

## Original Research

Effect of salicylic acid on seed germination and seedling growth of Moldavian balm (*Dracocephalum moldavica* L.) under salt stress

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## ABSTRACT:

Seed germination is considered as one of the critical stage in the growth cycle of plants. Salinity reduces germination and seedlings growth, and ultimately, the function of most medicinal plants. The aim of this research was to study the effect of salt stress (NaCl) and salicylic acid on some characteristics of *Dracocephalum moldavica* L. by measuring seed germination and seedling growth at various concentrations of NaCl and salicylic acid in order to improve salt tolerant capacity. Salicylic Acid (SA) plays a major role in regulating various physiological processes such as plant growth, ion absorption, photosynthesis and germination. In order to investigate the effect of pretreatment of SA under the condition of salt stress on features of germination and seedling growth characteristics, a factorial experiment in a complete randomized design with three replications was carried out. Treatments consisted of salicylic acid at five levels (0, 0.5, 1, 1.5, and 2mM) and salinity at six levels (0, 45, 90, 120, 150 and 200mM) were constructed and analyzed. The results showed that salinity stress significantly reduced all germination and growth parameters compared to the control. Application of salicylic acid (1mM) improved germination and growth parameters at all salinity levels compared to the control. The research out comes are positive towards using salicylic acid on salinity stress conditions and proved that, it can be used to increase the efficiency of Moldavian balm in different salinity conditions.

## Keywords:

Moldavian balm, Seed germination, Growth parameters, Salt stress, Salicylic acid.

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## INTRODUCTION

Moldavian balm is an annual herbaceous plant, belonging to the family Lamiaceae. The origin of this plant is reported to be southern Siberia and the Himalayan slopes (Omidbaigi, 2008; Galambosi and Holm, 1989) and naturally grows in the temperate regions of Europe and Asia (Galambosi *et al.*, 1989; Domokos *et al.*, 1994). The active substance of this plant is a volatile oil that has many uses in pharmaceutical, food, health and cosmetic industries (Borna *et al.*, 2007).

Plants are constantly exposed to biotic and abiotic stresses that have a negative effect on their growth, metabolism and yield. Factors such as drought, cold, heat, salinity and toxic are the major environmental stresses in the cultivation of this plant. Salinity is one of the abiotic stresses limiting the growth and production of agricultural products in arid and semi-arid regions (Marschner, 1995). In Iran, 8.6 million hectares of agricultural lands are affected by salinity (Momeni, 2010). Salinity would affect plant physiology, germination and growth by ionic toxicity, osmotic stress, and oxidative damage (Iterbe-Ormaetxe *et al.*, 1998). Furthermore, salinity stress increased cell respiration (Sudhir and Murthy, 2004), changed carbon and nitrogen metabolism (Kim *et al.*, 2004), as well as mineral distribution and also caused membrane instability (Marschner, 1995; Gupta *et al.*, 2002). Moreover, it reduced the chlorophyll biosynthesis (Khan, 2003) and the efficiency of photosynthesis (Munns, 2002), which them ultimately lead to a reduction in economic performance.

Germination is a critical stage in plant growth cycle because germination plays a major role in determining the final density of the plant. Plants with enough salinity resistance during the germination stage can successfully complete their early growth stage (Rezazadeh and Kochaki, 2005; Moradi and Rezvani-Moghaddam 2010). Salt affects seed germination and early growth seedling through osmotic effects (Khan and Ungar, 2001), ion toxicity or a combination of the two. Reduc-

tion in the germination properties can be attributed to the decrease in amount and rate of water absorption (Moradi and Rezvani-Moghaddam 2010). Plants show different mechanisms for resistance to salt stress. Various studies have been done on the use of plant hormones to increase the resistance of plants to salt stress (Hermann *et al.*, 2007). One of the compounds identified in this regard is salicylic acid. Salicylic Acid (SA) plays an important role in the regulation of various physiological processes such as cell growth, development, ion absorption, photosynthesis and enzyme activation (Tirani and Kalantari, 2007). In addition, the role of SA in defense mechanisms under biotic and/or abiotic stress suggests that it could also alleviate salt stress in plants (Terry and Joyce, 2004; Huijsduijnen *et al.*, 1986).

Many studies have shown that seed pretreatment with SA increases its resistance to various environmental stresses, such as salinity stress in *Cucumis sativus* (Baninasab, 2013), *Vicia faba* (Anaya *et al.*, 2015) and *Foeniculum vulgare* (Moradi and Rezvani-Moghaddam, 2010). However, there is a little information on salinity tolerance of plant species that are used for medicinal purposes when they are pretreated with SA. This study was initiated to determine the effect of pretreatment with salicylic acid on improving the resistance of a common medicinal plant species (Moldavian balm) in Iran.

## MATERIALS AND METHODS

### Seed treatment

Seeds of Moldavian balm were obtained from a commercial supplier. Seeds were stored in sealed plastic jars at 4°C until the start of the experiment. Seeds were surface-sterilized for 10 min in 15% (v/v) sodium hypochlorite solution, and rinsed thoroughly with deionized water (Talei *et al.*, 2011).

The experiment was carried out as a completely randomized design with a factorial arrangement of treat-

**Table 1. Analysis of variance (ANOVA) of treatments with NaCl, SA and their interaction (SA\*NaCl) on germination indices in *D. moldavica* seed**

S. No	Source variations	Df	Mean squares						
			Germination percentage (%)	MGT	Seed vigor	Seedling length (mm)	Root length (mm)	Stem length (mm)	Fresh weight (mg)
1	SA	4	2177.5**	40.1**	26742.2**	201.3**	79.9**	94.5**	52.7**
2	NaCl	5	6998.4**	50.8**	451564.4**	3767.7**	399.7**	1691.6**	447.5**
3	SA*NaCl	20	95.8**	4.5**	2440.5**	7.6*	4.2*	7.2**	1.4 <sup>ns</sup>

\*\* and \* respectively significant of 1 and 5% of probability. ns= non significant.

ments with three replications. The treatments included SA concentration at five levels (0, 0.5, 1, 1.5 and 2mM) and NaCl concentration at six levels (0, 45, 90, 120, 150 and 200mM). Seeds were soaked in different concentrations of SA for 12 h at 22°C under dark condition, and then three 25-seed replicates were placed in 11-cm petri dishes containing one disk of Whatman No. 1 filter paper, with 7 ml of NaCl solution. All petri dishes were placed in the germinator at the temperature of 25+1°C in the dark with a relative humidity of 70% for 14 days. Germinated seeds were counted every day (Standard germination is when the root tip was approximately 2mm) (Saberli and Moradi, 2017).

#### Treatments, estimations and formula

In this experiment germination percentage, mean germination time, seed vigor index, seedling length, stem and root length and fresh weight of seedlings were measured.

**Germination Percentage (GP)** (Camberato and Mccarty, 1999).

$$GP = (n/N) * 100$$

where, n: number of germinated seeds in the n<sup>th</sup> day;

N: total number of seeds

**Mean Germination Time (MGT)** (Walker and Sessing, 1990).

$$MGT = \sum(fi * ni) / N$$

where, fi: day of counting; ni: number of germinated seeds in the day; N: total number of germinated seeds

**Seed Vigor Index (SVI)** (Agraval, 2005)

$$SVI = \text{Germination Percentage} \times \text{Seedling Length}$$

Analysis of variance was carried out using SAS software (SAS, 2003) and means were compared using the Least Significant Differences (LSD) test at P= 0.05.

## RESULTS

### Germination percentage

The results of analysis of variance (Table 1) showed a high significant effect of NaCl and salicylic acid on the germination of Moldavian balm. Germination percentage was decreased with increasing salinity stress (Table 3). The highest and lowest germination percentage was observed in the control treatment and at

**Table 2. Effect of SA pretreatment on germination traits and seedling growth characteristics**

S. No	Salicylic acid (mM)	Germination percentage (%)	MGT (day)	Seed vigor	Seedling length (mm)	Root length (mm)	Stem length (mm)	Fresh weight (mg)
1	0	55.5±25.04	6.9±3.68	154.5±155.83	18.3±14.1	5.6±3.68	12.3±9.55	7.9±4.55
2	0.5	77.1±18.79	3.3±1.2	232.2±160.31	24.4±14.11	9.6±5.2	16.9±9.68	11.3±5.39
3	1	84.4±13.97	3.2±1.14	245.1±167.77	27.3±15.6	11.0±5.38	18.3±9.97	12.4±4.9
4	1.5	71.3±20.29	4.1±1.23	195.9±170.96	22.5±14.72	8.4±5.53	15.4±10.09	10.1±5.44
5	2	65.7±22.75	4.5±2.54	171.9±159.1	21.2±14.63	7.1±4.8	14.4±9.96	9.5±5.12

Table 3. Effect of NaCl on germination traits and seedling growth characteristics

S. No	Salinity (mM)	Germination percentage (%)	MGT (day)	Seed vigor	Seedling length (mm)	Root length (mm)	Stem length (mm)	Fresh weight (mg)
1	0	96.2±5.54	2.5±0.59	440.2±31.16	41.9±2.82	15.4±2.68	29.9±1.08	16.4±1.76
2	45	89.3±8.5	3.1±0.73	373.9±62.34	40.5±5.03	12.9±3.4	25.9±3.96	15.5±2.22
3	90	80.2±11.05	4.1±0.85	205.5±77.18	24.3±5.18	8.6±2.98	15.1±3.85	11.8±2.47
4	120	64.2±12.23	4.7±1.09	101.6±42.18	15.4±3.77	7.2±2.7	12.0±2.39	9.0±1.52
5	150	55.2±13.7	6.6±2.97	63.09±31.65	11.0±2.28	4.7±1.28	7.6±1.48	6.6±1.52
6	200	39.7±15.52	6.5±3.68	15.3±11.53	3.3±2.49	1.4±1.08	2.3±1.79	2.1±1.73

200mM NaCl, respectively. Several researchers also showed the negative effects of salinity stress on germination of different plant species and concluded that reduction in germination percentage of seeds under salt stress resulted from the absence of ethylene production during imbibition (Orlovsky *et al.*, 2011; Chang *et al.*, 2010). Application of salicylic acid pretreatment increased germination percentage significantly compared to control treatment (Table 2). The results showed that the lowest and the highest germination percentage were obtained at 0 and 1mM salicylic acid concentrations. Germination percentage was decreased by increasing the salicylic acid concentration from 1 to 2mM. However, There was a significant interaction ( $p<0.01$ ) between salinity and SA concentration (Table 1). Figure 1 shows the effect of SA on germination percentage at different NaCl concentrations. In the study of

the interaction between salinity and pretreatment with SA, it was found that at different levels of salinity, there is a significant difference between salicylic acid concentrations. At all salinity levels, priming seeds with 1mM concentration of SA had the highest percentage of germination compared to untreated ones. Shakirova and Bezrukova (1997) and Rajasekaran and Blake (1999), demonstrated that seed pretreatment with SA had a positive effect on seed resistance to salinity stress. Salicylic acid alleviated the inhibitory effects of salinity stress on germination.

#### Mean Germination Time (MGT)

The NaCl and salicylic acid concentration had significant effect on the MGT (Table 1). Increased salinity also significantly increased MGT and delayed the germination of seeds (Table 3). SA had a positive effect on MGT. The seed germinated faster under pre-

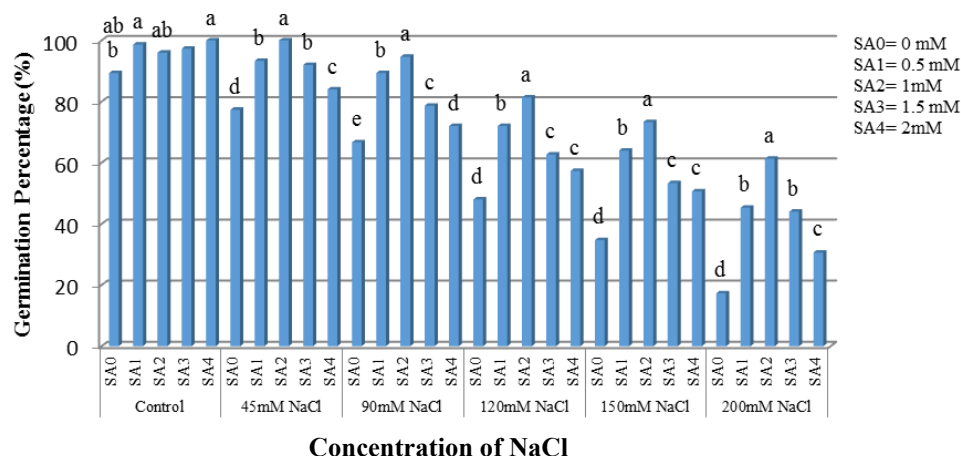


Figure 1. Interaction between SA pretreatment and salt stress on germination percentage

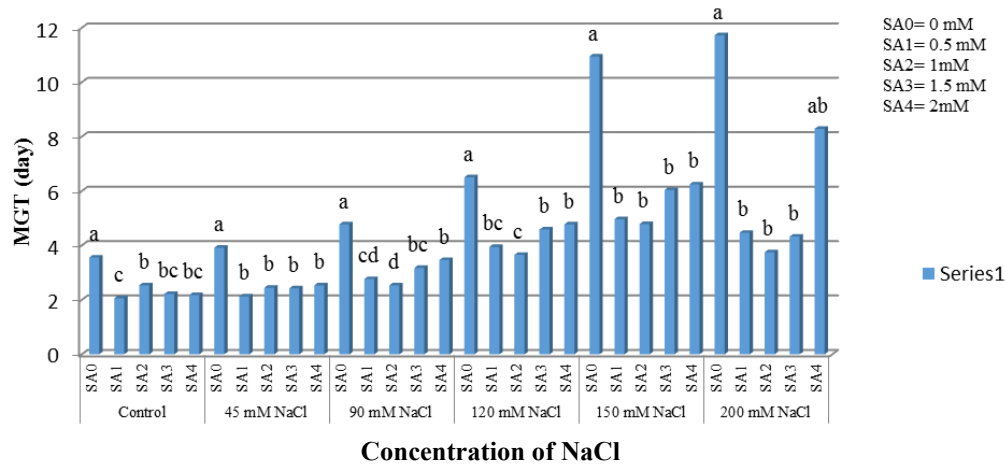


Figure 2. Interaction between SA pretreatment and salt stress on mean germination time

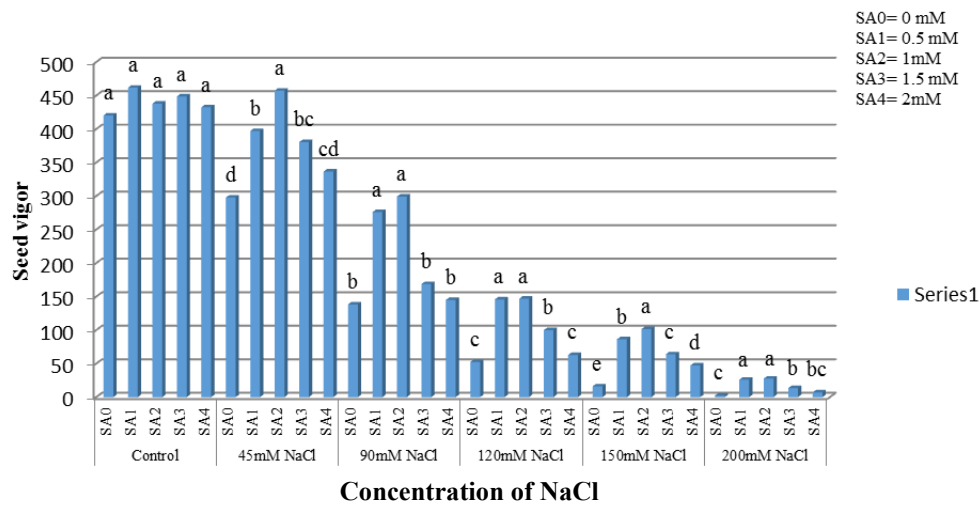


Figure 3. Interaction between SA pretreatment and salt stress on seed vigor

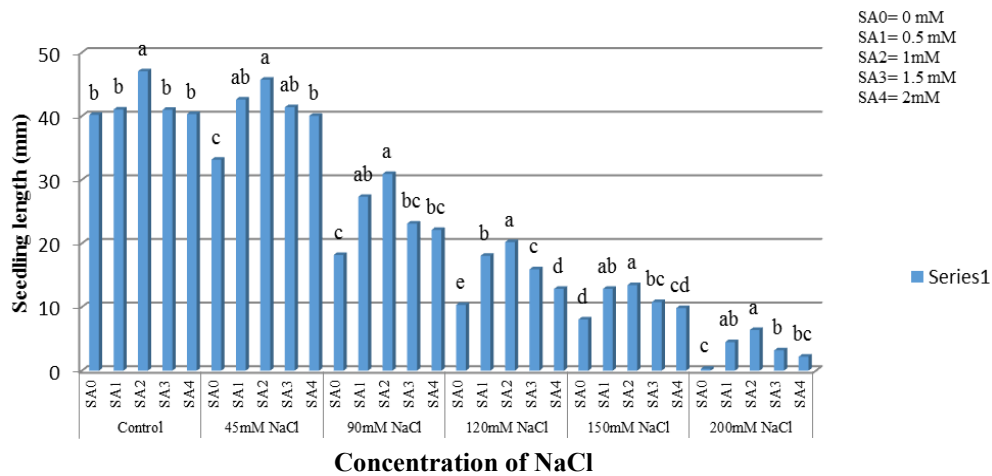


Figure 4. Interaction between SA pretreatment and salt stress on seedling length

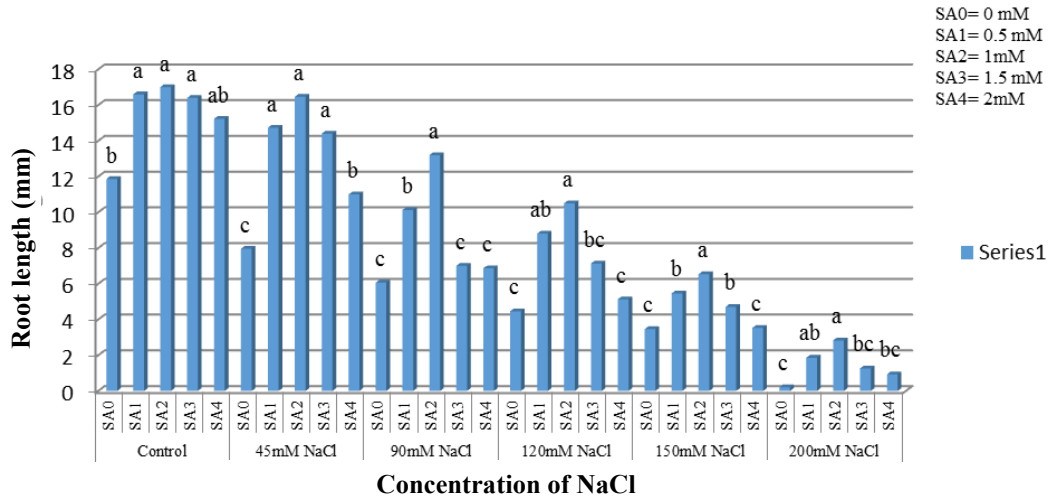


Figure 5. Interaction between SA pretreatment and salt stress on root length

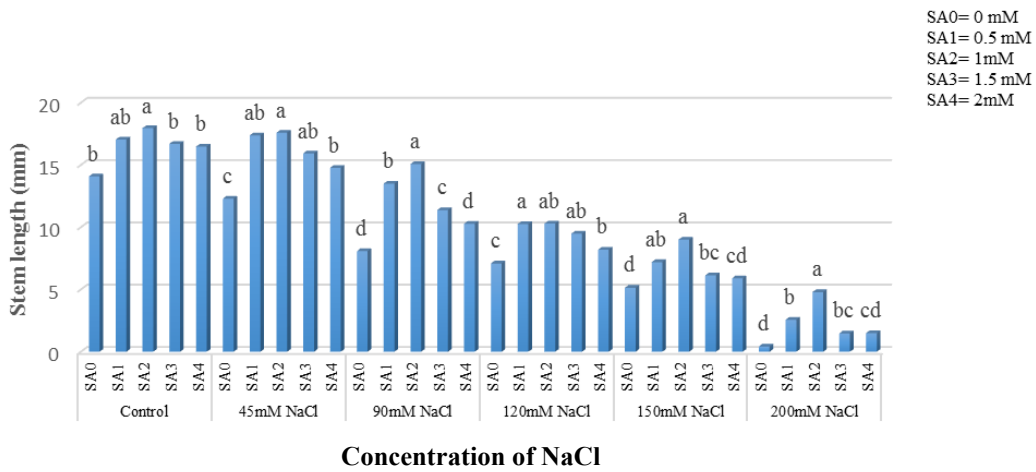


Figure 6. Interaction between SA pretreatment and salt stress on stem length

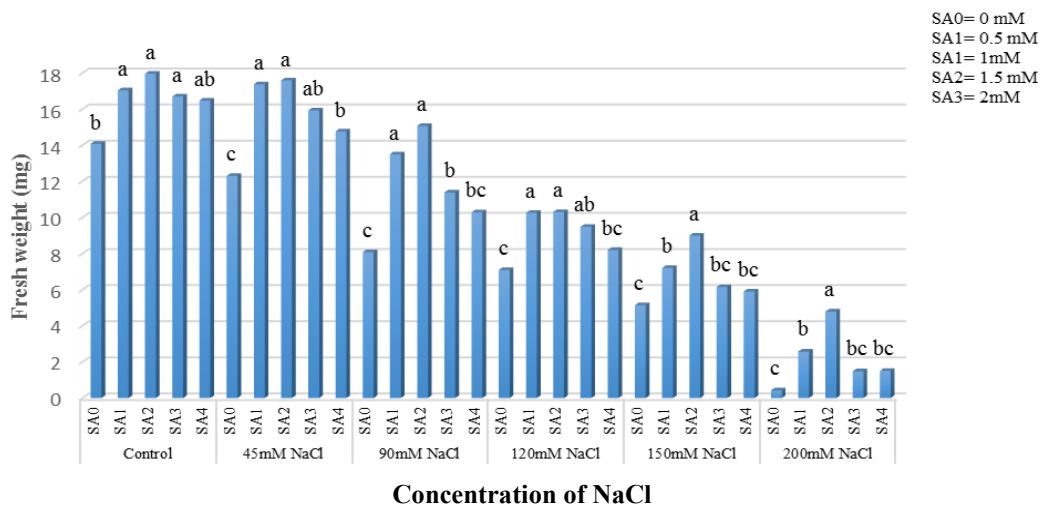


Figure 7. Interaction between SA pretreatment and salt stress on fresh weight

treatment with 1mM SA (3.2 days) in which germination time was 53.62% less than control seeds (Table 2).

There was a significant ( $p < 0.01$ ) interaction effect between salinity and SA concentration on MGT (Table 1). The effect of NaCl concentration and SA on the MGT is shown in Figure 2. The application of SA should significant effect on the MGT under all salinity levels. Salicylic acid treatments have a positive effect the MGT compared to the control. Nun *et al.* (2003) reported that SA could inhibit catalase activity and Reduction in catalase activity leads to increased activity of peroxide hydrogen, which can improve the germination of some seeds. It is likely that salicylic acid stimulates seed germination through gibberellin biosynthesis and thermogenesis (Shah, 2003). However, according to the above results, pretreatment of seeds with SA improved seed germination and decreased the MGT under salinity conditions.

#### Seed vigor

Seed vigor was significantly affected by both salinity and SA pretreatment (Table 1). Seed vigor index was decreased by increased concentration of NaCl (Table 3). All concentration of SA significantly increased seed vigor compared with the control treatment (Table 2). Our results showed that the lowest and highest seed vigor was obtained from 0 and 1mM SA concentrations, respectively. In addition, the interactive effect of salinity and SA showed that seed vigor of the treatments exposed to 0.5 and 1mM SA had a significant difference compared to the control treatments. In all salinity levels, seeds treated with 0.5 and 1mM SA showed the highest seed vigor and seeds in control and 2mM SA treatments had the lowest seed vigor (Figure 3). This result is in agreement with the results reported by Jobshahr and Khoramivafa (2012) in *Calendula officinalis*, they reported a positive effect of seed priming with salicylic acid on seed vigor under salinity stress. The positive effect of salicylic acid on seed vigor can be due to the effect on the production of hormones

that contribute to increase germination percentage and vegetative parameters.

#### Seedling length

Analysis of variance results showed a significant effect of NaCl and salicylic acid on the seedling length (Table 1). With increasing salinity, seedling length decreased significantly as with 200mM NaCl seedling length decreased by 92% compared to the control (Table 3). Application of SA increased seedling length (Table 2). The maximum and minimum seedling lengths were observed in the treatment with 1mM salicylic acid and control respectively. The plant length increased with the application of 0 to 1mM SA, and beyond these concentrations the length decreased. There was a significant interaction between salinity and SA concentrations (Table 1). At all salinity levels, 1mM pretreatment with salicylic acid had the highest seedling length, and control treatment had the lowest seedling length (Figure 4). It should be noted that at some salinity levels, no significant differences were observed between control treatment and 2mM salicylic acid. Many studies have demonstrated the positive effect of salicylic acid on growth parameter under salinity stress conditions Palma *et al.* (2009) and Noreen and Ashraf (2008). In addition, Khodary (2004) showed that salinity stress reduced growth parameters of corn, and application of seeds primed with salicylic acid increased salt resistance and increased vegetative traits compared to the control treatment. Salinity reduces the biosynthesis of hormones and enzymes by decreasing water availability and decreasing water absorption of seed (drought stress) and prevents seedling growth (Khan and Ungar, 2001).

#### Root and stem length

The root and stem length were significantly affected by salinity and SA pretreatment (Table 1). Root and stem length decreased with the increasing salinity stress (Table 3). Earlier studies have shown that NaCl treatment decreased some growth parameters such as shoot and root of plants (Zhu *et al.*, 2004; Mori *et al.*,

2011). Application of SA significantly increased root and stem length (Table 2). The maximum of root and stem length were obtained from seed pre-treatment with 1mM SA (11 and 18.3mm, respectively).

The result showed that pre-treatment with salicylic during stress has a positive effect on salinity resistance in seeds (Figure 5 and 6). The pretreatment with 1mM concentration of salicylic acid, affects root and stem length in the control and stress conditions, but its effect increases by increasing salinity stress. This finding showed an increase in the salicylic acid concentration more than 1mM and has a negative effect on the root and stem length. Salinity stress directly reduced growth by decreasing breakdown and translocation of seed reserves and finally decreased assimilate provision for embryo (Farhadi and Azizi, 2014). Moreover, Gutierrez- Coronado (1998) showed increased growth parameters such as stem and root lengths of soybean plant in response to salicylic acid treatments. Salicylic acid regulates the growth and division of the cells by affecting hormones such as auxin, cytokinin, gibberellin and abscisic acid. It has been reported that growth-promoting plant hormones increase the amount of cell division of the primary meristem, which ultimately leads to an increase in root and stem length growth (Shakirova *et al.*, 2003).

#### **Fresh weight**

Fresh weight of seedling grown in different concentrations of NaCl and SA are presented in Figure 7. Salinity decreased fresh weight of seedling compared to non-saline condition (Table 3). The applications of SA improve the fresh weight especially under 1mM (Table 2), which was 56.96% more than the control. Similar effects were reported by Anaya *et al.* (2015) on the seeds of bean and was grown under salinity stress and priming with salicylic acid.

#### **CONCLUSION**

In general, salinity stress reduced germination

and seedling growth and increased salinity level that increased the negative salinity effects on the germination and growth parameters. Furthermore, the application of salicylic acid at 1mM concentrations in all salinity levels increased the germination and growth properties compared to the control (0mM SA), but high levels of salicylic acid pre-treatment causes damage to seed germination and seedling growth characteristics. According to the results of this study, seed priming with relatively low and medium (0.5 and 1mM SA) salicylic acid concentrations in salt stress conditions increased seed germination and seedling growth compared to control treatment, which increased seedling initial deposition during planting and eventually increased the yield of the plant, especially in areas with salty water or soil resources.

The results of this experiment can be recommended for testing soil and water tests in different areas and comparing salinity levels. The findings of this study showed that salicylic acid could improve germination and plant growth at different levels of salinity with the least cost and loss during plant growth and environmental.

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