Biological Forum – An International Journal **7**(1): 1389-1394(2015)

ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

Investigation of the Effects of the Pre-Treatment of Seed with Ascorbic Acid on Some Physiological and Biochemical characteristics of Wheat Seedlings under Salinity Stress Conditions

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(Received 29 March, 2015, Accepted 07 May, 2015)
(Published by Research Trend, Website: www.researchtrend.net)

ABSTRACT: In order to investigate the effect of pre-treatment of seeds with ascorbic acid on the a, b and a+b chlorophyll content, proline and wet weight of wheat plumule (*Triticum aestivum* L.) under salinity stress, a study of Pishgam cultivar was performed three replication with a factorial experiment in the form of a completely randomized design. An acid ascorbic factor in three concentrations of 0, 50 and 100 mM and salinity in three levels of 0, 75 and 150 mM was used in crop plants in the laboratory of Islamic Azad University, Mahabad Branch. The results indicated that by increasing the ascorbic acid up to 100 mM, a, b and a+b chlorophyll content was significantly increased. Ascorbic acid treatment in concentrations of 50 and 100 mM moderated the decrease of wheat a, b and a+b chlorophyll content in salinity stress conditions. By increasing the concentration of ascorbic acid up to 100 mM, the proline content was significantly decreased. At the same time, the increase of saline concentration up to 150 mM increased the proline of wheat seedlings. In fact, the use of ascorbic acid decreases the proline activation and moderates the effects of salinity stress in wheat.

Keywords: Salinity, Proline, Chlorophyll, Ascorbic Acid

INTRODUCTION

One of the most important and critical stages in plant growth is germination, which is seriously limited under salinity stress conditions. Irrigation with inappropriate or saline water is one of the most important factors in salt increase; the soil becomes saline and as a result creates salinity in the plant (Mirmohammadi Meybodi and Ghare Yazi, 2002). Priming is a method that seeks the proper solutions to increase the percentage of seed germination rate and especially to create homogeneous germination and uniformity in the farms (Farooq *et al.,* 2005). The most significant effects of priming include the primary establishment of the plant, seed weight increase and biomass, improving the food quality and water use efficiency, and decreasing the damage caused by pathogens (Afzal *et al.,* 2005). One of the useful effects of priming on the seed and most of the plant is the increase in the germination percentage and rate, improving viability, increasing the germination in lower temperatures, and encouraging better germination in salinity conditions (Afzal *et al.,* 2005). Applying ascorbic acid before salinity stress leads to recycling and better survival of tomato seedlings (Shalata and Neumann, 2001).

Germination and the rapid emergence of seedlings are important factors in the successful establishment of plants. It has been reported that among the various techniques that can be used before planting, treatment of seed (priming) is simple, cheap, has a lower risk percentage, and is more effective. It leads to raised plant tolerance for germination and establishment in stressful environments (Ashraf and Harris, 2004). Harris *et al.,* (2007) stated that in soils that lack moisture and micronutrients, seed priming can accelerate the emergence of seedlings, encourage uniformity, and improve seed performance. The growth hormones that are normally used for seed priming include auxins (NAA-IBA-IAA), gibberellins (GA), kinetin, abscisic acid, polyamines, ethylenes, salicylic acid and ascorbic acid (Ashraf and Foolad, 2005). Ascorbic acid is a little that can be dissolved in water and has strong antioxidant properties. It is used as an enzyme substrate in cycles, for detoxification, and to neutralize superoxide radicals and singlet oxygen (Noctor and Foyer, 1998). It is known that ascorbic acid has some roles in plants growth aspects such as cell division and enlargement, development of cellular wall, and other physiological procedures (Buettner and Schafer, 2004).

The purpose of the present research is to investigate the effect of ascorbic acid on some physiological properties of wheat under salinity stress conditions.

MATERIALS AND METHODS

The present research was done in 2013 using Pishgam cultivar with a factorial experiment in the form of a completely randomized design in three replication. The study was done three times with acid ascorbic factors in the research laboratory of the Agriculture Faculty of Islamic Azad University, Mahabad Branch. In order to perform the study, healthy and similar seeds were first chosen and disinfected by fungicides. After disinfection, the seeds were immediately leached with distilled water three times. Salinity treatment was applied by sodium chloride salt (NaCl) in three levels of 0 (control), 75 and 150 mM, and three concentrations of ascorbic acid 0 (control), 50 and 100 mM were added to the Petri dishes containing wheat seeds. Treatment with ascorbic acid continued for 24 hours. To carry out the test, disposable and disinfected 12 cm Petri dishes were used. Then, the ascorbic acid treatments were separately applied to each Petri. Next, the treated Petri dishes were kept in that condition for 24 hours. After ending the treatment, the seeds were taken out of the Petri dishes and leached with distilled water. A sterile filter paper was placed in each Petri dish and different salinity concentrations ranging from 3 to 5 ml were added. Finally, the Petri dishes were closed with Para film and placed in a germinator. The a, b and a+b chlorophyll, proline, and wet weight of the wheat plumule were measured. Chlorophyll measurement was done using the method of Sestak and Catsky (1996), and proline measurement was performed using the method of Bates *et al.*, (1973). Comparison of means was done with a Duncan test at the 5 % level. Data analysis was done with SPSS software version 13.0 (SPSS, 2004), and the diagrams were drawn using Excel software.

RESULTS AND DISCUSSION

A. Chlorophyll a

The results of variance analysis showed that there was a significant difference ($p<0.01$) between the concentrations of ascorbic acid, salinity and interaction effect ($p<0.05$) on chlorophyll a content (Table 1).

Table 1: Analysis of variance of the properties of the wheat being investigated under ascorbic acid and salinity.

Source of variation	df	Mean Square				
		Chlorophyll a	Chlorophyll b	Chlorophyll a+b	Proline	Wet weight of plumule
Ascorbic acid (A)	2	1.98 **	1.099 **	5.30 **	797.57 **	314.41 **
Salinity (B)	2	5.92 **	0.96 **	13.11 **	2678.9 **	1020.6**
A×B	4	0.139 *	0.145	0.446 *	47.19	24.24
error	18	0.041	0.055	0.099	23.35	13.95
C.V (%)		12.30	25.44	12.29	14.87	18.46

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

The results of comparing the mean of treatment combinations of ascorbic acid and salinity indicated that the application of 100 mM of ascorbic acid and the non-application of salinity led to the maximum extent of chlorophyll a. The minimum extent of chlorophylla was observed at the concentration of 150 mM of salinity with no application of ascorbic acid (Fig. 1).

Sairam *et al.*, (2002) stated that in salinity conditions there was a significant correlation between the extent of chlorophyll and the performance of the seedling, and that the chlorophyll content was decreased by increasing the salinity.

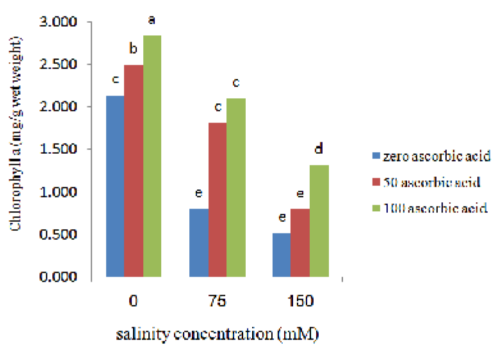

Fig. 1. Effect of ascorbic acid and salinity on chlorophyll a content in wheat (Duncan 5 %)

The activation extent of chlorophyllase increases under salinity stress (Ranjan *et al.,* 2001). The chlorophyll a extent is decreased due to the stress effect on the wheat's Pishtaz (Tale Ahmad and Haddad, 2010).

B. Chlorophyll b

The results of variance analysis of the data indicated that there was a significant difference ($p<0.01$) between the concentrations of ascorbic acid and salinity in terms of effect on chlorophyll b content. The interaction effect of ascorbic acid and salinity was not significant. In fact, the simultaneous application of ascorbic acid and salinity is not effective on chlorophyll b content (Table 1).

The results of comparing the means of ascorbic acid levels indicated that by increasing the concentration of the ascorbic acid up to 100 mM, the content of chlorophyll b could be significantly increased (Fig. 2), while by increasing the salt concentration up to 150 mM the chlorophyll b content of the wheat seedlings was significantly decreased (Fig. 3).

Under stress conditions, the decrease of photosynthesis extent includes a disruption of biochemical reactions (Oncel *et al.,* 2000). Under stress conditions, the light-receiving complexes become more damaged, and this leads to a serious decrease of chlorophyll b in chloroplast and the increase of the a-to-b ratio under stress conditions (Oncel *et al.,* 2000).

Salinity stress disrupts the absorption of some essential elements such as iron and magnesium, which are essential in chlorophyll synthesis (Neocleus and Vasilakakis, 2007).

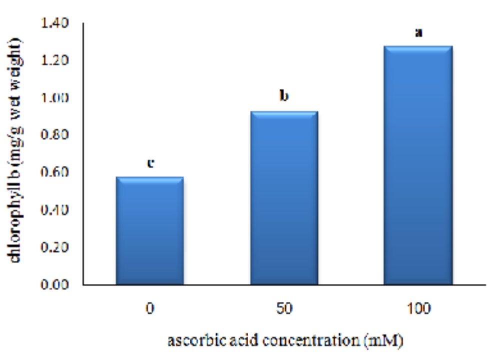

Fig. 2. Effect of ascorbic acid on chlorophyll b content in wheat (Duncan 5 %)

C. Chlorophyll a+b

The results of variance analysis of the data indicated that there was a significant difference ($p<0.01$) between the concentrations of ascorbic acid, salinity and interaction effect ($p<0.05$) on chlorophyll a+b content. The results of comparing the mean of treatment combinations of ascorbic acid and salinity showed that the application of 100 mM ascorbic acid along with the non-application of salinity leads to the maximum content of chlorophyll a+b.

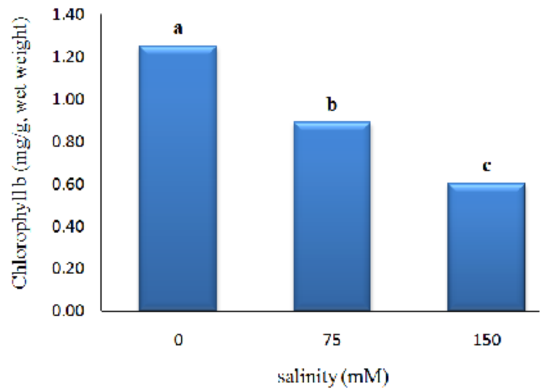

Fig. 3. Effect of salinity on chlorophyll b content in wheat (Duncan 5 %)

The minimum content of chlorophyll a+b was observed in salinity of 150 mM with no application of ascorbic acid (Fig. 4).

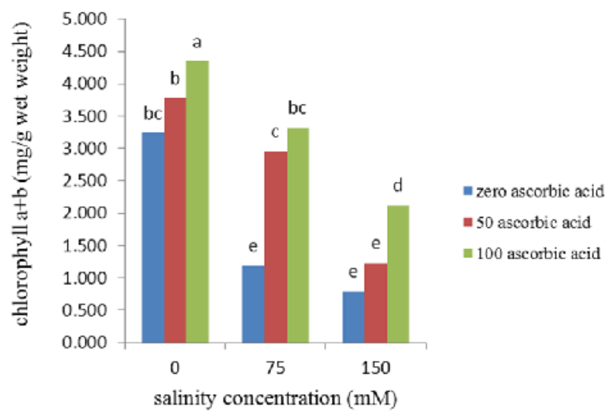

Fig. 4. Effect of ascorbic acid and salinity on chlorophyll a+b content in wheat (Duncan 5 %).

Sairam *et al.,* (2002) reported when confronted with salinity stress can be altered using mechanisms to avoid salinity, such as maintaining higher contents of RWC and increasing the chlorophyll content.

In investigations of the effect of salinity on the chlorophyll content in wheat, it has been reported that the chlorophyll decrease in resistant varieties is far smaller than in salinity-sensitive varieties. Similar results to those found in this study were reported by Sairam and Saxena (2000) in relation to the effect of salinity on the chlorophyll content of whole wheat. One of the reasons for this decrease is the increase in the activation content of chlorophyllase enzyme which is induced under salinity stress conditions (Ranjan *et al.,* 2001).

D. Proline

The results of variance analysis of the data indicated that there was a significant difference ($p<0.01$) between the contents of ascorbic acid and salinity in terms of effect on proline content. It should be mentioned that the interaction effect of ascorbic acid and salinity was not significant (Table 1).

The results of comparison of the means of ascorbic acid levels showed that the maximum content of proline exists at a concentration of 0 mM, while the minimum content of proline amino acid exists in 100 mM ascorbic acid concentration (Fig. 5). Increasing the salt concentration up to 150 mM leads to a significant accumulation of wheat proline content (Fig. 6).

Decrease in chlorophyll content can be due to change in nitrogen metabolism in connection with the construction of combinations such as proline which are important in osmotic changes (Borzouei *et al.*, 2011). The decreasing content of protein under salinity stress conditions due to the reaction of protein with free radicals and the resulting amino acid changes is related to increased activity of protein parser enzymes and decreased protein synthesis, as well as the accumulation of free amino acids such as proline. The results showed that the proline content was increased with salinity treatment.

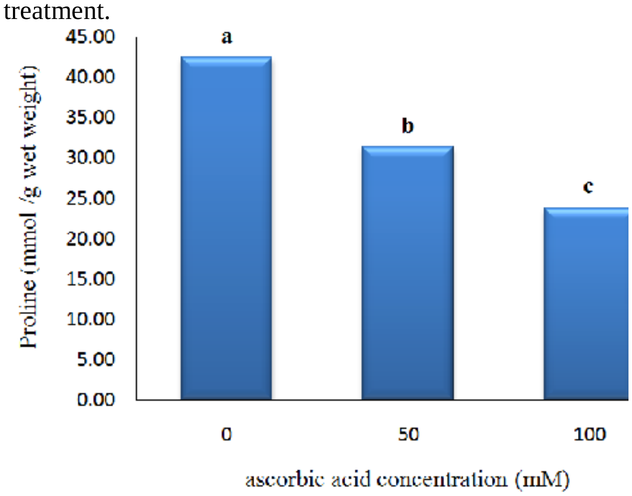

Fig. 5. Effect of ascorbic acid on proline content in wheat (Duncan 5 %).

E. Wet weight of plumule

The results of variance analysis of the data indicated that there was a significant difference ($p<0.01$) between the concentration of ascorbic acid and salinity in terms of effect on wet weight of plumule and interaction effect of ascorbic acid and salinity was not significant (Table 1). Considering the results of mean comparison, it can be noted that by increasing the concentration of ascorbic acid up to 100 mM, the wet weight of the plumule was significantly increased (Fig. 7). However, by increasing the salt concentration up to 150mM, the wet weight of wheat seedling plumule was significantly decreased (Fig. 8).

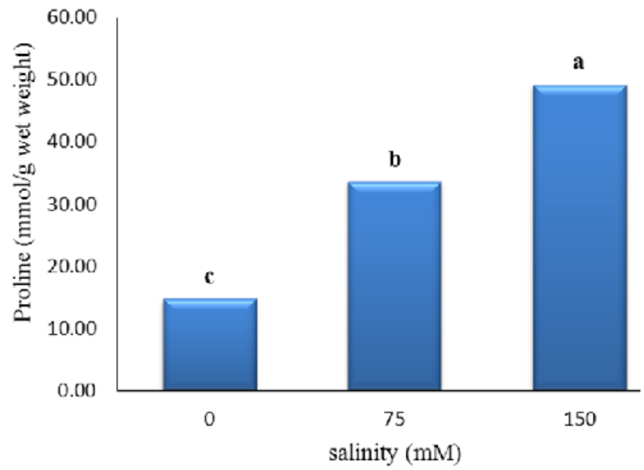

Fig. 6. Effect of salinity on proline content in wheat (Duncan 5 %).

The performed researches indicate that by increasing the salinity, the length of radical, plumule, and dry weight of the seedling all significantly decrease (Alebrahim *et al.*, 2004). In the case of irrigation with saline water or with water containing high concentrations of salt, the plant produces more sugar and organic acid by expending energy, and sends them to the root in order to maintain the high concentration of solution inside the root. If this continues the plant will die down, will be unable to manage the concentration of the soil solution and will ultimately die (Mirmohammadi Meybodi and GhareYazi, 2002).

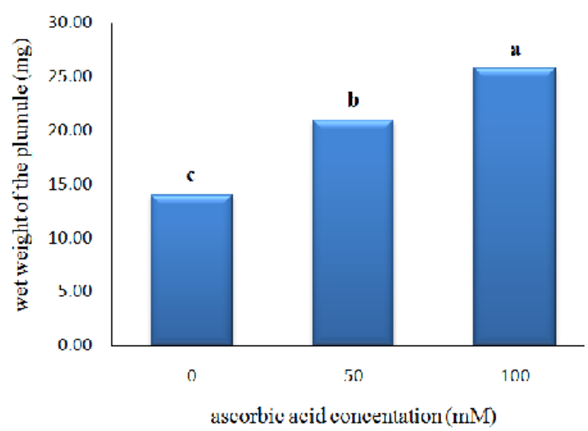

Fig. 7. Effect of ascorbic acid on wet weight of plumule in wheat (Duncan 5 %).

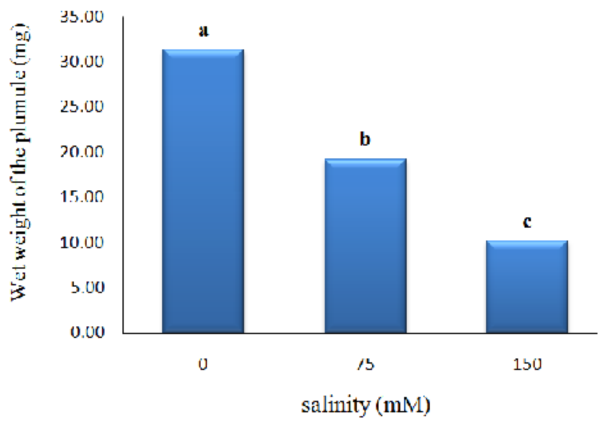

Fig. 8. Effect of salinity on wet weight of plumule in wheat (Duncan 5 %).

CONCLUSION

Considering the obtained results, it can be stated that the wheat seeds use some physiological mechanisms such as decreasing chlorophyll and accumulating proline amino acids in order to handle salinity stress, and as a result this leads to a decrease in the wet weight or biomass of the wheat. An insufficient increase in the activation of antioxidant enzymes leads to a decrease in the ability of the plant to tolerate the damage caused by salinity stress. In such conditions, ascorbic acid acts as an antioxidant, prevents the increase of activity of the mentioned enzymes, and substitutes for their activity. In addition, ascorbic acid prevents proline increase, and in this condition the assimilation of the plant is applied to increase the biomass, which will finally increase the performance.

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