Influence of salicylic acid pre-treatment on germination, vigor and growth parameters of garden cress (Lepidium sativum) seedlings under water potential loss at salinity stress

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ABSTRACT: One of the critical stages of plant growth cycle, stages of germination and seedling establishment because it plays a major role in determining the final density of plants. Low seed germination and seedling emergence is one of the main problems in saline areas. Seed priming technique has been known as a cause to improve germination and seedling emergence under different environment stresses. The other side, Salicylic acid or orthohydroxybenzoic acid play a major role in regulation of many physiological processes e.g. growth, development, ion absorption and germination of plants. The objective of this study was to evaluate the effectiveness of seed priming in improving seed germination, seedling vigor index and growth characteristics of Garden cress (Lepidium sativum), in response to 4 levels of NaCl (0, 50, 100 and 150 mM) and to 4 levels of priming by Salicylic acid (0, 500, 1000 and 1500 µM). An experiment was conducted as factorial based on CRD with three replications in research laboratory of agriculture faculty of Maragheh University. The results showed that plumule length and radicle length were significantly (P<0.01) decreased by increasing salinity for unprimed seed (control) compared with primed seeds but plumule weight and radicle weight were non-significantly. Increased salinity significantly increased plumule to radicle ratio of seedlings. Also, Salinity conditions reduced seed viability and seed germination percent. Non-stress and 150 mM NaCl Treatment had the maximum (95.3%) and had the minimum (89.7%) seed germination percent, respectively. Also in priming by salicylic acid control treatment and 500 µM had the maximum and the treatment 1000 and 1500 µM had the minimum seed germination percent. In conclusion seed priming by salicylic acid improved the germination, but this increase was not significant compared to control treatment.

Keywords: Seed priming; Germination; Salinity; Salicylic acid; Lepidium sativum.

Abbreviations: SA-Salicylic Acid; SGP-Seed Germination Percentage; RL-Radicle Length; PL-Plumule Length; SL-Seedling Length; RFW-Radicle Fresh Weight; PFW-Plumule Fresh Weight; SFW-Seedling Fresh Weight; SVI-Seedling Vigor Index.

INTRODUCTION

Salinity in farms that are in arid and semi-arid areas in almost all the regions of the world is a major problem (Munns and James, 2003; Khan and Qaiser, 2006). Among these stresses, high concentrations of salt in the soil can result in severe detrimental factors, such as poor germination, seedling establishment and crop yield. Reduced plant growth under salt stress is a commonly occurring phenomenon (Iqbal and Ashraf, 2006; Hosseini and Thengane, 2007). This is mainly due to low soil water potential and an imbalance in the uptake of mineral nutrients and their accumulation within the plant. Also, salt stress can disturb growth and photosynthetic processes by causing changes in the accumulation of Na⁺, Cl⁻ and nutrients, and disturbance in water and osmotic potential (Nafees et al., 2010). It also mitigates the effects of salinity by reducing the uptake of toxic ions and maintaining cell membrane integrity (Maria et al., 2000).

Seed priming is an efficient method for increasing seed vigor and synchronization of germination, as well as the growth of seedlings of many crops under stressful conditions (Carvalho et al., 2011). Generally priming would cause an effective invigoration of the dry seed which is the inception of metabolic processes that normally occur during imbibition and which are subsequently fixed by drying the seed (Hanson, 1973). Seed
pre-treatment is recommended in farming as a simple, inexpensive technique for counteracting the salinity of soils.

Salicylic acid is a phenolic plant growth regulator having a role in regeneration of physiological processes in plants (Sakhabutinova et al., 2003). Salicylic acid and polyamines as signal molecules may play a role in improving the growth and development of the crops (Krantev et al., 2008). Salicylic acid or orthohydroxybenzoic acid belongs to a group of phenol compounds that are known as the mitigating factors of plant responses to environmental stresses (Senaratna et al., 2000). It has been reported that SA has improved some abiotic stresses, e.g. heat stress (Dat et al., 1998; He et al., 2002), chilling stress (Kang and Saltveit, 2002) and heavy elements stress (Metwally et al., 2003). Karthiresan et al. (1984) and Zhang et al. (1999) reported that increase in emergence percentage in seeds primed with SA under saline conditions may be due to enhanced oxygen uptake, increased α-amylase activity and the efficiency of mobilizing nutrients from the cotyledons to the embryonic axis and increased the contents of soluble sugar, protein and free amino acids. According to these researches, SA pre-treatment have improved growth and resulted in higher resistance of plants to salinity, so that it have increased germination percentage, seed vigor index and growth parameters of the seedlings. So salicylic acid is an effective priming factor and increases some of the growth parameters.

Therefore, given the medicinal value of garden cress (Lepidium sativum), the objective of the current research was to study the responses of garden cress seedlings to salinity stress and to investigate the possibility of mitigating the effects of salinity stress on this crop by pre-treating its seeds with salicylic acid.

**MATERIALS AND METHODS**

The current study was carried out in research laboratory of Department of Agronomy and Plant Breeding, Faculty of Agriculture, University of Maragheh, Maragheh, Iran in 2012 as a factorial experiment based on a randomized complete block design with three replications on garden cress (Lepidium sativum).

The experimental treatments included salinity at four levels (0, 50, 100 and 150 mM) and salicylic acid at four levels (0, 500, 1000 and 1500 µM). The seeds and petri dishes were sterilized by 10% hypochlorite solution and then, were rinsed with distilled water. The seeds were soaked in 500, 1000 and 1500 µM SA at 25°C for 4 hours. Distilled water was used as control treatment. Afterwards, the primed seeds were rinsed with distilled water and dried between two layers of paper (23±2°C with relative humidity of 60%).

Thirty pre-treated seeds were included in each petri dish and received the same required amount of salinity solutions and distilled water (as control). After 24 hours, the seeds were daily checked for 7 days and the number of germinated seeds was recorded. A seed was considered germinated upon emergence of radicle about 2 mm in length. Seed germination percentage (SGP) was determined at the end of the test. Germination potential of the garden cress (Lepidium sativum) seeds was estimated in accordance with the International Rules for Seed Testing (ISTA, 1985).

To determine the radicle length (RL), plumule length (PL), seedling length (SL), radicle fresh weight (RFW), plumule fresh weight (PFW), seedling fresh weight (SFW) after the 7th day, normal plumules and radicles produced in each petri dish were separated from the seeds, and their lengths and fresh weight were measured. Seedling vigor index (SVI) was calculated by multiplying the germination percentage by the seedling length and then dividing the product by 100 (Abdul-Baki and Anderson, 1970).

For statistical analysis, a factorial experiment with completely randomized design (CRD) with three replications was used. Analysis of Variance was based on ANOVA procedure by software SAS. Differences among the means of the treatments were estimated using the Duncan's multiple range tests at the 5% probability level.

**RESULTS AND DISCUSSION**

The results of analysis of variance showed that salinity significantly affected radicle length, plumule length, seedling length, plumule to Radicle ratio, radicle fresh weight, plumule fresh weight, seedling fresh weight, seed germination percentage and seed viability (Table 1). Also, the results of analysis of variance showed that salicylic acid significantly affected seed germination percentage and seedling vigor index (Table 1 and 2). However, a non-significant interaction occurred between salinity and SA concentration.

Table 1. Analysis of variance of the effects of salt condition, seed priming by salicylic acid and their interactions on germination and seedling growth parameters.

<table>
<thead>
<tr>
<th>S.O.V</th>
<th>Mean Square</th>
<th>RL</th>
<th>SL</th>
<th>PFW</th>
<th>RFW</th>
<th>SFW</th>
<th>P/R</th>
<th>SGP</th>
<th>SVI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salt condition (SC)</td>
<td>3</td>
<td>40.4</td>
<td>29.1 **</td>
<td>121 **</td>
<td>335 *</td>
<td>34.4</td>
<td>445 *</td>
<td>2.96 *</td>
<td>62.4 *</td>
</tr>
<tr>
<td>Salicylic acid (SA)</td>
<td>3</td>
<td>10.9 *</td>
<td>2.31</td>
<td>19.6</td>
<td>187</td>
<td>14.3</td>
<td>150 *</td>
<td>0.64 *</td>
<td>178 **</td>
</tr>
<tr>
<td>SC×SA</td>
<td>9</td>
<td>8.59 *</td>
<td>0.41</td>
<td>9.18</td>
<td>162</td>
<td>9.89</td>
<td>212 *</td>
<td>0.66 *</td>
<td>20.2 *</td>
</tr>
<tr>
<td>Error</td>
<td>32</td>
<td>315</td>
<td>39.5</td>
<td>442</td>
<td>3947</td>
<td>386</td>
<td>4680</td>
<td>25.1</td>
<td>482</td>
</tr>
</tbody>
</table>

Table 1. Analysis of variance of the effects of salt condition, seed priming by salicylic acid and their interactions on germination and seedling growth parameters.
ns, * and **: Non significant, significant at 5 % and 1 % levels of probability, respectively.

Plumule Length (PL), Radicle Length (RL), Seedling Length (SL), Plumule Fresh Weight (PFW), Radicle Fresh Weight (RFW), Seedling Fresh Weight (SFW), Plumule to Radicle ratio (P/R), Seed Germination Percentage (SGP) and Seedling Vigor Index (SVI).

The seedling fresh weight and seedling length were significantly affected by salinity (Table 1). Seedling fresh weight and seedling length were significantly decreased with the increasing salinity stress (Fig. 1c). Earlier studies have shown that NaCl treatment decreased the some growth parameters such as fresh weight of shoot and root of plants (Yildirim et al., 2008; Mori et al., 2011). In this study, we found that the presence of NaCl reduced the plumule fresh weight and radicle length of the garden cress (Lepidium sativum) compared to the control as a consequence of salt osmotic effects, which reduced water availability (Fig. 1a, b). During imbibitions, water entry occurs through aquaporins, which have reduced expression in the presence of salt (Boursiac et al., 2005).

![Figure 1](image-url)

**Figure 1.** Effect of salinity levels on plumule and radicle length (A), plumule and radicle fresh weight (B), seedling length and seedling fresh weight (C) in garden cress (Lepidium sativum). Within each salt level, means with different letters are significantly different (p<0.05).
Application of SA no significantly increased seedling fresh weight and seedling length (Table 1) and that there was no statistically significant difference between various levels of salicylic acid. Our results in against with those of Baninasab and Baghbanha (2013) in cucumber, Stevens et al. (2006) in tomato and Gunes et al. (2005) in maize who showed that SA treatments ameliorated the negative effect of salt stress on fresh and dry weight of plants. Shakirova et al. (2003) indicated that SA treatments reduced the damaging action of salinity on wheat seedlings growth, rising indoleacetic acid content and enhancing of cell division and extension of root cell. Khan et al. (2003) reported that SA stimulated the root formation of some crops. Gunes et al. (2005) recorded that increases in fresh and dry matter of salt stressed plants in response to SA might be related to the induction of antioxidant response and protective role of membranes that increase the tolerance of plant to damage.

Increased salinity significantly increased plumule to radicle ratio of garden cress (Lepidium sativum) seedlings (Fig. 2a). Sharp and Oavies (1989) stated that when seedlings were put under low water potential (-1.6 MPa), radicle growth was often less limited than the growth of hypocotyl which was a mechanism for the adaptation of crop to water stress. In control and salinity stress conditions a negative correlation was found between plumule to radicle ratio and the PL (r=-0.56*), PFW (r=-0.50*) and SFW (r=-0.57*) (Table 3).

![Figure 2](image.png)

Figure 2. Effect of salinity levels on plumule to radicle ratio (A) and vigor index (B) in garden cress (Lepidium sativum). Within each salt level, means with different letters are significantly different (p<0.05).

Seed vigor index is also called power of seed or seed viability, it is the power and ability of seed emergence in difficult conditions (stress). Seedling vigor index were significantly decreased with the increasing salinity stress (Fig. 2b). Maximum seed vigor is related to low level of salt stress and control treatment. By increasing osmotic potential due to NaCl, seed vigor is decreased. Some of the researchers announced that in the stress condition, growth of plumule decrease more than radicle. Also, decreasing in weight is more than length (Shalheret et al., 1995). On the other hand, there are some reports that under stress condition, root growth affected more than shoot and may be root weight do not affected significantly (Van-De-Vanter, 2001). Also, it has been reported root growth can be consider as a screening scale for seed vigor, because if seedling can't make a powerful root system, their survival ability reduce significantly (Makawi et al., 1999). By increasing the stress level plants will allocate more assimilates to root in order to growth more and be able to absorb more water.

In control and salinity stress conditions a positive correlation was found between SVI and the plumule length (r=0.93**), radicle length (r=0.84**), seedling length (r=1**) and seed germination percentage (0.66**) (Table 3).

The germination percentage was significantly affected by salinity and SA pretreatment, but not their interaction (Table 1). Salinity led to the severe loss of germination percentage, so that 50, 100 and 150 mM salinity levels decreased it by 2.6, 2.9 and 5.9%, respectively (Fig. 3). Adequate water absorption and performance of metabolic and biochemical process in phases I and II germination may be reasons of germination and seedling weight improvement (Jorjandi and Sharifi-Sirchi, 2012). A negative effect of salinity on germination and emergence has been reported for several vegetables species (Sivritepe et al., 2003; Jamil et al., 2006). Such a response might be related to the inhibitory effect of the solution low osmotic potential and/or to ionic toxicity (Zhu, 2002).
Application of SA significantly decreased seed germination percentage (Table 2). The most germination percentage was obtained from seedlings pre-treated with 500 µM Salicylic acid but no-signification control treatment and seed germination percentage decreased with increasing concentrations of SA. Similar, Farahbakhsh (2012) reported that the concentration of 0.25 and 0.5 mM of salicylic acid on measured traits was more effective compared with the other levels. There are conflicting reports about the effects of salicylic acid on seed germination. Sharafizad et al. (2013) reported that after the control treatment (priming with distilled water), the highest percentage of germination was for low concentrations of Salicylic acid (0.7 mM) and germination percentage decreased with increasing concentrations of SA. Moradi and Rezvani-Moghaddam (2012) reported that the highest radicle to hypocotyle ratio was obtained at 1.5 mM salicylic acid, and 4 mM salicylic acid didn’t have significant effect on all maintained parameters. With no salinity stress (control), salicylic acid didn’t have any significant effect on all parameters, but when there was salinity stress, pre-priming increased all studied characteristics significantly. Jamshidi-Jam et al. (2012) and Madah (2005) reported that low concentrations of salicylic acid increased the germination percentage, but this increase was not significant compared to control treatment. Also, Shakirova et al. (2003) reported soaking wheat seeds at a low concentration of salicylic acid (0.05 mM) for 3 hours activate germination. But, our results in against with those of Torabian (2010) have investigated the effect of SA pre-treatment of seeds (at three levels of 0, 0.1 and 0.5 mmol) on germination and growth of seedlings of alfalfa under salinity stress conditions (at three levels of 0, 10 and 15 ds.m⁻¹). Rajaskaran et al. (2002) showed the external application of salicylic acid stimulates seed germination.

Table 1 analysis of variance shows that there is no significance difference in this trait between the treatments. Comparison between the average levels of seed priming (Table 2) shows that by increasing the concentration of SA, seedling vigor index is decreased.

Table 2. Mean comparison of seed priming by salicylic acid on germination and seedling vigor index in garden cress (Lepidium sativum).

<table>
<thead>
<tr>
<th>Traits</th>
<th>Germination percentage (%)</th>
<th>Seedling vigor index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salicylic acid (µM)</td>
<td>95.8 a 9.90 a</td>
<td>96.8 a 8.79 ab</td>
</tr>
<tr>
<td>0</td>
<td>95.8 a</td>
<td>96.8 a</td>
</tr>
<tr>
<td>500</td>
<td>90.3 b</td>
<td>88.3 b</td>
</tr>
<tr>
<td>1000</td>
<td>90.3 b</td>
<td>6.65 b</td>
</tr>
<tr>
<td>1500</td>
<td>90.3 b</td>
<td>7.12 ab</td>
</tr>
</tbody>
</table>

Means followed by the same letter within columns are not significantly different (P < 0.05) according to Duncan’s test.

Table 3. Correlation coefficients among germination and seedling growth parameters in garden cress (Lepidium sativum) under different salinity levels.

<table>
<thead>
<tr>
<th>Traits</th>
<th>PFW</th>
<th>RFW</th>
<th>PL</th>
<th>RL</th>
<th>SL</th>
<th>SFW</th>
<th>P/R</th>
<th>SGP</th>
<th>SVI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plumule Fresh Weight (PFW)</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Radicle Fresh Weight (RFW)</td>
<td>0.26</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plumule Length (PL)</td>
<td>-0.06</td>
<td>-0.10</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Radicle Length (RL)</td>
<td>0.44</td>
<td></td>
<td>0.58*</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Seedling Length (SL)</td>
<td>0.16</td>
<td>0.02</td>
<td>0.93**</td>
<td>0.84**</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Seedling Fresh Weight (SFW)</td>
<td>0.97**</td>
<td>0.49*</td>
<td>-0.08</td>
<td>0.45</td>
<td>0.15</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plumule to Radicle ratio (P/R)</td>
<td>-0.50*</td>
<td>-0.43</td>
<td>0.30</td>
<td>-0.56*</td>
<td>-0.05</td>
<td>-0.57*</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Seed Germination Percentage (SGP)</td>
<td>0.12</td>
<td>0.33</td>
<td>0.51*</td>
<td>0.60*</td>
<td>0.61*</td>
<td>0.19</td>
<td>-0.19</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Seedling Vigor Index (SVI)</td>
<td>0.14</td>
<td>0.04</td>
<td>0.93**</td>
<td>0.84**</td>
<td>1.00**</td>
<td>0.14</td>
<td>-0.06</td>
<td>0.66**</td>
<td>1</td>
</tr>
</tbody>
</table>

* and ** Significant at the 5 and 1 percent levels, respectively and another no significant.
CONCLUSION

According to our results and with comparing primed and non-primed seeds it is clear that seed priming could not enhance seed germination in stress conditions. Generally, a decreasing trend in all traits except plumule to radicle ratio was observed for increasing concentrations of salt. Low concentrations of salicylic acid (500 μM) increases the germination percentage, but this increase was not significant compared to control treatment, and germination percentage decreased with increasing concentrations of SA. Therefore it can be concluded that priming is a simple, cheap and unsophisticated tool that has a practical importance and could be recommended to farmers to achieve higher germination and uniform emergence under field conditions.

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