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PUTRESCINE IMPROVE LOW TEMPERATURE TOLERANCE OF FENNEL (*FOENICULUM VULGARE* MILL.) SEEDS

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ABSTRACT. The effects of polyamine priming on the germination behaviour of fennel at low temperatures were investigated. For preparing the putrescine pretreatments, seeds were divided into four parts. Two samples primed into putrescine (10 or 20 ppm) for 24 h, other samples were as controls. In order to eliminate the effect of water from test results, seeds were soaked in water only. After the priming, seeds were dried and used for germination test at 10 and 20 °C. Except for seedling dry weight, all of the priming treatments improved germination performance and seedling growth of fennel seeds. Maximum germination percentage was achieved by 10 ppm Put application and lower value was observed in control seeds. About the energy of germination and mean germination time, polyamine treatments had better effect than hydropriming, but similar results was observed from seeds treated by 10 ppm Put and hydroprime on root and shoot length. Results showed that adequate presence of Put in the priming media had better than priming with water only. However, high concentrations of Put had not significant effect as well as 10 ppm Put. These results indicated that 10 ppm Put priming could be as an effective method to improve low temperature tolerance of fennel seeds.

Key words: Fennel; Germination performance; Low temperature; Putrescine; Seedling growth.

INTRODUCTION

Fennel (*Foeniculum vulgare* Mill.) is one of the most important medicinal plants that grow in many parts of the world, especially on dry soil near the coasts. Fennel seed is used in the food and flavor industry for addition to meats, vegetable products, fish and tea. The essential oils are used in condiments, soaps, creams. Fennel mainly cultivated in north-west of Iran. In this regions fennel seeds are sowed directly in the field at March, the soil temperature is often lower than the optimal temperature (15-20 ºC), causing delayed or non uniform seedling emergence. Failure of obtaining fast and uniform emergency is a major

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limiting factor for fennel production. Arin and Kiyak (2003) reported that emergence percentage decreased under stressful temperatures because of difficulty in water uptake. Abbas (2011) reported that cold temperature during the imbibition phase of germination leads to the increase of escape of solutes from the seeds, such as carbohydrates, which has been attributed to the disturbance of plasma membrane integrity during imbibition at low temperature.

Several approaches have been used to decrease the adverse impact of low temperature on the germination performance of seeds. Seed priming has proved to be an effective method in imparting stress tolerance to plants. Rapid and uniform emergence enhancement by seed priming technology has been found by (Basra *et al.,* 2005; Kaur *et al*., 2005). According to McDonald (2000), primed seeds get the potential to rapidly imbibe and revive the seed metabolism thus enhancing the germination rate and at the end of uniform emergence. Seed performance of various crops can be improved by inclusion of plant growth regulators and hormones during priming and other pre-sowing treatments (Lee *et al*., 1998). The polyamines (PAs) are small aliphatic, polycationic nitrogenous compounds that can associate with anionic membrane components, such as phospholipids, thereby stabilizing the bilayer surface and retarding membrane deterioration under stress conditions (Basra *et al*., 1994). There is now conclusive evidence that polyamines accumulate in plants and are involved in their protection against various environmental stresses (Bouchereau *et al*., 1999; He *et al*., 2002). In the mild stress period, their accumulation act as a type of hardening and results in better survival in the case of subsequent stress (Simon-Sarkadi *et al*., 2006). Exogenous PAs were effective in positive responses to abiotic stress, including water stress, hypoxia stress and salt stress in plants (Kubis, 2006; Sanchez *et al*., 2005; Chattopadhayay *et al*., 2002). Xu *et al.* (2011) reported that during chilling stress at 11°C, seed priming with 0.01 mM and 0.1 mM putrescine (Put) for 48 h, significantly, increased germination percentage, germination index, seedling length and dry weight of both varieties, compared to the controls without Put treatment. The objectives of this study were to investigate effects of hormonal priming with putrescine (Put) on subsequent germination and seedling emergence of fennel at low temperature.

MATERIALS AND METHODS

The used seeds for this purpose were collected from same age plants. A number of two umbels was used. Seeds were screened out based on size. For priming, seeds were priming in putrescine (Put) solutions of different concentrations (10 and 20 ppm), for 24 h. Each gram of fennel seeds was primed in 15 ml Put solution, under aerated conditions at 20°C, with three replicates. Untreated seeds were used as control 1. Seeds primed in water at 20°C, for 24 h, were used as control 2 (hydropriming) for eliminating the effect of water from test results. After pre soaking, the seeds were surface dried on filter paper and then allowed to air-dry for 12 h, at room temperature (25°C).

Primed and unprimed seeds were surface sterilized by soaking in 10% sodium hypochlorite, for 5 minutes, followed by three 60 seconds rinses in the sterile distilled water, and then dried at room temperature. Germination trials were conducted in Petri dishes between layers of papers. Seeds were germinated at constant temperatures (T) of 10 and 20°C, the dark in an incubator. Treatments were arranged in factorial on the base of randomized completely block, with three replicates of 25 seeds for each plot. Germination percentage, root and shoot length and other traits about germination behaviour were calculated.

The main germination time (MGT) was calculated according to the following formula:

$$MGT = \frac{\sum Dn}{\sum n},$$

where n is the number of seeds, which were germinated on day D, and D is the number of days, counted from the beginning of germination.

Energy of germination (EG) was recorded on the fourth day after plantation relative to the total number of seeds tested (Farooq *et al.,* 2005).

Germination data was analyzed using SPSS software (version 16). One-way analysis of variance (ANOVA) was conducted and Duncan's multiple range tests at 5% level of significance, was used to separate means.

RESULTS AND DISCUSSION

Germination percentage (GP) was significantly affected by priming techniques. The highest GP was achieved by priming with 10 ppm Put, followed by hydropriming that had not significantly difference with 20 ppm Put (*Fig. 1*). It seems that high concentration of Put (20 ppm) had not positive effect on seed germination as well as 10 ppm. Application of 10 ppm Put increased approximately 37% GP, compared with control. Between the temperatures, GP was higher in 20ºC (*Tab. 1*). Omidbaigi and Hornok (1992) reported that optimum temperature for fennel germination is 15-20ºC; under extreme temperature, germination is not occurred or is delayed (Bennet, 2004).

Putrescine treatments enhanced the energy of germination and had significant differences with hydropriming and control (*Fig.1*). All priming treatments resulted in lower values of MGT (*Fig. 2*), compared with the control. Between the priming techniques, earlier germination was observed in seeds treated with putrescine. Interaction between priming techniques and temperature on MGT was significant. Under relatively optimum temperature (20ºC), MGT of treatment seeds with 10 ppm Put was the slightly better than those for other treatments, but under low temperature, 20 ppm Put solution was the best from this viewpoint (*Fig. 2*).

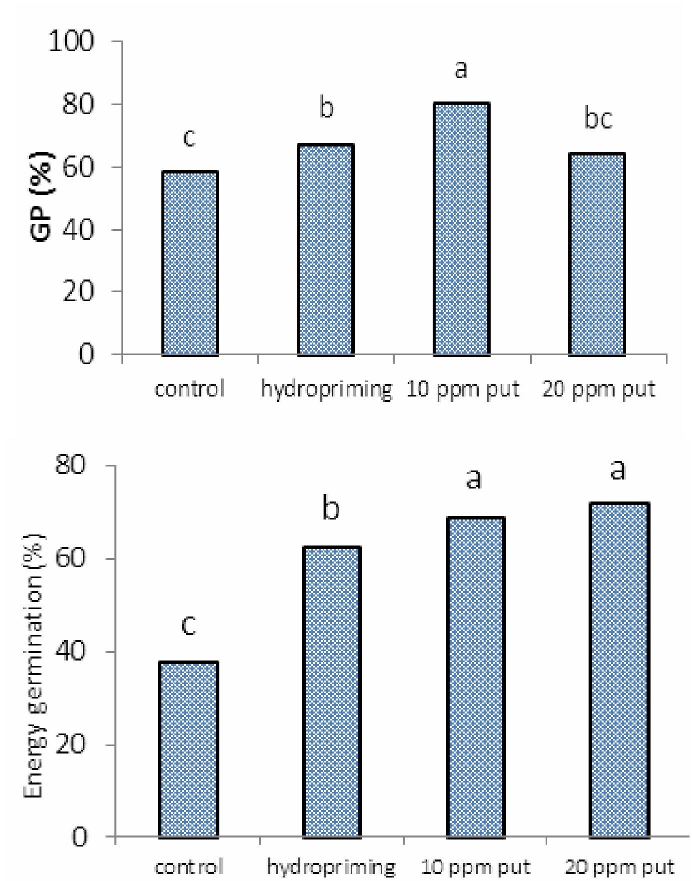

Figure 1 - Influence of seed priming treatments on germination percentage (GP) and energy germination

Table 1 - Changes in germination percentage (GP), energy germination (EG), root length (RL), shoot length (SL), seedling length (SeL), seedling dry weight (SDW) and mean germination time (MGT) at different temperatures

Temp. (^{0}C)	GP (%)	EG (%)	RL (cm)	SL (cm)	SeL (cm)	SDW (mg)	MGT (day)
10	60.33b	56.91b	2.94b	3.11b	6.06b	44.16b	10.50b
20	75.25a	64.08a	3.40a	3.99a	7.39a	53.69a	7.66a

Different letters in each column indicate significant difference at p≤0.05.

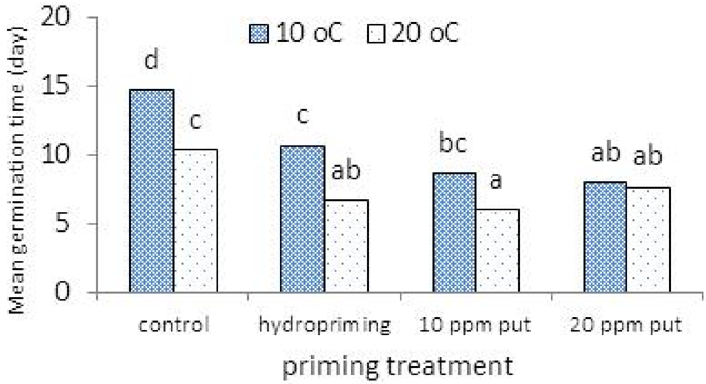

Figure 2 - Influence of priming treatments on mean germination time under different temperatures

Germination performance improvement after treatment might be the effect of processes like breakdown of reserve food materials, increased cell division and expansion of embryonic axis. Farooq *et al.* (2008) reported that seed priming with 10 ppm Put was the best concentration in improving the germination and early seedling growth in fine rice.

Root and shoot length also significantly affected by priming treatments. Priming with 10 ppm of Put had highest root and shoot length; however had not significantly difference with hydropriming (*Fig. 3 a,b*). Large seedlings were observed by 10 ppm Put and small ones achieved by control and 20 ppm Put (*Fig. 3 c*). Maximum seedling dry weight was observed from seed soaked in 10 ppm Put solution (*Fig. 3d*), and minimum values for this trait was observed by seed soaked in 20 ppm Put which was similar to control. Between the temperatures, 20ºC had better effect on these traits (*Tab. 1*). Similar results were reported by Shengchun *et al.* (2011). Han *et al.* (1992) suggested that root was more easily affected by Put treatments during seed germination.

Several reports had been presented that hydropriming improved germination performance of seeds (Basra *et al.*, 2002; Shahbaz *et al.*, 1998). It is reported that priming technique increased the polyamines accumulation in the seeds (Basra *et al.,* 1994), which caused vigor improvement. Farooq *et al.* (2007) reported that the effectiveness of seed priming can be enhanced by the incorporation of polyamines in the priming medium. It seems that polyamines seed treatments contributed towards enhanced buildup of polyamines that resulted in improved germination performance. PAs have been suggested to afford protection against environmental stresses, including chilling (Rodríguez-Kessler *et al*., 2006). Cao *et al*. (2008) showed that seed soaking with Put can improve chilling tolerance of maize seeds. Membrane is one of the major regions where early affected by stress conditions. It seems that Put by rigidifying the membrane, decrease adverse effect of low temperatures on membrane, change from the gel phase to the liquid crystalline phase during water uptake, so improve germination behavior of fennel seeds.

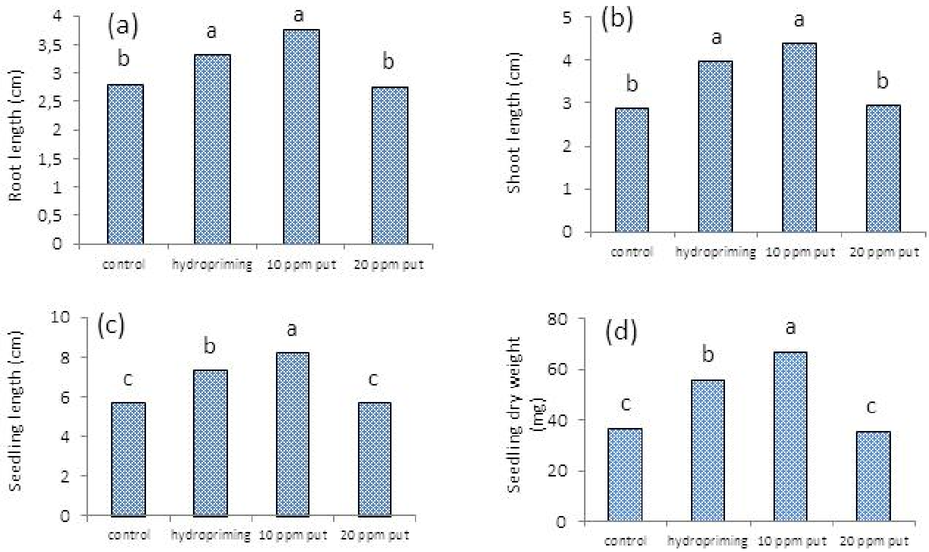

Figure 3 - Influence of seed priming treatments on: a) root length, b) shoot length, c) seedling length; d) seedling dry weight

CONCLUSION

Priming technique can be alleviating the adverse effect of low temperature on germination behavior of fennel, and also application of polyamine such as putrescine in priming media was more effective than hydropriming. Beneficial effect of Put depends on their concentration; in this study, 10 ppm Put was more effective than 20 ppm.

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