Biological Forum – An International Journal **7**(1): 1226-1232(2015)

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

Investigation of the effects of Pre-Treatment of Seed with Ascorbic Acid on the Activities of Antioxidant Enzymes and Destructive Biomarkers on Wheat Seedlings under Salinity Stress Conditions

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(Received 22 March, 2015, Accepted 26 April, 2015)
(Published by Research Trend, Website: www.researchtrend.net)

ABSTRACT: In order to investigate the effect of the pre-treatment of seed with ascorbic acid on antioxidant enzymes under salinity stress, some wheat amino acids (*Triticum aestivum* L.) of Pishgam cultivar were studied with a factorial experiment in the form of a completely randomized design. The study was performed three replication, with acid ascorbic factors in three concentrations of 0, 50 and 100 Mm and in three levels of 0, 75 and 150 mM in crop plants in a laboratory at Islamic Azad University, Mahabad Branch. The results indicated that by increasing the concentration of ascorbic acid, the content of catalase, superoxide dismutase, glutathione peroxidase, malondialdehyde and D-tyrosine was significantly decreased. At the same time, the increase in salt concentration to 150 mM significantly increased the content of catalase, superoxide dismutase, glutathione peroxidase, malondialdehyde and D-tyrosine of the wheat seedlings. In fact, applying ascorbic acid decreased the activation of catalase enzyme, superoxide dismutase, glutathione peroxidase, malondialdehyde and D-tyrosine, and moderated the effects of salinity stress.

Keywords: Salinity, Wheat, Antioxidants, Ascorbic Acid, Destructive Biomarkers.

INTRODUCTION

The existence of soil under salinity conditions has been reported in almost all climate zones and at different heights above sea level (Rao *et al.*, 2006). Salinity is a factor that seriously limits the production of crops in different regions including arid and semi-arid regions. Irrigation with inappropriate or saline water is one of the most important factors of salt increase. The soil becomes saline, and salinity is created as a result.

Bread wheat (*Triticum aestivum* L.) is one of the important crops in the world and the main food source of people living in arid and semi-arid regions. In these regions, water lack as the primary factor of soil salinity is considered the secondary factor in the reduction of plant growth and the functioning of the seed (Munns *et al.*, 2006). Priming is a method that seeks the proper solutions to increase the percentage of germination rate, and especially to create homogeneous germination and uniformity in farms (Farooq *et al.*, 2005). Nowadays, pre-treatment (priming) of the seed is used as a practical method in agriculture that has positive effects on plant germination and growth. The most significant effects of priming include the primary establishment of the plant, increase of seed weight and biomass, improvement of the food quality water use efficiency, and decrease in the damage caused by pathogens (Afzal *et al.*, 2005). Some of the useful effects of priming on the seed and the plant more generally include increase in the germination percentage and rate, improved viability, increased germination in lower temperatures, and better germination in salinity conditions (Afzal *et al.*, 2005). Germination and rapid emergence of seedlings are important factors in the plants' successful establishment. It has been reported that among the various techniques used before planting, the treatment of seed (priming) is simple, cheap, and has a lower risk percentage, as well as being more effective, leading to raised plant tolerance for germination and establishment in stressful environments (Ashraf and Harris, 2004). Growth hormones that are 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). Soaking the wheat seed in ascorbic acid has desirable effects on growth and transpiration of the plant. Ascorbic acid is a small molecule 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 role in aspects of plant growth, such as cell division and enlargement, development of cellular wall, and other physiological procedures (Buettner and Schafer, 2004).

Treatment by ascorbic acid with a fairly concentration has significantly increased the percentage and rate of some physiological actions in cereals (Buettner and Schafer, 2004). In this regard, it has been reported that treatment with ascorbic acid significantly increased the germination percentage and rate in *Puccinellia distans* (Saberi and Tavili, 2010). In addition, ascorbic acid has been introduced as the best treatment to improve the quality of the seed of Canola Okapi (Alivand *et al.*, 2012).

The purpose of the present research is to investigate the effect of ascorbic acid on physiological and biochemical changes of wheat during germination in salinity stress conditions.

MATERIALS AND METHODS

This study was done in 2013 using Pishgam cultivar wheat (*Triticum aestivum* L.) with a factorial experiment in the form of a completely randomized design in three replication at the laboratory of Islamic Azad University, Mahabad Branch.

In order to perform the test, healthy and similar seeds were first chosen and disinfected by fungicides. Then, they were kept in 20 % sodium hypochlorite solution in order to be disinfected. Salinity treatment was applied by sodium chloride salt (NaCl) in three concentrations of 0 (control), 75 and 150 mM, and ascorbic acid were separately applied to each Petriin three levels of 0 (control), 50 and 100 mM. Then, the treated Petri dishes were kept in these conditions for 24 hours. A sterile filter paper was placed in each Petri dish and different salinity concentrations ranging from 3 to 5 ml were added to the dishes. Finally, the Petri dishes were closed with Parafilm and were put in a germinator. Catalase, superoxide dismutase, glutathione peroxidase, malondialdehyde and D-tyrosine of the wheat plumule were measured. The measurement of the activation of catalase enzyme and glutathione peroxidase were done using the method of Paglia and Valentine (1987), and the measurements of superoxide dismutase was performed with Misraand Fridovich (1972) method. Means comparison was done with a Duncan test at5 % level. Data analysis was done with SPSS softwareversion 13.0 (SPSS, 2004), and the diagrams were designed using Excel software.

RESULTS AND DISCUSSION

A. Catalase

The results of variance analysis showed that there was a significant difference ($p<0.01$) between the concentration of ascorbic acid and salinity and interaction effect ($p<0.05$) in terms of effect on catalase activity (Table 1).

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

		Mean Square				
Source of variation	**d.f**	**Catalase**	**Superoxide dismutase**	**Glutathione peroxidase**	**Malondialdehyde**	**D-tyrosine**
Ascorbic acid (A)	2	5.54 *	4.89 **	0.75 **	652.9 **	179.76 **
Salinity (B)	2	10.33 **	12.21 **	3.49 **	2401.8 **	407.12**
A*B	4	0.232 *	0.52 **	0.273 **	44.14	30.75 *
Error	18	0.074	0.066	0.021	38.11	7.26
C.V (%)		13.29	11.52	14.55	17.54	20.03

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

The results of comparing the means of treatment combinations of ascorbic acid and salinity indicated that the application of 150 mM salinity and the non-application of ascorbic acid led to the maximum activation of catalase enzyme. The minimum extent of enzyme activation was observed at the concentration of 100 Mm of ascorbic acid with no application of salinity (Fig. 1). In fact, using ascorbic acid in stress conditions leads to a decrease in the catalase enzyme activation and moderates the effects of salinity stress.

Like environmental stress,salinity stress causes the increase of active oxygen species such as superoxide O_2, oxygen peroxide H_2O_2 and hydroxyl radical OH in the cell and damage to the membrane lipids, proteins and nucleic acids (Noctor and Foyer, 1998).

The ability of ascorbate to decrease electrons in order to produce MDHA is the basis of the biological advantage of its antioxidant capacity (Buettner and Schafer, 2004). Various researches have indicated that there is a strong connection between the tolerance to oxidative stresses that is created as a result of environmental stresses, and the increase in the concentration of antioxidant enzymes in photosynthetic plants (Sairam and Srivastava, 2002).

Catalase enzyme is able to removeH_2O_2 from the environment, but this enzyme is located at the peroxisome of leaf cells and is not found in chloroplast (Tale Ahmad and Haddad, 2010). Increasing the catalase activity helps to decrease the photorespiration and compensation point of the CO_2 (Brisson *et al.*, 1998).

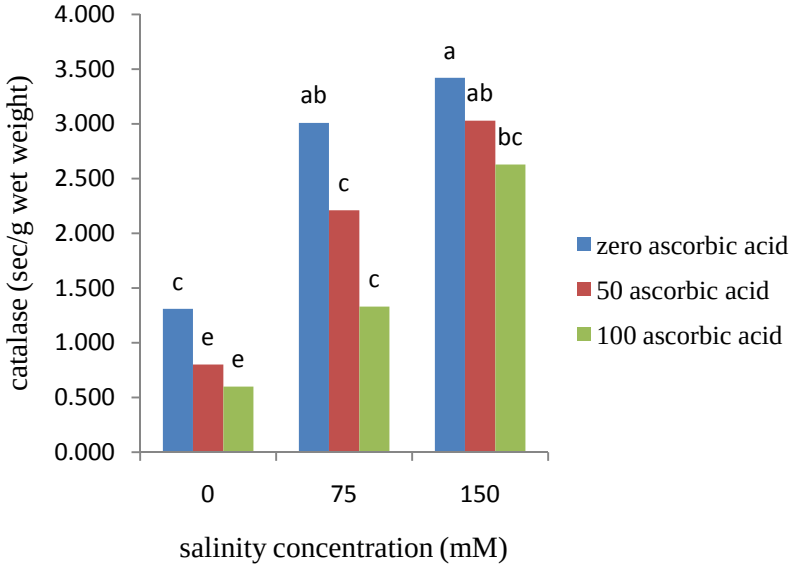

Fig. 1. Effect of ascorbic acid and salinity on the catalase activity (Duncan 5 %).

B. Superoxide dismutase (SOD)

The results of analysis of variances indicated that there is a significant difference ($p<0.01$) between the levels of ascorbic acid and salinity, and also interaction effect of ascorbic acid and salinity with regard to the activity of superoxide dismutase (Table 1). The results of the comparison of ascorbic acid treatment combinations and salinity indicated that application of 150 mM salinity and non-application of ascorbic acid led to the maximum activation of superoxide dismutase enzyme. The minimum activation of superoxide dismutase enzyme was observed at 50 and 100 mM concentrations of ascorbic acid and with no application of salinity (Fig. 2).

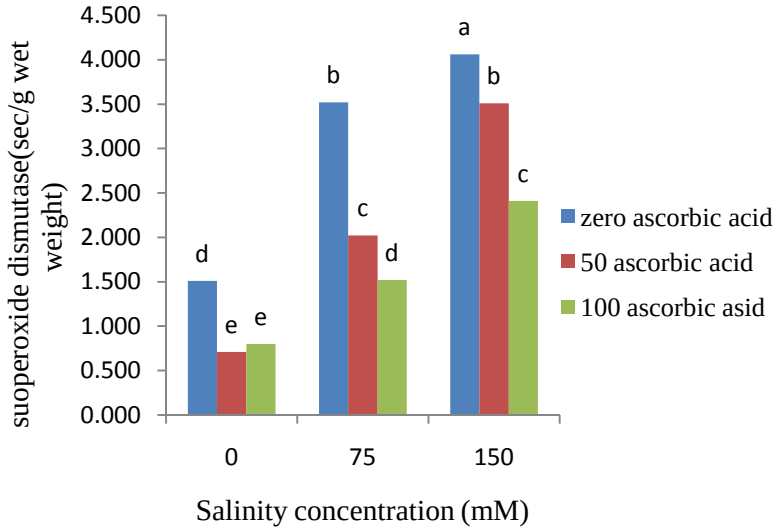

Fig. 2. Effect of ascorbic acid and salinity on the activity of superoxide dismutase enzyme (Duncan 5%).

In fact, applying ascorbic acid under stress conditions causes a decrease of superoxide dismutase enzyme activation and as a result decreases the effects of salinity stress.

In accordance with the present results, Borzouei *et al.* (2011) declared that the activation of superoxide dismutase enzyme (SOD) in conditions of irrigation with saline water was significantly increased. By decreasing the membrane lipid peroxidation, ascorbic acid leads to resistance against stress. One of the reasons for improving the germination by ascorbic acid is the existence of its antioxidant property and the limiting of free radicals (Buettner and Schafer, 2004). According to the research results of Borzouei *et al.* (2011), as a result of an increase in the activation of the superoxide dismutase enzyme (SOD), the content of radicals is kept at lower levels, and this leads to a decrease of oxidative damage created by salinity stress. Superoxide dismutase enzyme (SOD) is the first defensive line of the cell against the attack of free radicals.

C. Glutathione peroxidase (GPX)

The results of variance analysis showed that there was a significant difference ($p<0.01$) between the concentration of ascorbic acid and salinity, and also the interaction effect of ascorbic acid and salinity with regard to effect on glutathione peroxidase (GPX) (Table 1).

The results of comparison of ascorbic acid and salinity treatment combinations indicated that applying 150 mM salinity and non-application of ascorbic acid led to the maximum activation of glutathione peroxidase. The minimum activation of glutathione peroxidase was obtained in ascorbic acid concentrations of 0, 50 and 100 mM and without salinity application (Fig. 3). The results indicate that applying ascorbic acid in stress conditions decreases the activation of glutathione peroxidase, and as a result leads to a decrease of the effects of the salinity stress.

Together with glutathione and some other antioxidant enzymes, ascorbic acid has a role in neutralizing the free oxygen radicals, including superoxide ion, resulting from various non-biological stresses such as salinity (Buettner and Schafer, 2004). The lack of activation of antioxidant enzymes leads to a decrease in the ability of the plant to tolerate the damage resulting from salinity stress (Borzouei *et al.*, 2011). Sairam and Srivastava (2002) declared that salinity stress is the indicator of significant differences between various wheat genotypes with regard to the partial extent of the leaf water, chlorophyll amount, membrane stability index, extent of hydrogen peroxide ascorbate, and activation of superoxide dismutase peroxidase and glutathione reductase.

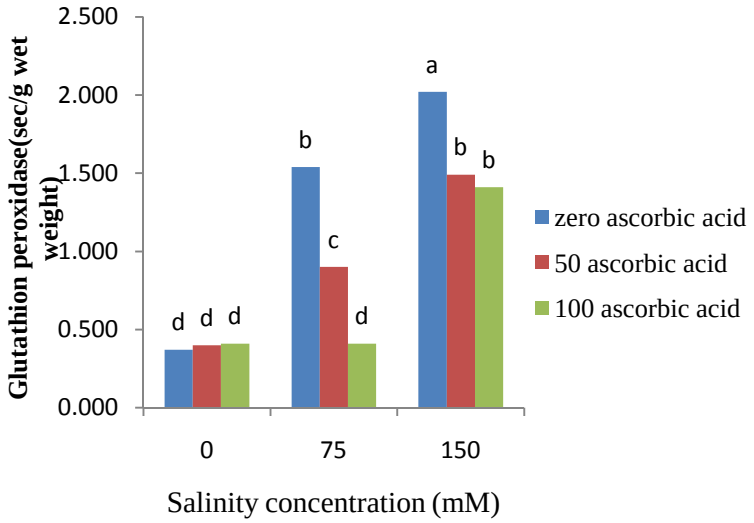

Fig. 3. Effect of ascorbic acid and salinity on the activation of glutathione peroxidase (Duncan 5 %).

D. Maldondialdehyde (MDA)

The results of variance analysis showed that there was a significant difference ($p<0.01$) between the concentration of ascorbic acid and salinity with regard to effect on the content of maldondialdehyde (Table 1).

The obtained results showed that by increasing the concentration of ascorbic acid up to 100 mM, the extent of malondialdehyde was significantly decreased (Fig. 4). Increasing the saline concentration up to 150 mM led to a significant increase in the content of wheat malondialdehyde (Fig. 5).

Borzouei *et al.* (2011) declared that the content of malondialdehyde was significantly increased in conditions of irrigation with saline water. Moreover, Sairam and Srivastava (2002) reported that malondialdehyde increases in salinity stress conditions and results in the decrease of concentration of the stability index of cell membrane. Therefore, the results of the present study are in accordance with the reports of the abovementioned researchers.

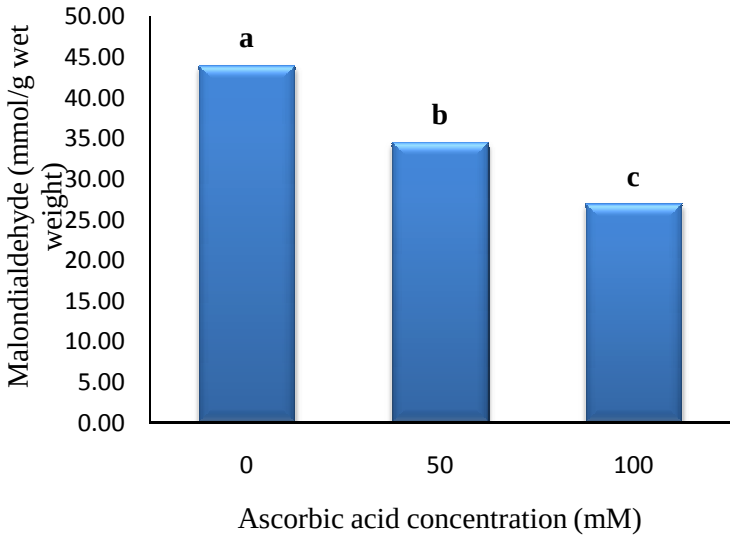

Fig. 4. Effect of ascorbic acid on the content of malondialdehyde (Duncan 5 %).

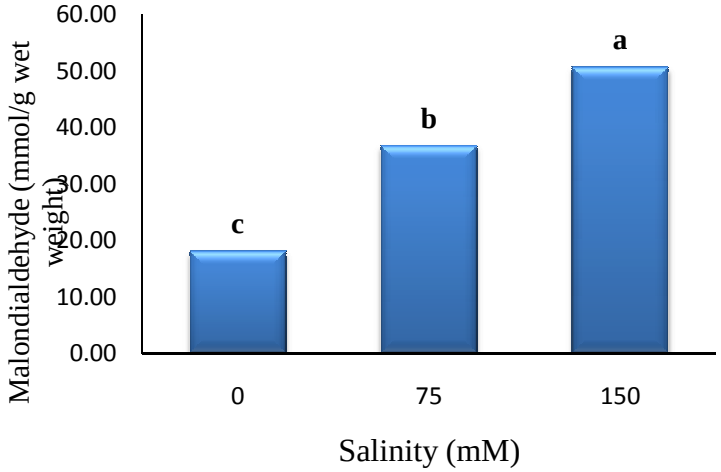

Fig. 5. Effect of salinity on the content of malondialdehyde (Duncan 5 %)

E. D-tyrosine

The results of variance analysis indicated that there was a significant difference ($p<0.01$) between the concentration of ascorbic acid and salinity and interaction effect ($p<0.05$) in terms of effect on the effect on the activation of D-tyrosine (Table 1). The results of comparison of ascorbic acid and salinity treatment combinations indicated that applying 150 mM salinity and non-application of ascorbic acid led to the maximum activation of D-tyrosine. The minimum activation of D-tyrosine was obtained with ascorbic acid concentrations of 0, 50 and 100 mM and without salinity application (Fig. 6). In fact, it can be concluded that applying ascorbic acid under salinity stress conditions causes the decrease of the effects of salinity stress, and as a result decreases the activation of D-tyrosine. Pre-treatment of seed causes changesin the proteins such as D-tyrosine, but the type of protein remains constant (Hill, 1999; Olave and Guzman, 2004).

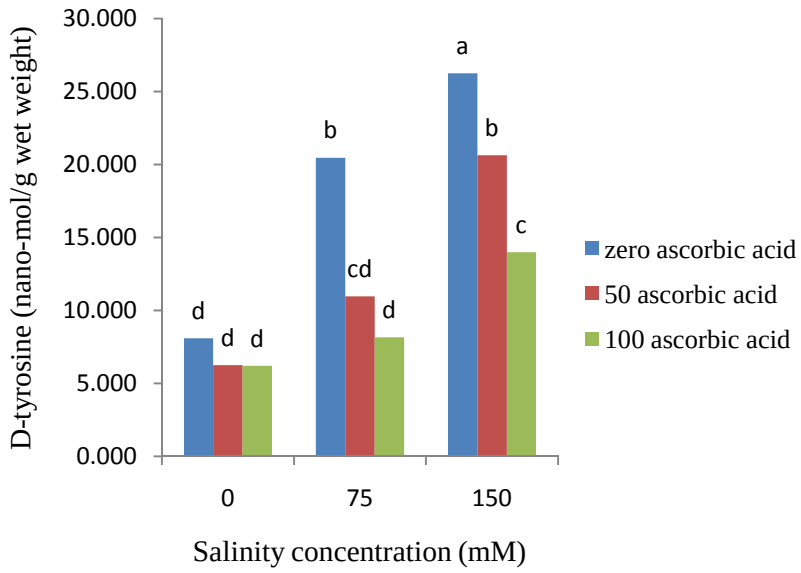

Fig. 6. Effect of ascorbic acid and salinity on the activation of D-tyrosine (Duncan 5 %)

CONCLUSION

Considering the results obtained from wheat seeds, it can be concluded that in order to confront salinity stress, some physiological characteristics, including decreasing chlorophyll, increasing activation of catalase, superoxide dismutase, glutathione peroxidase and malondialdehyde enzymes, and the accumulation of D-tyrosine amino acids are deployed that cause decrease of the wet weight or biomass of wheat. A lack of activation of antioxidant enzymes leads to a decrease in the ability of the plant to tolerate damage resulting from salinity stress. In these conditions, ascorbic acid acts as an antioxidant, prevents the activation increase of the mentioned enzymes and substitutes for their activation. On the other hand, ascorbic acid prevents an increase in the amount of D-tyrosine, and in these conditions assimilate of the plant is applied to increase the biomass, which will finally increase performance.

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