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Effect of Different Concentration of Salt and PEG Solution on *Dracocephalum moldavica* Seed Germination and Seedling Early Growth

Shima Alaei*, Zahra Moradi Khibary* and Amin Ahmadi Rad*
**Young Researchers and Elite Club, Kermanshah Branch,*
Islamic Azad University, Kermanshah, IRAN.

(Corresponding author: Shima Alaei)
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ABSTRACT: An experiment was carried out in a completely randomized design with 12 treatments and 3 replications. Polyethylene glycol was used at 0, 5, 10, 15, 20, 25 and 30 % concentrations with water potentials of 0, -0.03, -0.19, -0.41, -0.67, -0.99 and -1.35 MPa respectively. Salinity levels were 50, 75, 100, 150 and 200 mM. Seeds treated with -1.35MPa did not germinate. The seeds in -0.99 MPa water potential and 150 and 200 Mm salinity, did not produce seedlings. Increasing of salinity and water stress levels caused significant reduction in germination percentage, germination rate, seed vigor index and radicle and plumule length. The highest and least of seed germination percentage was concerned to control and 200Mm salinity treatment respectively. Germination rate showed significant reduction at -0.41, -0.67, -0.99 MPa water potential and 150 and 200 Mm of salinity.

Keywords: Dragonhead (*Dracocephalum moldavica*), water stress, salinity, Germination, Seedling Growth.

INTRODUCTION

Dragonhead with scientific name of *Dracocephalum moldavica* from Lamiaceae family is one of the medicinal and aromatic plants that its essential oil uses widely in medicinal, food, cosmetic and health industrials. Dragonhead cultivated in different regions of Iran. It is a considerable plant for its high amount of essential oil. Its essential oil has antiseptic and antibacterial properties and is used for stomach-ache and bloat (Omidbaigi, 2000).

Water and salinity stresses are two of the most environmental factors that regulate plant growth and development, and limit plant production. According to the results of Hassani's research (2006) if the water potential reduced, seed germination will be delayed or prevented.

The result of salinity stress on morphological characters of *Cumimum. cyminum* and *Valeriana officinalis* showed that by increasing salinity level percentage of germination, root length, shoot length, root dry weight, shoot dry weight, biomass and shoot/root ratio decreased. *Cumimum cyminum* had a high tolerance to salinity in their germination and seedling stages phase but *Valeriana officinalis* showed sensitive to increased salinity in this phase (Salami, 2006). Germination stage is an importance of growth plant stage that often effective by environmental stress including water and salinity stress (Hosseini 2006). With regard to development of saline and drought lands and the shortage of agricultural land it comes into great importance to make use of salt and tolerant plant. Since dragonhead is an important plant in medicine, the investigations in to the cultivation aspects of this plant are essential.

MATERIAL AND METHODS

An experiment was carried out in order to study the effect of different salinity and osmotic potential levels on germination and seedling growth of *Dracocephalum moldavica* in a completely randomized design with 12 treatments and 3 replications. Seeds were subjected to water stress using polyethylene glycol (PEG 6000) at seven concentrations (0, 5, 10, 15, 20,25and 30%) representing water potentials of 0, -0.03, -0.19, -0.41, -0.67, 0.99 and -1.35 MPa. Salinity levels applied were 50, 75, 100, 150 and 200 mM. The seeds of the dragonhead (*D. moldavica* L.) were used in this investigation were provided from Medicinal and Aromatic Plants, Department of Cornivus University in Budapest. The experimental trial was carried out at Islamic Azad University of Kermanshah, situated in the west of Iran. The seeds were imbibed in the distilled water, solutions of Polyethylene Glycol (PEG6000) or NaCl. Hypochlorite Sodium was used at a concentration of 2.5 % for 5-7 minute to disinfection. The germination percentages were average of three replications of 25 seeds. The seeds were placed on a Watman paper moistened 13 ml distilled water (control), NaCl solutions of 50, 75, 100, 150 and 200 mM and PEG-6000 solutions of -0.03, -0.19, -0.41, -0.67, 0.99 and -1.35 MPa in 9.0 cm diameter Petri dishes.

The Petri dishes were tightly sealed with colorless microfilm in order to avoid water losses during the experiment and incubated in growth chamber at 25°C, 16 h light and 8 h dark for 15 days. The germination was considered to have occurred when the primary root was 2 mm long. After 14 days of germination test, in the entire germinated seedling, germination percentage, germination rate, seed vigor index, radicle and plumule length were determined.

Germination rate = Number of germinated seed in first day/ day number of experiment +………+ Number of germinated seed in last day/ day number of experiment.

Seed vigor index = (plumule length + radicle length)* germination percentage / 100.

At the end of experiment, data were analyzed by using MSTATC computer software and mean were compared by statistical test. Statistical differences were calculated according to duncan's multiple range tests. We considered differences significant at p values 0.05%.

RESULTS

A. Water stress

The results showed that increasing of water stress, decreased significantly germination percentage, germination rate, seed vigor index and radicle and plumule length ($p<0.05$). Seeds treated with -1.35MPa did not germinate. The results indicated that by increasing water stress level, radicle and plumule length decreased that was statistically significant ($p<0.05$) at -0.03 MPa level. Radicle growth was more than plumule growth at all of the water stress treatments (Fig. 1).

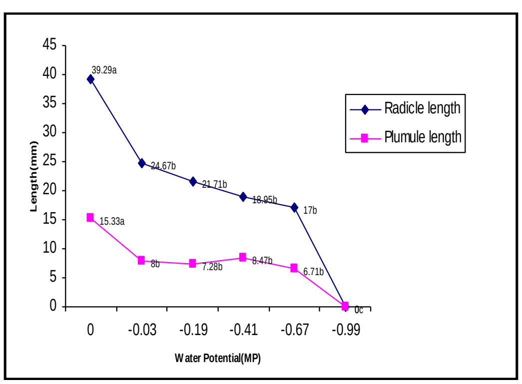

Fig. 1. Effect of water stress on radical and plumule length of *Dracocephalum moldavica.*

The seeds that germinated in -0.99 MPa water potential did not produce seedlings. Decreasing of germination percentage in -0.99 MPa water potential was statistically significant at P≤0.05. The highest and least of germination percentage concerned to lack (control) and -0.99MP of water stress treatments. 0 - -0.67 MPa was the best range for seed germination on *Dracocephalum moldavica* (Fig. 2).

Also water stress affected on germination rate severely. This factor showed significant reduction at -0.41, -0.67, 0.99 MPa water potential levels. The highest and least of this concerned to -0.03 and -0.99 MPa water stress treatments. Between -0.41 and -0.99 MPa level was significant different at 5% level (Fig. 3).

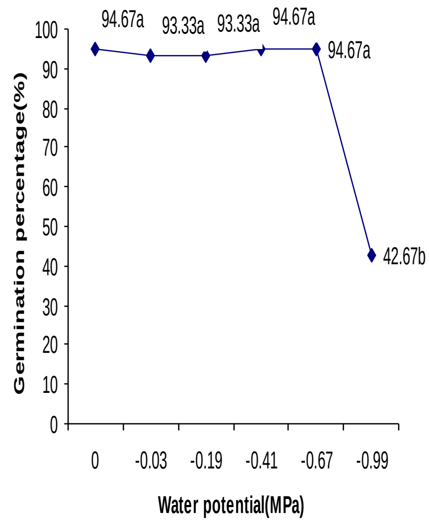

Fig. 2. Effect of water stress on germination of *Dracocephalum moldavica.*

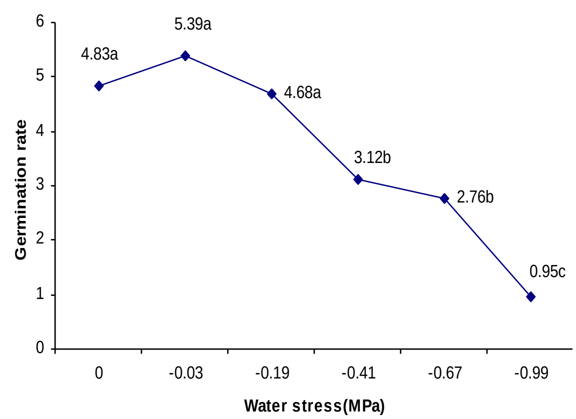

Fig. 3. Effect of water stress on germination percentage of *Dracocephalum moldavica.*

Increasing of water stress at -0.03 level decreased seed vigor index significantly at P≤0.05. Decreasing of seed vigor index in water potential of less than -0.03 MPa depend to decreasing of radicle and plumule length in this level. The highest of seed vigor index was 51.9mm at control treatment (Fig. 4).

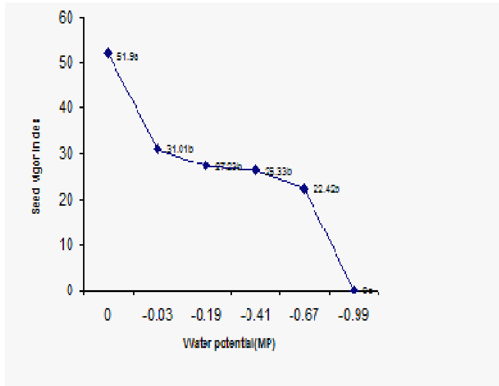

Fig. 4. Effect of water stress on seed vigor index of *Dracocephalum moldavica.*

B. Salinity stress

Increasing of salinity levels caused significant reduction at 5% level in germination percentage, germination rate, seed vigor index and radicle and plumulelength. The results showed that salinity have significant effects on seed germination of dragonhead. The seeds that germinated in 150 and 200 mM salinity did not produce seedlings. Decreasing of germination percentage in 150 and 200 mM of salinity was statistically significant at P≤0.05. The highest and least of seed germination percentage was concerned to control and 200 mM salinity treatment respectively (Fig. 6).

Decreasing of radicle length in 150 mM and plumule length in 100 mM of salinity was significant at 5% level. The highest of plumule length (22mm) and radicle length (24.13) was concerned to 50mM salinity treatment. Plumule length was more sensitivity than radicle length at excessive salinity condition (Fig. 5).

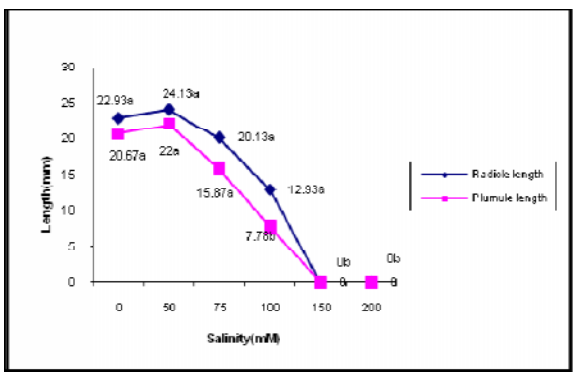

Fig. 5. Effect of salinity stress on radicle and plumule length of *Dracocephalum moldavica.*

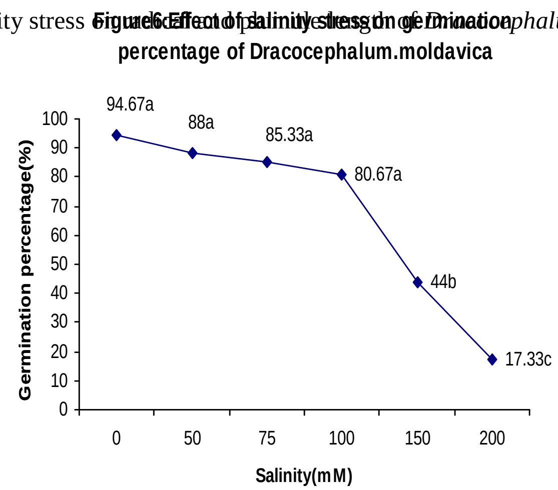

Fig. 6. Effect of salinity stress on germination percentage of *Dracocephalum moldavica.*

Germination rate showed significant reduction at 150 and 200 mM of salinity. The highest (4.92 number per day) and least (0.55 number per day) of seed germination rate was concerned to control and 200Mm salinity treatment respectively (Fig. 7). Seed vigor index in more than 100 mM of salinity decreased that it was significant at 5% level. Seed vigor index was 41.28mm control treatment (Fig. 8).

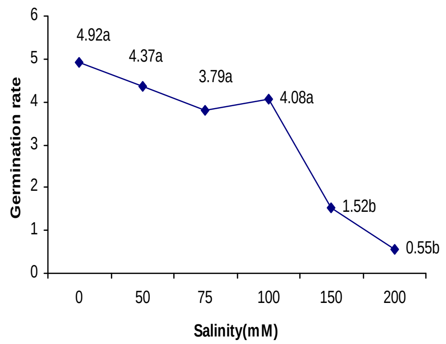

Fig. 7. Effect of salinity stress on germination rate of *Dracocephalum moldavica.*

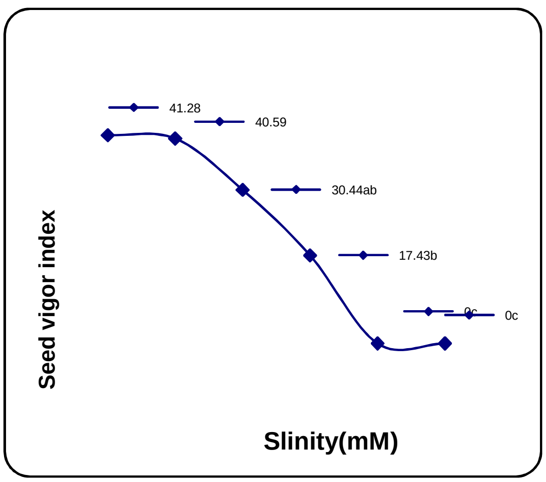

Fig. 8. Effect of salinity stress on seed vigor index of *Dracocephalum moldavica.*

DISCUSSION

The results showed that water stress affected on seed germination of dragonhead so that with decreasing of water potential in seed medium, seed germination and seedling growth significantly decreased. Negative effects of water stress on seed germination of rice (Alam *et al.*, 2002), basil (Hassani, 2006), pepper (Demir, 2008), Isabgol (Hosseini, 2006) have reported. Moreover water potential gradient (reduced water availability) between the external environment and the seeds also inhibits the primary root emergence (Eneas Filho *et al.*, 1995).

It seems that in water stress condition between total characters, plumule length was more sensitive because at -0.03 MP decreased significantly but germination percentage was more tolerance and decreased at -0.99MP water potential. This results are in agreement with the other studies like Hassani (2006) and Alam *et al* (2002) that have expressed plumule growth need to more turgecence pressure in comparison with germination process.

Hassani (2006) found that water stress have significant effects on seed germination of basil. Also germination rate showed significant reduction at -0.41 MPa water potential. On the other hand, the results of that study showed that different levels of water potential have significant effects on seedling growth. With decreasing water potential on *Ocimum basilicum*, plumule length, fresh weight of radicle, plumule and seedling decreased that it is agreeing with these conclusions. But opposite of my results, radicle length increased that could be concluded that in water stress condition, root growth of basil was affected lower than shoot growth. Salinity is a limiting factor for plant growth and crop production.

Hosseni's results (2006) are in agreement with the studies that explained with increasing water and salinity stress decreased significantly germination rate, germination percentage, plumule and radicle length ($P \leq 0.05$). 0 - -8 bar was the best rang for seed germination on Isabgol. Also seem that seed germination on Isabgol has more tolerance in salinity stress condition than to water stress condition. Despite the salt resistance varies according to ontogeny, the first exposure of the crop to salinity stress usually occur at the germination stage in direct sowing and transplanting production (Passam and Kakouriotis, 1994).

As water absorption of seed is disordered or become slow, germination metabolic activities in seed will retard (Livington, 1990).

One of the reasons of plumule length decreasing in water stress condition, decreasing or lack of nutrition transmission from store tissues to embryo has explained (Uniyal, 1998). Decreasing of water absorption by seed in water stress condition cause decreasing of enzymes and hormones excretion consequently seedling growth disorder (Hosseini, 2006).

Demir *et al* (2008) reported that the higher salt and osmotic stress concentrations the lower was the germination percentage and seedling fresh weight of pepper. The seed germination was higher and sensitivity index were lower in NaCl than in PEG at the same water potential. The results showed that the inhibition of the germination at the same water potential of NaCl and PEG resulted from osmotic effect rather than the salt toxicity.

The seed require higher amount of water uptake during the germination under salt stress due to the accumulation of the soluble solutes around the seeds which increases the osmotic pressure. This causes excessive uptake of ions which results in toxicity in the plant (Jones, 1986).

Both the osmotic and toxic effects of the salts have been implicated in the inhibition of the germination (Machado Neto *et al.*, 2004).

Higher germination percentages obtained from NaCl compared to those of PEG at the same concentrations proved that the adverse effect of PEG on the germination was due to an osmotic effect rather than specific ion accumulation (Demir, 2008). These results were consistent with Demir (2008) and Murrillo-Amador *et al.,* (2002) in cowpea who affirmed that growth medium salinity or drought could affect the seed germination by decreasing the ease of uptake water. The result of salinity stress on morphological characters of *Cumimum cyminum* and *Valeriana officinalis* showed that by increasing salinity level percentage of germination, root length, shoot length, root dry weight, shoot dry weight, biomass and shoot/root ratio decreased. *Cumimum cyminum* had a high tolerance to salinity in their germination and seedling stages phase but *Valeriana officinalis* showed sensitive to increased salinity in this phase (Salami, 2006).

CONCLUSION

Results of two experiment showed that increasing water and salinity stress decreased significantly germination rate, germination percentage, plumule and radical length. 0- 75 mM salinity was the best range for seed germination. At 100mM salinity, seeds 80.67% germinated and decreasing of germination percentage was not significant. 150 and mM salinity treatments were not suitable for seed germination of dragonhead.

It seems that in water and salinity stress condition between total characters, plumule length was more sensitive to water and salinity stress. Germination percentage of dragonhead seed was tolerant and decreased at -0.99MP water potential and 150mM salinity stress. Plumule growth need to more turgecence pressure in comparison with germination process. Decreasing of germination process in water stress condition depend to decreasing of water absorption. Decreasing of water absorption by seed in water stress condition cause decreasing of enzymes and hormones excretion consequently seedling growth disorder.

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