The Effect of Seed Priming with Different Concentrations of Ascorbic Acid on Germination and Vigor of Cumine (*Cumin cyminum* L.) under Saline Conditions

M. Ghiyasi*, R. Amirnia, M. Fazelimanesh and M. Tajbakhsh

Department of Agronomy, Faculty of Agriculture, Urmia University, Urmia, Iran

**Corresponding author, Email: mahdighiyasi@gmail.com, m.ghiasi@urmia.ac.ir*

Abstract

In order to evaluate the effects of seed priming with ascorbic acid at different concentrations on germination and seedling growth of Cumin (*cuminum cyminum*) under salt stress, an experiment was conducted at the Department of Agronomy, Faculty of Agriculture, Urmia University, West Azerbaijan, Iran in 2015. The two treatment factors were salinity stress in four levels (0, 50, 100, 150 Nacl mM) and seed priming with ascorbic acid at four concentrations (0, 15, 20 and 25 mM). The experimental design was two factor factorial arranged in completely randomized design (CRD) with three replications. Final germination percentage (FG%), mean germination time (MGT), shoot and root length, seedling length (SL), the fresh and dry weight of the seedlings (FWS) (DWS) and seedling vigor index (SVI) were evaluated at the end of the experiment. Factors were statistically significant on all studied traits. Moreover, a significant of two way interaction (salinity stress × seed priming) was found in final germination percentage (FG %), mean germination time (MGT) and seedling fresh weight (SFW). The results clearly showed that pre-sowing treatments with ascorbic acid can improve the germination process seed vigor, especially under saline conditions and the optimum concentrations of ascorbic acid in order to promote germination and seedling growth of cumin seeds were 20mM and 25mM ascorbic acid.

Keywords: ascorbic acid, salt stress, seed priming, cumin (*cuminum cyminum*), germination

Introduction

Cumin *(cuminum cyminum*) is one of the most important medicinal plants in Iran. It is an annual, herbaceous plant, which belongs to the family of Umbeliferae with aromatic compounds that is used as a spice in cooking and food industry. *Cumin cyminum* L. has been used in folk medicine for the treatment of indigestion, strengthening the immune system, stomach strengthening and etc. (Roodbari et al., 2013).

Environmental stresses are the most limiting factors for different agricultural production. Salinity as one of the significant environmental stresses leads to decreasing the productivity of crops (Ghiyasi et al, 2008). All types of ecological stresses impact on various phonological stages and the severity of these damages in various stages of growth are not the same. Seed germination and seedling growth are critical phonological stages that are deeply influenced by environmental stresses such as salinity. There are also reports that salt may disrupt rapidity, uniformity and seedling establishment of the farm (Tajbakhsh and Ghiyasi, 2009). As with other environmental stresses, salinity reduces the rate of germination and Seedling establishment stages and delays them. Oxidative stress is a consequence of salinity that leads to the creation of free radicals in germinated seeds and growing seedlings (Ghorbanli et al., 2010).

Plants use various methods for modulating oxidative damage like using some antioxidants. Ascorbic acid is one of the most powerful

antioxidants that inhibits the revival of free radicals and reduces the damage of them (Fecht chirtoffers et al, 2003).

Several studies have clearly indicated the effects of ascorbic acid pre-sowing treatment on plants. The use of this pre-treatment can lead to a significant reduction in salinity damages on seed germination and establishment of different plants (Ajmal khan et al., 2006).

The main objective of this study was to evaluate the impact of seed priming with ascorbic acid at different concentrations on germination and seedling growth of cumin (*Cumin cyminum* L.) under salt stress.

Materials and Methods

In order to evaluate the effects of seed priming with ascorbic acid at different concentrations on germination and seedling growth of Cumin *(cuminum cyminum*) under salt condition, an experiment was conducted at the Department of Agronomy, Faculty of Agriculture, Urmia University, West Azerbaijan, Iran in 2015. Research was conducted in the laboratory and the experimental design was two factorial (3×4×4) arranged in a completely randomized design (CRD) with three replications. The two treatment factors were salt stress and priming [salinity stress in four levels (0, 50, 100, 150 Nacl mM) × seed priming with ascorbic acid in four levels (0, 15, 20 and 25 mM)].

For germination tests, Seeds were fully disinfected by using fungicide VITAVAX-T in a ratio (g:L) of 2:1. After disinfecting, Seed priming immediately was carried out with ascorbic acid at different conditions. The ratio of seed weight to solution volume was 1:5 (g-mL) (Barsa et al., 2004). To avoid low-oxygen condition during the experiment, the solution was aerated by aquarium pumps. Cumin seeds were immersed in solution for 16 h and after this period for reaching initial moisture, the treated seeds were dried for 24 h at (22±1°C) under the shade. Treated seeds were placed in 9 cm diameter sterile petri dishes with filter paper and 5ml of salt solution was added to each Petri dish. Four petri dishes of 25 seeds were used for each replication and total seeds in one replication was 100.

Irrigation with salt solution was done every other day. To prevent the accumulation of salt, filter papers were changed every 3 days. Then petri dishes were put in germinator with alternating temperature cycle (24±1°C, 16h dark/8h light): relative humidity %70. Seeds with 2 mm radical emergence were considered germinated and daily observations were recorded during 14 days. To calculate the indices of seedling growth, 10 germinated seeds from each treatment were randomly selected and were grown among the filter papers for 14 days.

Final germination percentage (FG%), mean germination time (MGT), shoot and root length, seedling length (SL), the fresh and dry weight of the seedlings (FWS) (DWS) and seedling vigor index (SVI) were evaluated at the end of the experiment.

Mean germination time (MGT) was calculated based on the following formula: Where n is the number of seeds germinated on each day and D is the day of counting.

$$MGT = \frac{\sum (Dn)}{\sum n}$$

The following formula was used to calculate the seedling vigor index:

SVI = [Mean seedling length (cm)] [Germination (%)]

The fresh and dry weight of the seedlings were measured after 14 days. Before weighing, seedlings were dried on a paper towel to remove their surface moisture. Then they were kept in an oven maintained at 70°C for 48 h and were weighed to calculate the dry matter production.

All data were statistically analyzed by using MSTATC and results were measured by means of variance analysis followed by the Duncan's multiple range test. Charts were plotted with Excel software.

Results and Discussion

Final Germination Percentage (FG %)

According to the results, salinity and seed priming showed a significant effect on all studied characters. Furthermore, a significant of two way

interaction (salinity stress×seed priming) was found for final germination percentage (FG%), mean germination time (MGT) and seedling fresh weight (SFW). Mean comparison of interaction for final germination percentage demonstrated seeds primed with ascorbic acid in contrast to untreated seeds had the highest germination at all salinity levels. The highest germination rate with a value of 88.21% was obtained from seeds primed with 20mM ascorbic acid in non-stress conditions.

This treatment compared to other treatments except seeds primed with 25 mM ascorbic acid was statistically significant in normal condition. The lowest germination rate with a value of 26.6% was recorded at untreated seeds under saline condition of 150 mM and at all salinity levels, seeds treated with 20mM ascorbic acid had the most germination rate (Figure 1).

Mean Germination Time (MGT)

Mean comparison of interaction for mean germination time illustrated seeds treated with 20 mM ascorbic acid had the minimum germination time (7.6 days) and the maximum mean germination time was achieved from untreated seeds under saline condition of 150 mM during 13.22 days. Statistically, none of the other treatments were similar to this treatment. Based on statistical data, salinity significantly decreased mean germination time of seeds primed with 20 mM ascorbic acid at all levels. In salinity levels of 50 mM, 100 mM and 150 mM, the treatments of ascorbic acid in concentrations of 20, 25 and 15 mm caused by the increase in the speed of germination and consequently the average reduction in germination respectively (Figure 2).

Seedling Length (SL)

Mean comparison was performed on these two factors separately, due to an insignificant interaction of salinity stress × seed priming on seedling length. Results indicated that increasing in salinity levels significantly reduced the seedling length. None of salinity levels were statistically the same. The value of this trait for saline levels of 50mM, 100mM and 150mM were 8.04, 7.30, 5.80 and 4.90 respectively (Figure 3).Mean comparison of seed priming factor indicated that different concentrations of 15, 20 and 25 mM compared to non-primed condition increased seedling length

Table 1 Mean square from analysis of variance for measured seedling characteristics in primed and non-primed seeds of cumin(*Cumin cyminum L.)* in response to salt stress

		Mean square					
Source of variation	Degree of freedom	Final germination percentage	Mean germination time	Seedling length	Seedling fresh weight	Seedling dry weight	Seedling vigor index
Salinity	3	3502.2**	31.1**	24.7**	116.6**	0.15**	476666.6**
Priming	3	780.6**	13.1**	1.7**	5.3**	0.01**	58489.1**
Salintiy×Priming	9	32.8*	0.31**	0.07^{ns}	0.32**	0.001^{ns}	532.1^{ns}
Error	32	3.9	0.01	0.05	0.031	0.002	810.3
CV%	6.53%	3.09%	1.02%	3.4%	1.58%	10.8%	6.53%

* Indicates significance at p<0.05 level; ** Indicates significance at p<0.01 level; ^{ns} not significant

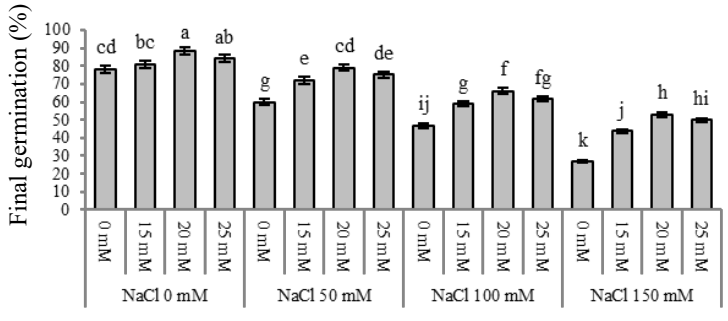

Figure 1 Effects of ascorbic acid treatments on final germination percentage of cumin at different salt levels. Different letters indicating significant differences at P≤0. 01.

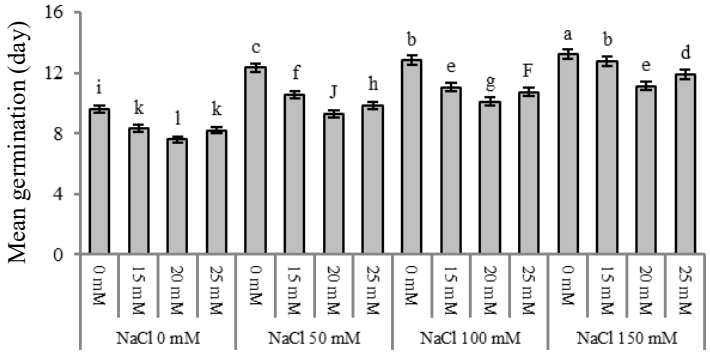

Figure 2 Effects of ascorbic acid treatments on MGT of cumin at different salt levels. Different letters indicating significant differences at P≤0. 01.

significantly. However, the highest effect was measured from primed seeds with 20 mM ascorbic acid and the value of this treatment was 7.0 cm. Primed seeds with 15 and 25mM ascorbic acid were significantly at the same level. In this level the value of this character were 6.4 and 6.5 cm respectively. The lowest seedling length with a value of 6.1cm was obtained from untreated seeds (Figure 4).

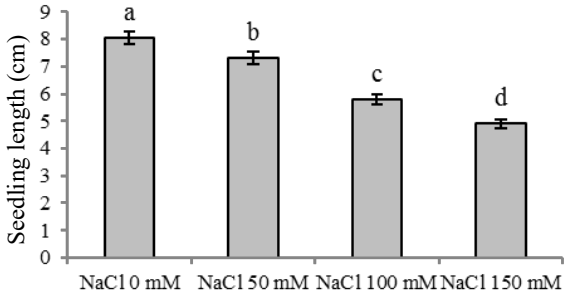

Figure 3 Effects different NaCl concentrations on seedling length of cumin. Different letters indicating significant differences at p≤0. 01.

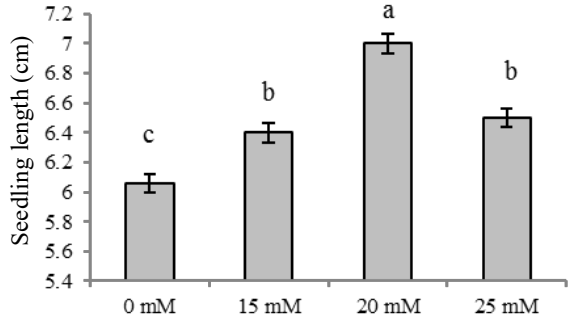

Figure 4 Effects of ascorbic acid treatments on seedling length of cumin. Different letters indicating significant differences at p≤0. 01

Seedling Fresh Weight (SFW)

Mean comparison of interaction for final germination percentage showed that seeds primed with 20 mM ascorbic acid Led to a significant increase in seedling fresh weight under non-stress condition. The value of this trait was 14.7 mg. In normal condition, no differentiations were observed among the non-primed treatments and primed treatments with 15 mM and 25 mM ascorbic acid. Seeds primed with 20 mM ascorbic acid had significantly more weight, in the salinity level of 150 mM and the value of seedling fresh weight for non-primed and primed seeds with 15 mM, 20 mM and 25 mm ascorbic acid were 11.10, 12.80, 13.34 and 12.10 respectively. Although pre-sowing treatment with 15 mM and 25 mM comparisons to control treatment resulted in a significant increase in fresh weight in saline conditions, but they didn't have statistically differences and compared to primed seeds with 20 mM ascorbic acid were on the lower level. In the salinity level of 100 mM, all three concentrations of ascorbic acid (15, 20 and 25 mM) compared to non-primed treatment, Enhanced one trait significantly. In salinity level of 100 mM, the value of ascorbic acid in concentrations of 20, 25 and 15 mM were 10.1, 11.2 and 10.74 mg respectively. However, the fresh weight was 9.05 mg in non-treated condition. In normal condition, seeds primed with 20 mM had the highest rate. Increasing the saline level to 150 mM, had a

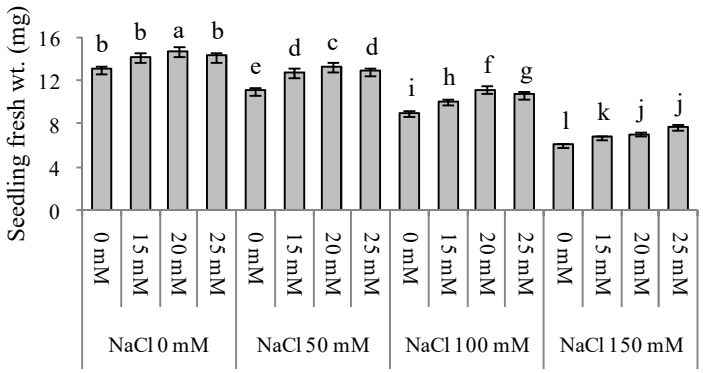

Figure 5 Effects of ascorbic acid treatments on seedling fresh weight of cumin at different salt levels. Different letters indicating significant differences at p≤0. 01

positive effect on seedling fresh weight of primed seeds with 3 concentrations of ascorbic acid. In saline level of 150 mM, pre-sowing treatments with concentrations of 20 and 25 mM improved seedling fresh weight and the value of this trait for each concentration were 7.10 and 7.73 respectively. The value of this trait for untreated and treated seeds with 15 mM ascorbic acid were 6.03 and 6.80mg respectively (Figure 5).

Seedling Dry Weight (SDW)

Regarding the fact that the interaction between the two factors was not significant, the mean interaction was performed separately. Results showed that increasing levels of salinity reduced seedling dry weight, so that all salinity levels were statistically significant differences. In salinity levels of 50 mM, 100 mM and 150 mM, the value of seedling dry weight were 0.52, 0.41, 0.32 and 0.25 mg (Figure 6). Mean comparison of priming factor for seedling dry weight indicated that priming with ascorbic acid compared to non-primed condition caused by the increase in seedling dry weight and the value of this trait for non-primed and primed seeds with 15mM, 20mM and 25mm ascorbic acid were 0.35, 0.37, 0.41 and 0.37 respectively. The best result was obtained from seeds primed with 20mM ascorbic acid (Figure 7).

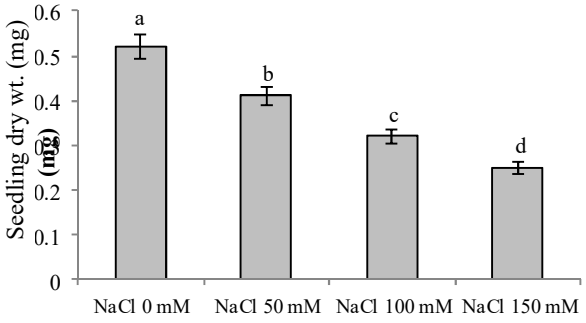

Figure 6 Effects different NaCl concentrations on seedling dry weight of cumin. Different letters indicating significant differences at p≤0. 01

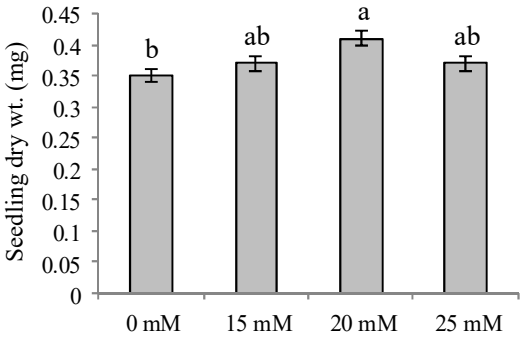

Figure 7 Effects of ascorbic acid treatments on seedling dry weight of cumin. Different letters indicating significant differences at p≤0. 01

Seedling Vigor Index (SVI)

There is an insignificant interaction of salinity stress × seed priming on seedling vigor index. However, main effects of two factors were significant. According to the mean comparison of main effects, increasing salinity levels were significantly reduced seedling vigor index. The

value of this trait for concentrations of 50 mM, 100 mM and 150mm were 668.3, 528, 339.0 and 215.6, under normal condition. According to (Figure 8), all levels of salinity of statistics in relation to this trait were independent team. Mean comparison of seed priming factor for seedling vigor index showed that all three concentrations of 15, 20 and 25mM were statistically different from control treatment. The value of this trait in non-primed condition and primed seeds with 15, 20 and 25mM ascorbic acid were 346.3, 424.5, 512.4 and 460.5 respectively (Figure 9).

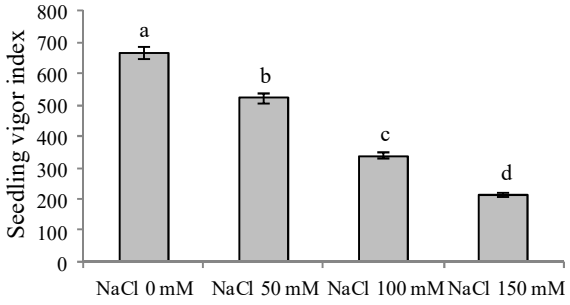

Figure 8 Effects different NaCl concentrations on seedling dry weight of cumin. Different letters indicating significant differences at p≤0. 01

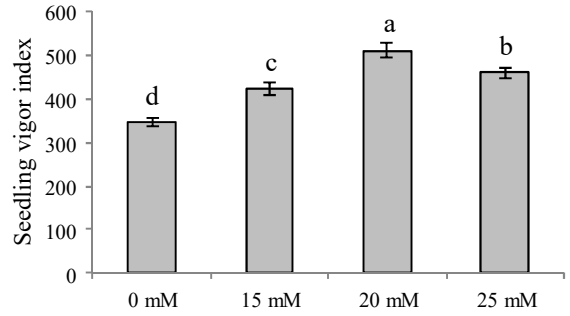

Figure 9 Effects of ascorbic acid treatments on seedling dry weight of cumin. Different letters indicating significant differences at p≤0. 01

Discussion

The study suggests that seed vigor can be increased by using seed priming techniques under saline condition. This impact is more tangible by increasing salinity levels. Moreover, the results showed that pre-sowing with ascorbic acid can lead to improve some germination indices such as germination percentage (FG %), mean germination time (MGT), seedling fresh and dry weight even in normal condition. Different reasons by which priming might improve germination indices have been proposed. In general, pre-sowing treatments are accompanied by soaking seeds in aquatic environments that compared to untreated seeds stimulates the germination process and improves seed vigor (Salehzade, 2009). However, the results of this experiment in relation to traits demonstrated that different concentrations of ascorbic acid do not have the same answer (replication-response) under stress and non-stress conditions.

It seems that ascorbic acid has specific characteristics which impacts on seeds. Ascorbic acid is a water soluble antioxidant that detoxifies hydrogen peroxide and superoxide species and other free radicals. Furthermore, it plays a particular role in plant growth processes such as cell division and the expansion of the cell wall. Oxidative stress on germinated seed and seedling is possible under salt stress condition. Therefore, the use of ascorbic acid as seed priming due to its antioxidant properties can lead to a reduction in oxidative stress damage and improve germination indices (Azooz, M.M., 2009).

The difference between various concentrations of ascorbic acid is due to the need of seeds and seedlings to it. Thus, it can be said there is an optimum concentration of ascorbic acid in every seed, which the most improved germination and seedling growth are achieved in it under stress conditions. This influence in many different combinations of materials such as plant growth regulators is true (Afzal et al., 2005). The results showed that the optimum concentrations of ascorbic acid in order to promote germination and seedling growth of cumin seeds were 20mM and 25 mM ascorbic acid.

References

Ajmal Khan, M., M.Z. Ahmed and A. Hameed. 2006. Effect of sea salt and L-ascorbic acid on the Seed germination of halophytes. J. Arid Environ. 67: 535-540.

Azooz, M.M. 2009. Salt stress mitigation by seed priming with salicylic acid in two faba bean genotypes differing in salt tolerance. Intern. J. Agri. Biol. 11: 343-350.

Basra, S.M.A., M. Farooq, K. Hafeez and N. Ahmad. 2004. Osmohardening: A new technique for rice seed invigoration. Intern. Rice Res. Notes. 29: 80-81.

Fecht christoffers, M.M., P. Maier and W.J. Horst. 2003. Apoplastic peroxidases and ascorbate are involved in managance toxicity and tolerance of *Vigna unguiculata*. J. Plant Physiol. 117: 237-244.

Ghiyasi, M., A.A. Seyabjani, M. Tajbakhsh, R. Amirnia and H. Salehzadeh. 2008. Effect of osmopriming with polyethylene glycol (8000) on germination and seedling growth of wheat (*Triticum aestivum*) Seeds under salt stress. Res. J. Biol. Sci. 3: 1249-1251

Ghorbanli, M., N. Adib-Hashemi and M. Peyvandi. 2010. Study of salinity and ascorbic acid on some physiological responses of *Nigella sativa* L. Iranian J. Medicinal and Aromatic Plants. 26: 370-388.

Roodbari, N., M. Lahooti, S. Roodbari, A. Aein and A. Ganjali. 2013. The effect of salinity stress on germination and seedling growth of cumin (*Cumin cyminum L.)*. J. Agri. Food Tech. 3: 1-4

Salehzade, H., M.I. Shishvan, M. Ghiyasi, F. Forouzin, and A.A. Siyahjani. 2009. Effect of seed priming on germination and seedling growth of wheat (*Triticum aestivum* L.). Res. J. Biol. Sci. 4: 629-631.

Tajbakhsh, M. and M. Ghiyasi. 2009. Seed Ecology. Jahade- Daneshghahi press, Urmia University.

Manuscript received 3 April 2016, accepted 15 June 2016