Germination and seedling growth in un-primed and primed seeds of Fenel as affected by reduced water potential induced by NaCl

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ABSTRACT: Germination is a critical stage in the life cycle of plants. Successful establishment of plants largely depends on successful germination. This study investigated the effects of salicylic acid (0, 0.25, 0.5 and 0.75 mM) on germination, germination rate, seed stamina index, hypocotyl length, radical length, seedling fresh and dry weight of Fenel (Foeniculum vulgare) under different water potentials (0, -2, -4 and -6 bar) induced by NaCl. The experimental design employed was a factorial arrangement based on completely randomized design with three replicates and performed at the laboratory of agriculture faculty of Shahid Bahonar University of Kerman, Iran, from October 2011 to March 2011. All traits responded negatively with lowering water potential but using salicylic acid ameliorated the harmful effect of induced stress on measured traits. The concentration of 0.25 and 0.5 mM of salicylic acid on measured traits was more effective compared with the other levels. Therefore, seed priming with salicylic acid could be a suitable tool for improving germination characteristics of Fenel under low water potential.

Keywords: Fenel, Germination, Salicylic Acid, Water Potential.

INTRODUCTION

The theory of seed priming was proposed by Heydecker (1973). It is a technique for controlling seed slow absorption and post-dehydration (Heydecker and Coolbear, 1977). After treatment with initiators, plant seeds exhibit not only enhanced emergence rate and even emerge of seedlings (Harris et al., 1999; Bradford, 1986) but also improved resistance or tolerance to cold (Li and Fu, 1990), drought (Wang et al., 2004) and salt (Ruan et al., 2003). Initiator has obvious effects on seed germination and osmotic stress resistance (Wang and Shen, 1991; Liao and Sun, 1994), during which the contents of compatible solutes, proline and soluble sugar, and the activity of protective enzymes, such as superoxide dismutase (SOD), peroxidase (POD), catalase (CAT) (Mittal and Dubey, 1995; Bohnert and Shen, 1999) as important indicators will increases. The effects of initiators on seeds germination and resistance to unfavorable growth conditions have been studied in legumes (Gu et al., 2000), wheat (Triticum aestivum L.) (Li et al., 2002), and onion (Allium cepa L.) (Heydecker et al., 1973).

Salicylic acid (SA) plays an important role in abiotic stress tolerance, and more interests have been focused on SA due to its ability to induce a protective effect on plants under adverse environmental conditions. SA may affect directly on specific enzymes function or may activate the genes responsible for protective mechanisms (Hayat and Ahmad 2007; Horvath et al. 2007). Shruti and Singh (2009) showed that salt-induced deleterious effects in maize seedlings were significantly eliminated by the pretreatment of SA. It is concluded that 0.5 mM salicylic acid improves the adaptability of maize plants to NaCl stress. Gunes et al. (2007) reported that SA could be used as a potential growth regulator to improve plant salinity tolerance. Previous results suggest that SA could be a promising compound for the reduction of abiotic stress sensitivity in plants, since under certain conditions it has been found to mitigate the damaging effects of various stress factors in plants (Horvath et al., 2007). Several methods of application (soaking seeds in SA prior to sowing, adding SA to the hydroponic solution, irrigating or spraying with SA solution) have been shown to protect various plant species against abiotic stress, such as salinity (El-Tayeb, 2005; Szepesi et al., 2009). It has also been shown that pre-soaking seeds in SA can protect the...
plants against abiotic stresses (Szepesi et al., 2008; Popova et al., 2009), but the background of this effect is not well understood.

Successful establishment of plants largely depends on successful germination (Gorai and Neffati, 2007). Germination is a crucial stage in the life cycle of plants and tends to be highly unpredictable over space and time. Several environmental factors such as temperature, salinity, light, and soil moisture simultaneously influence germination (Ungar, 1995; Huang et al., 2003; El-Keblawy and Al-Rawai, 2005, 2006; Gorai and Neffati, 2007). In arid and semi-arid areas in almost all the regions of the world, saline soils are becoming a major problem, due to a variety of natural and man caused factors (Khan and Qaiser, 2006). Initial establishment of species in salt deserts is related to germination response of seeds to salinity and early establishment usually determines if a population will survive to maturity (Tohe et al., 2000; Huang et al., 2003; Song et al., 2005).

This study was conducted to evaluate the effects of different water potential, salicylic acid concentration, and their interaction on germination percentage, germination rate and seedling growth of Fenel (*Foeniculum vulgare*). Information of this study provides a direct reference of seed priming technique in Fenel that can be used for re-establishing projects.

**MATERIALS AND METHODS**

1. **Plant material**
   Seeds of Fenel (*Foeniculum vulgare*) used in the experiment were provided from seed and plant research center. The seed moisture ranged between 6 and 7%. The seeds were less than 12 months old and had been previously stored in paper bags under conditions of 4°C and 20% relative humidity.

2. **SA treatment:**
   Seeds were surface-sterilised with a 3% sodium hypochlorite solution, rinsed in distilled water for three times and dried before the experiment. Afterwards, the seeds divided into four groups, equally. The first group was soaked for 12 h in distilled water to serve as control while three other groups were soaked in 0.25, 0.5 and 0.75 mM of salicylic acid respectively.

3. **Salt solutions:**
   After a preliminary test for low water potential tolerance (caused by NaCl), four water potential levels were selected and used for germination trials. The saline solutions had water potential of zero (control), −0.2, −0.4 and −0.6 Bar and electrical conductivity (EC) values of 0, 5.3, 10.3, 22.0 and 28.4 dS m⁻¹, respectively. The EC was measured with a portable conductivity meter (Mod. CyberScan CON 400/410, Eutech Instruments, Pte Ltd).

4. **Germination test:**
   Thirty seeds of each lot evenly placed on Whatman filter paper No.1 in sterilized 9-cm Petri dishes separately and 5 ml of each saline solution were added to related treatment. All Petri dishes were sealed to prevent the loss of moisture and avoid contamination, and then placed in a Conviron PGR-15 plant growth chamber for 6 days. The seed were allowed to germinate at 25/22°C with 16/8-h light/dark periodicity. The photosynthetic photon flux density was 340-mol m⁻² s⁻¹, provided by metal halide lamps, with a relative humidity of 60%. Germination was determined by counting the number of germinated seeds at 24-h intervals over a 6-d period and expressed as total percent germination. Seeds were considered to be germinated at the emergence of the radicle (Bewley and Black, 1994). Radicle and hypocotyl lengths were measured 6 days after germination. The total dry weight were determined by drying the plant material in an oven at 60°C for 24-h prior to weighing.

5. **Data analysis:**
   A factorial experiment based on a completely randomized design with three replicates was carried out at the laboratory of agriculture faculty of Shahid Bahonar University of Kerman, Iran, from October 2011 to March 2011.

Germination percentage, germination rate and seed stamina index were calculated using following formula.

- \( G = \left( \frac{n}{N} \right) \times 100 \)  
  (Jefferson and Penachchio, 2003)
- \( RG = \sum \left( \frac{Ni}{Di} \right) \)  
  (Jefferson and Penachchio, 2003)
- \( SSI = \left[ G \times (HL+RL) \right] / 100 \)  
  (Abdul-baki and Anderson 1970)

G: germination percentage, \( n \): number of seeds germinated, \( N \): total number of seed in each petri dishes, \( RG \): rate of germination (seed /day), \( Ni \): germinated seeds in each numeration, \( Di \): day of each numeration, \( SSI \): seed stamina index, \( HL \): average of hypocotyls length, \( RL \): average of Radicles length.

The data were processed using the GLM procedure of statistical analysis system (SAS). (SAS Inst., 1992).
RESULTS AND DISCUSSION

The results presented in Fig.1 indicate that the germination percentage (GP) reduced with decreasing water potential so that the lowest GP was recorded for the -6 bar water potential. Interaction of SA and water potential on GP was significant. SA at the levels of 0.25 and 0.5 mM caused a significant increase in germination percentage at the level of control (zero water potential). The same response was observed at -2 and -4 bar water potential. But the highest concentration of SA (0.75 mM) at the mentioned levels of water potential did not affect GP significantly compared to the control. The effect of all SA concentrations on GP in the lowest water potential (-6 bar) were the same statistically. The germination rate of Fenel at the level of control (zero water potential, without SA) was 4.4 seed day\(^{-1}\). Significant reduction in germination rate started at -2 bar water potential and decreased more as water potential reduced. Application of first level of SA (0.25 mM) affected significantly the germination rate of Fenel in all levels of water potential and it could lessen the effects of low water potential on this trait. In general, the highest germination rate recorded for 0.25 and 0.5 mM of SA at the zero water potential (no salinity), while the lowest amount of this trait was belonged to the two lower level of water potential (-4 and -6 bar) that pre-treated with 0 and 0.75 mM of SA. However, SA pre treatment had a different effect on germination rate at different levels of water potential (Figure 2).

The reason of reduction in GP at the lower water potential caused by NaCl may be due to slower rate of imbibition. From present investigations, it is quite clear that seeds primed with various concentrations of salicylic acid proved to be effective in inducing stress tolerance at the germination stage in Fenel. The mechanism of seed priming is to initiate the repairing system for membrane and the metabolic preparation for germination through controlling water absorption rate of seed (Mittal and Dubey, 1995; Bohnert and Shen, 1999; Li et al., 2002). As a result, the germination capability and resistance to unfavorable conditions of seed can be promoted obviously.

Primed presumably allowed some repairs of damaged to membrane caused by deterioration. It has been reported that primed seeds showed better germination pattern and higher vigor level than non-primed (Ruan et al., 2002). Nascimento and West (1998) indicated that the improvement in germination and vigor of normal/low-vigor seed might be due to reserve mobilization of food material, activation and re-synthesis of some enzymes DNA and RNA synthesis start during priming. Priming can repair some damages that have been arisen from seed erosion and improve seed quality (Arif et al., 2008).

The interaction of water potential and SA on hypocotyl length is presented in figure 3. The response of this trait varied at different levels of water potential with different concentrations of SA. Hypocotyl length decreased with reducing the water potential at the all levels of SA. The highest hypocotyl length at each of water potential levels recorded for 0.25 and 0.5 mM SA. In addition, the results showed that the higher concentration of SA (0.75 mM) often had a negative effect on this trait (Figure 3).

The response of radicle length to different levels of water potential and SA was almost the same as hypocotyl length. The maximum radicle length (6.95 cm) obtained when 0.25 mM SA applied at zero water potential while the minimum of this trait (0 cm, no radicle emerged) resulted from the lowest level of water potential (-6 bar) that pre-treated with 0 and 0.75 mM SA (Figure 4).

Werner and Finkelstein (1995) indicated that elevated salinity (lower water potential) slowed down water uptake by seeds, thereby inhibited their germination and root elongation. The probable reason for early emergence of the primed seed maybe due to the completion of pre-germination metabolic activities making the seed ready for radicle protrusion and the primed seed germinated soon after planting compared with untreated dry seed (Arif, 2005). Like germination percentage, prime seeds had higher hypocotyl and radicle length compared with un-primed seeds. These positive effects are probably due to the stimulatory effects of priming on the early stages of germination process by mediation of cell division in germinating seeds (Hassanpouraghdam et al., 2009; Sivritepe et al., 2003). The seedling fresh weight, respond differently to different levels of water potential and SA. SA at the 0.25 and 0.5 mM increased SFW significantly and had a same effect at the all levels of water potential but the highest concentration of SA did not affect this trait significantly compared to the control (Figure 5). The stimulatory effect of SA levels on SDW was almost the same with SFW. The results showed that the concentration of 0.25 and 0.5 mM SA were more effective than the two other levels in improving the harmful effects of low water potential caused by NaCl. At the zero level of water potential (control), 0.25 mM SA had the highest effect on SDW while in -2 and -4 bar water potential, 0.5 mM SA was more effective than the other levels. At the lowest water potential (-6 bar) the effect of 0.5 and 0.75 mM SA were the same statistically (Figure 6).

Our findings are in line with those of Ghoulam et al. (2001), who showed that salinity caused a marked reduction in growth parameters of sugar beet plants. Shoot fresh and dry weight and root fresh weight
were increased in seedlings raised from seeds primed with 50 ppm salicylic acid. It was also found that SA application increased the dry mass of wheat seedlings under water stress (Singh and Ushu, 2003). The protective and growth promoting effect of SA are presumably due to increased level of cell division within the apical meristem of seedling root, which caused an increase in plant growth.

Seed stamina index increased with the application of SA significantly. The higher concentration of SA did not significantly affected SSI when compared to the control. Although all the levels of water potential reduced the SSI significantly, but the lowest and highest reduction was belonged to -2 and -6 bar, respectively (Figure 7).

Seed priming is a regular step before sowing in a few vegetables and flower crops in some countries. As a result, the germination capability and resistance to unfavorable conditions of seed can be promoted obviously.

However, osmopriming has been shown to activate processes related to germination, for instance, by affecting the oxidative metabolic such as increasing superoxide dismutase (SOD) and peroxidase (POD) (Jie et al. 2002) or by the activation of ATPase as well as acid phosphatase and RNA syntheses (Fu et al. 1988).

Our finding revealed that inhibition of germination at lower water potential probably resulted from osmotic effect. Seeds primed with SA gave better performance than control (unprimed) under low water potential. It seems priming increased the tolerance of seeds to water stress, 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.

![Figure 1. The effects of SA on germination percentage of Fenel (Foeniculum vulgare) Under Salinity Stress](#)

![Figure 2. The effects of SA on germination rate of Fenel (Foeniculum vulgare) Under Salinity Stress](#)
Figure 3. The effects of SA on hypocotyl length of Fenel (Foeniculum vulgare) Under Salinity Stress

Figure 4. The effects of SA on radicle length of Fenel (Foeniculum vulgare) Under Salinity Stress

Figure 5. The effects of SA on seedling fresh weight of Fenel (Foeniculum vulgare) Under Salinity Stress
References


