrid maize. Agron. Crop Sci. 2008;194:152–160.
9. Jafar MZ, Farooq M, Cheema MA, Afzal I, Basra SMA, Wahid MA, Aziz T, Shahid M. Improving the performance of wheat by seed priming under saline conditions. Agron Crop Sci. 2012;198:38–45.
10. Khan A, Long I, Amjid M, Shah A, Ahmad I, Nawaz H. Inducing salt tolerance in growth and yield of sunflower (*Helianthus annuus* L.) by applying different levels of ascorbic acid. J. Plant Nut. 2013;36:1180-1190.
11. Behairy R, El-Danasoury TM, Craker L. Impact of ascorbic acid on seed germination, seedling growth, and enzyme activity of salt-stressed fenugreek, J. Med. Active Plants. 2012;1(3):106-113.
12. Kris ME. Does Ascorbic Acid Reduce Salt Stress in *Lactuca sativa* Plants? California State Science Fair; 2007.
13. Mohsen AA, Ebrahim MKH, Ghoraba WFS. Effect of salinity stress on *Vicia faba* productivity with respect to ascorbic acid treatment. Iranian J. Plant Physio. 2013;(3):725-736.
14. Saeidnejad A. H, Pasandi Pour A, Pakgohar N, Farahbakhsh H. Effects of Exogenous Nitric Oxide on Germination and Physiological Properties of Basil under Salinity Stress. J. Med. Plants By-products. 2013;1:103-113.
15. Jefferson LV, Penachchio M. Allelopathic effects of foliage extracts from four *Chenopodiacea* species on seed germination. J. Arid. Enviro. 2003;55:275-285.
16. Abdul-baki AA, Anderson JD. Viability and leaching of sugars from germinating barely. Crop Sci. 1970;10:31-34.
17. Weatherley PE. Studies in water relations of cotton plants. I. the field measurement of water deficit in leaves. New Phytol. 1950;49:81-/87.
18. SAS Institute Inc, SAS Users Guide, Statistical Analysis Institute Inc. Cary, North Carolina, SAS Users Guide; 1997.
19. Tejera NA, Campos R, Sanjuan J, Lluch C. Nitrogenase and antioxidant enzyme activities in Phaseolus vulgaris nodules formed by Rhizobium tropici isogenic strains with varying tolerance to salt stress. J. Plant Physiol. 2004;161(3):329-338.

20. Tejera NA, Campos R, Sanjuan J, Lluch C. Effect of sodium chloride on growth, nutrient accumulation, and nitrogen fixation of common bean plants in symbiosis with isogenic strains. J. Plant Nut. 2005;28:1907-1921.
21. Arshi A, Abdin MZ, Iqbal M. Ameliorative effects of $CaCl_2$on growth, ionic relations and proline content of senna under salinity stress. J. Plant Nut. 2005;28:101-125.
22. Younis ME, Hasaneen MNA, Kazamel AMS. Exogenously applied ascorbic acid ameliorates detrimental effects of NaCl and mannitol stress in Vicia faba seedlings. Protoplasma. 2010;239:39-48.
23. Manchanda G, Garg N. Salinity and its effects on the functional biology of legumes. Acta Physiol. Plant. 2008;30:595-618.
24. Ruiz-Lozano J, Porcel R, Azco´n C, Aroca R. Regulation by arbuscular mycorrhizae of the integrated physiological response to salinity in plants: new challenges in physiological and molecular studies. J. Exp. Bot. 2012;63(11):4033–4044.
25. Smirnoff N. Ascorbate, tocopherol and carotenoids: metabolism, pathway engineering and functions. In: Smirnoff N, (ed) Antioxidants and Reactive Oxygen Species in Plants. Blackwell Publishing Ltd., Oxford; 2005.
26. Davey M, Montagu WMV, Inze D, Sanmartin M, Kanellis A, Smirnoff N, Benzie IJJ, Strain JJ, Favell D, Fletcher J. Plant l-ascorbic acid: chemistry, function, metabolism, bioavailability and effects of processing. J. Sci. Food Agric. 2000;80:825–860.
27. Al-Hakimi AM, Hamada AM. Counteraction of salinity stress on wheat plants by grain soaking in ascorbic acid, thiamine or sodium salicylate. Biol. Plant. 2001;44:253–261.
28. Cabo RC, Gonza-lez-Reyes JA, Cordoba F, Navas P. Rooting hastened in onions by ascorbate and ascorbate free radical. J Plant Growth Regul. 1996;15:53–56.
29. Citterio S, Sgorbati S, Scippa S, Sparvoli E. Ascorbic acid effect on the onset of cell proliferation in pea root. Physiol. Plant. 1994;92:601–607.
30. Chen Z, Gallie DR. The ascorbic acid redox state controls guard cell signaling and stomatal movement. Plant Cell. 2004;16:1143–1162.
31. Buettner GR, Schafer FQ. Ascorbate as an antioxidant in vitamin C. In: Asard H, May JM, Smirnoff N. (ed) Functions and Biochemistry in Animals and Plants. Bios Scientific Publishers, Oxford; 2004.

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