

## Full Length Research Paper

# Effect of seed priming on seed germination properties of two medicinal species in the presence of salinity

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**Germination properties of two medicinal species including sage (*Salvia officinalis* L.), and marsh mallow (*Althaea officinalis*) evaluated under different antioxidant compounds and salinity levels by using a factorial based on completely randomized design with 4 replication at Special Crops Laboratory of Ferdowsi University of Mashhad. The studied factors for each plant consisted of seed priming at 4 levels including control (distilled water), ascorbic acid (40 mM), gibberlic acid (75 mg/L) and salisilic acid (1.5 mM), and five salinity levels according to electrical conductivity by adding NaCl to distilled water (control, 5, 10, 15 and 20 dS/m<sup>-1</sup>). Results showed that, pretreatment with gibberlic acid improved germination percentage and rate in two species under higher salt concentration. However, increased in salt concentrations sharply declined the germination of both species. Generally, seed priming with gibberlic acid could improve germination and seedling properties of these two species in the presence of high salinity.**

**Key words:** Antioxidants, germination percentage, gibberlic acid, salinity

## INTRODUCTION

Approximately 15.2% (25 million hectare) of surface area of Iran are affected by salinity (Postini and Salmani, 1995). Salinity reduces water availability for seed germination and prevents normal germination of seeds by increasing oxidative stress (Khan et al., 2006). Medicinal plants are in use by many people in developing countries, considering their low costs, effectiveness, the frequently inadequate provision of modern medicine, and cultural and religious preferences (Bannayan et al., 2011).

Sage (*Salvia officinalis* L.) Lamiaceae is a perennial woody sub-shrub native to the Mediterranean and widely distributed in Iran (Glisic et al., 2010). It is cultivated in several countries mainly to obtain dried leaves to be used as raw material for medicine, perfumery, and food industry (Gomes et al., 2002). Marsh mallow (*Althaea officinalis* L.), a popular herb from the Malvaceae family, is a perennial plant native throughout salty marshes of Iran and now is cultivated throughout the world. It has

been known from ancient time as a medicinal plant (Sutovska et al., 2009). Increasing salinity levels significantly reduced the percentage and rate of germination, in different medicinal plants (Khan and Ungar, 2001). Some plant hormones and antioxidants such as gibberellic acid, ascorbic acid, and salicylic acid enhanced the germination of medicinal plants under saline conditions (Kafi et al., 2011).

Zhang et al., (2010) reported salt tolerate plants decrease the salt absorption in germination phase with compare to drought tolerate plant species. Baes and Arechiga (2007) Showed that seed treatment with gibberellic acid improved germination percentage under saline conditions. Ascorbic acid plays an important role in removing toxic hydrogen peroxide in plant cells. Ascorbic acid concentration of 40 mM/lit significantly increase germination of halophyte species under salinity conditions (Khan et al., 2006). Salicylic acid alleviated the negative

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**Table 1.** Effects of different seed priming and salt concentrations on germination percentage and rate of sage (*Salvia officinalis* L.) and marsh mallow (*Althaea officinalis*).

| Priming type                           | <i>Salvia officinalis</i> L. |                                 | <i>Althaea officinalis</i> |                                 |
|--|------------------------------|---------------------------------|----------------------------|---------------------------------|
|  | Germination percentage (%)   | Germination rate (seed per day) | Germination percentage (%) | Germination rate (seed per day) |
| Control                                | 61 <sup>b</sup>              | 5 <sup>ab</sup>                 | 35.8 <sup>ab</sup>         | 6.3 <sup>a</sup>                |
| Ascorbic acid                          | 71 <sup>a</sup>              | 7 <sup>a</sup>                  | 41.0 <sup>a</sup>          | 7.4 <sup>a</sup>                |
| Gibberellic acid                       | 58 <sup>b</sup>              | 4 <sup>b</sup>                  | 28.8 <sup>b</sup>          | 3.9 <sup>b</sup>                |
| Salicylic acid                         | 40 <sup>c</sup>              | 3 <sup>b</sup>                  | 15.6 <sup>c</sup>          | 1.2 <sup>c</sup>                |
| <b>Salt levels (dS/m<sup>-1</sup>)</b> |                              |                                 |                            |                                 |
| 0                                      | 72 <sup>a</sup>              | 7 <sup>a</sup>                  | 43 <sup>a</sup>            | 7 <sup>a</sup>                  |
| 5                                      | 68 <sup>a</sup>              | 7 <sup>ab</sup>                 | 38 <sup>ab</sup>           | 7 <sup>a</sup>                  |
| 10                                     | 64 <sup>a</sup>              | 5 <sup>ab</sup>                 | 31 <sup>b</sup>            | 4 <sup>b</sup>                  |
| 15                                     | 54 <sup>a</sup>              | 3 <sup>b</sup>                  | 23 <sup>c</sup>            | 3 <sup>c</sup>                  |
| 20                                     | 43 <sup>b</sup>              | 2 <sup>c</sup>                  | 15 <sup>c</sup>            | 1 <sup>c</sup>                  |

negative effects of active oxygen which produced during photosynthesis and seed germination of wheat under salinity (Doulatabadian et al., 2008).

The aim of this research was to determine gibberellic acid, ascorbic acid, and salicylic acid impacts on germination properties of marsh mallow and sage under different salinity levels.

## MATERIALS AND METHODS

### Seed source and treatments

The mature seeds of Sage and Marsh mallow were collected from natural ecosystems of Northeast of Iran in 2010. After collection, immature seeds and those damaged by insects were removed. The seeds were surface sterilized by soaking in 1% sodium hypochlorite (NaOCl) for 5min and subsequently rinsed thoroughly with distilled water prior to applying any treatment. All germination experiments were conducted using three replications of 25 seeds per each treatment. Seeds were placed on double layered Whatman No.1 filter paper moistened with 5ml of distilled water in sterilized Petri dishes.

### Experimental design

This experiment was carried out using a factorial based on completely randomized design with four replications at Special Crops Laboratory of Ferdowsi University of Mashhad. Seed priming was at four levels including control (distilled water), ascorbic acid (40 mM), gibberellic acid (75 mg/L) and salicylic acid (1.5 mM), and five salinity levels according to electrical conductivity by adding NaCl to distilled water (control, 5,10,15 and 20 dS/m<sup>-1</sup>).

### Experiment conditions

Seeds were soaked by distilled water, ascorbic acid, gibberellic acid, and salicylic acid for 12 h and dried under room temperature conditions (25°C) for 12 h before transfer to the germination test process. After each treatment, seeds were transferred to germinators with continuous darkness, constant temperature of 20±1°C and relative

humidity between 70 and 75%. Germinated seeds were counted and removed every 24 h for 15 days. A seed was considered germinated when the tip of the radicle grown free of the seedcoat. The germination rate was calculated by (Maguire, 1962):

$$Rs = \sum_{i=1} \frac{Si}{Di}$$

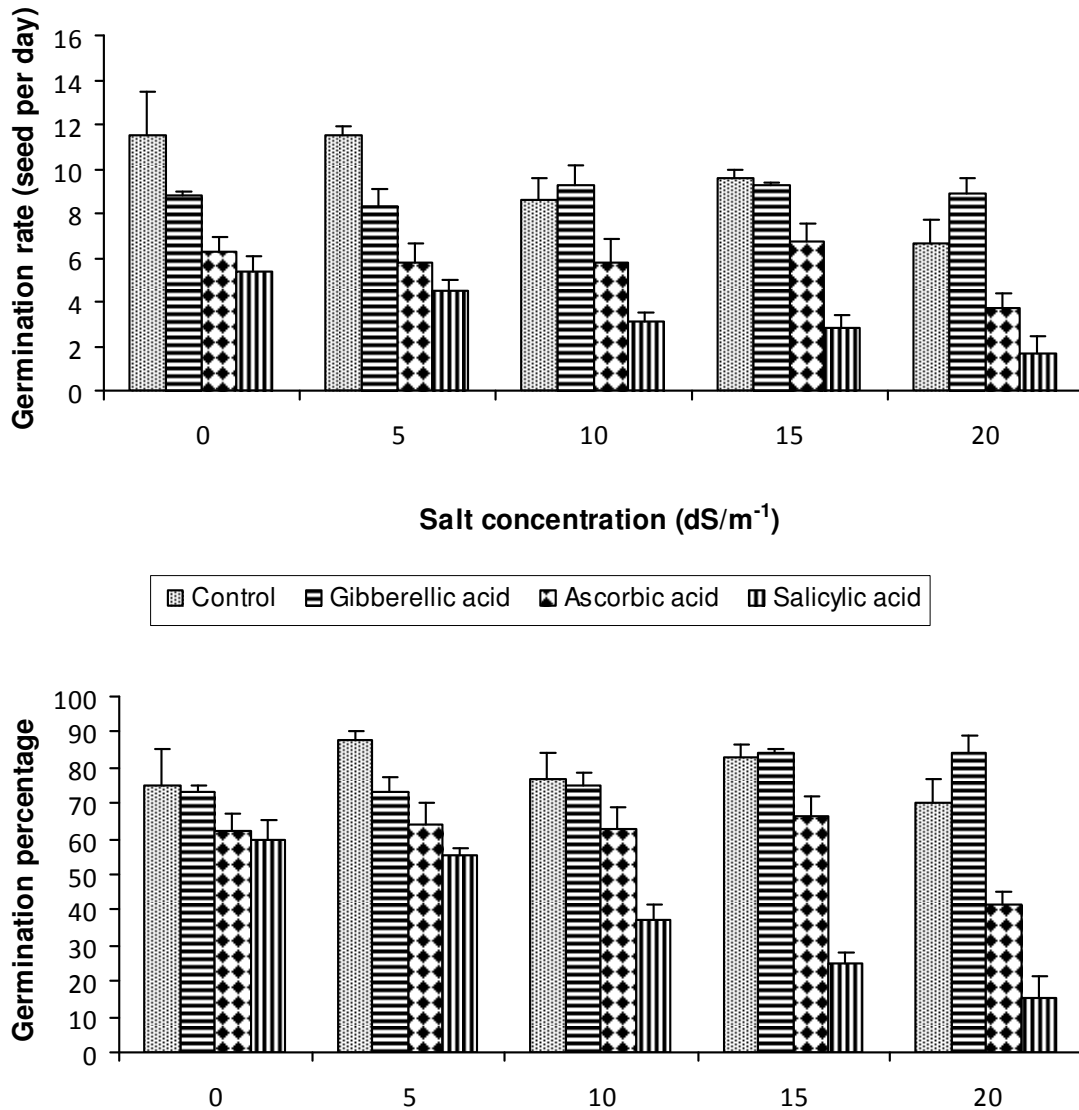
Where Rs is germination rate, Si is germinated seed number in each counting, and Di is counting day's number. Germination percentage and rate calculated in this study.

### Statistical analysis

In order to compare the treatments impacts on study parameters, analysis of variance (ANOVA) was performed as standard procedure for factorial based on completely randomized design. The t-test was used to find significant differences among treatments. The significant differences between treatments were compared by Duncan's multiple range tests at 5% probability level.

## RESULTS AND DISCUSSION

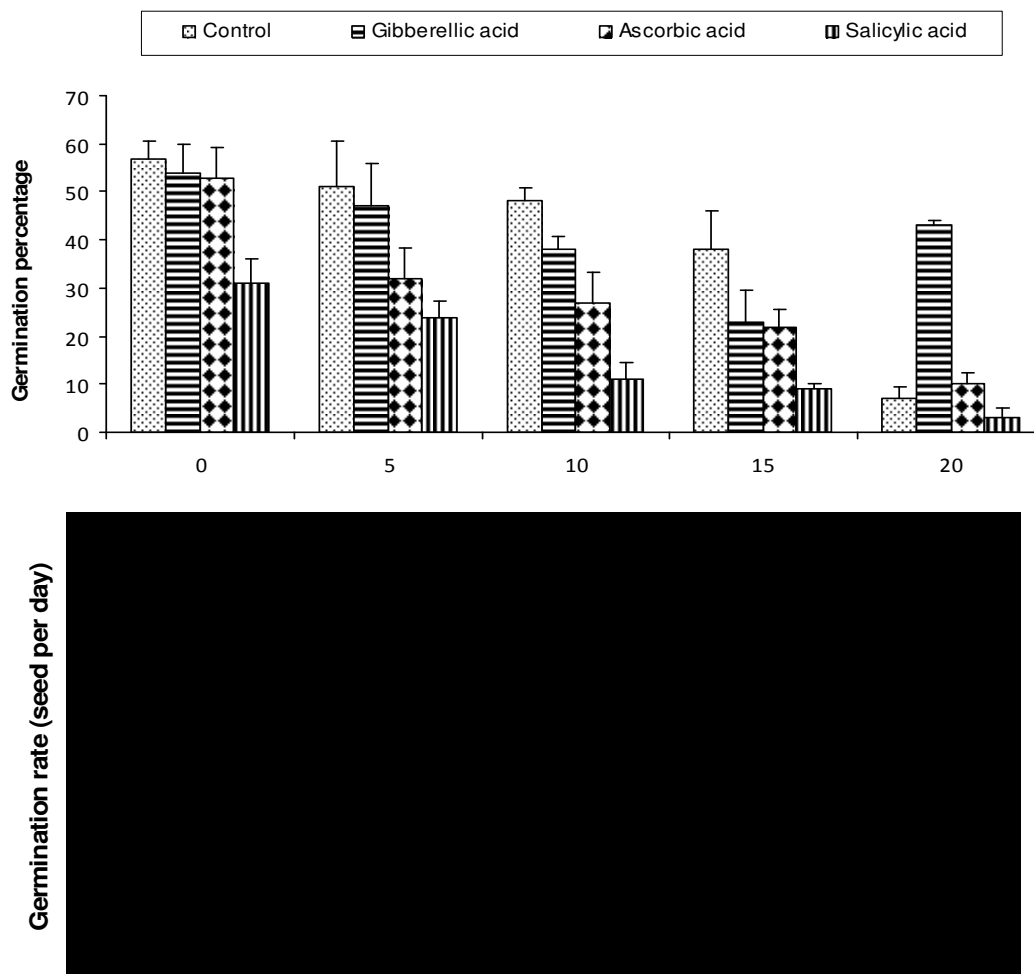
Analysis of variance showed a significant difference among priming treatments ( $p>0.01$ ) in germination percentage and rate across both species (Table 1). Highest values of germination percentage and rate were obtained under ascorbic acid priming treatment. Sage seeds showed higher germination percentage and rate (71% and 7 seeds.day<sup>-1</sup>) in comparison with marsh mallow (41% and 7.4 seeds.day<sup>-1</sup>) seeds under ascorbic acid priming (Table 1). On the other hand, salicylic acid priming application indicated lowest values of germination in both species (Table 1). Reactive oxygen species (ROS) are generated mainly during depletion of food reserves and oxidative phosphorylation at germination level. It has been reported that seeds enclose various antioxidants in small amounts and compounds (Khan et



**Figure 1.** Interactive effects of different seed priming and salt concentrations on germination percentage and rate of sage (*Salvia officinalis* L.).

al., 2006). Thus under stress situations such as salinity, this protective antioxidant system becomes inefficient and may lead to germination failure and even seed death. An artificial raise in cellular level of an antioxidant such as ascorbic acid should be helpful in improving stress tolerance at germination (Khan et al., 2006). The results illustrated that increasing in salt stress levels statistically influenced ( $p > 0.01$ ) germination percentage and rate in sage and marsh mallow (Table 1). Maximum germination percentage and rate was reached in non-saline controls. Many reports showed similar results of salt on seed germination (Khan and Ungar, 2001; Jamil et al., 2006). Germination decrease under saline condition has been recognized to lowering of water potential and/or ionic toxicity but newly this impact was also approved to oxidative stress induced by salinity (Khan et al., 2006).

Impacts of different seed priming on seed germination under various salt stress levels were significant and were similar for both species (Figures 1 and 2). Non-saline controls showed highest germination percentage and rate up to 15 ( $\text{dS/m}^{-1}$ ) salt concentrations across all seed priming treatments in both species (Figures 1 and 2). Gibberellic acid priming alleviated the inhibitory impacts of salt levels on seed germination percentage and rate of sage and marsh mallow even at 20  $\text{dS/m}^{-1}$  salt concentration. Gibberellic acid priming showed more influence on germination percentage and rate of marsh mallow seeds compared to sage seeds in 20  $\text{dS/m}^{-1}$  salt concentrations (Figures 1 and 2). It seems likely that gibberellic acid plays fundamental role in germination process of seeds especially on releasing carbohydrate and proteins degrading enzymes in seeds under salt



**Figure 2.** Interactive effects of different seed priming and salt concentrations on germination percentage and rate of marsh mallow (*Althaea officinalis*).

stress (Baes and Arechiga, 2001). In addition, Kim and Park (2008) reported that salt stress was prevented gibberellic acid induced genes activity on seed germination process.

In conclusion, it seems that marsh mallow seeds and sage seeds were tolerant to salt stress until 15 (dS/m<sup>-1</sup>) salt concentrations. Moreover, genes coding for the synthesis of gibberellic acid might deactivated under higher concentration of salt consequently, seed germination was promoted by additional supplies of gibberellic acid at higher salinities in both species.

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