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Effect of osmopriming on germination characteristic of *Valeriana officinalis* L. seed under drought stress

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ABSTRACT: In order to study on effect of osmopriming on seed germination characteristic of Valeriana officinalis L. under drought stress, a factorial experiment in completely randomized design carried out with four replications and 2 treatments at the Research Laboratory of Shahrekord University, 2013. Four levels of drought stress (0, -2, -4, -6 bar) as main factor and four levels of priming (0, -4, -8, -12 bar) as secondary factor were considered. The results indicated that osmopriming, drought stress and their interaction had significant effect on germination percent, germination rate, mean germination time, rootlet length, and shootlet length. The osmopriming had an effect on increased germination percent, germination rate, and mean germination time. Meanwhile, the highest levels for the mentioned traits were obtained with treatment of -8 bar. Also, the drought stress made all germination traits to be decreased. And the highest levels were obtained with control treatment. Overall, regarding our research results, to achieve the highest levels for germination components in similar condition, treatment level of osmopriming as -8 bar is recommended with control level of drought.

***Keywords*:** Osmopriming, drought stress, Valeriana officinalis L.

INTRODUCTION

The diversity of plant species grown in plains and mountains of Iran makes fame for our country as one of the valuable genetic reservoirs of pharmaceutical and aromatic herbs in the world. One of the valuable pharmaceutical herbs is valerian with a huge importance in Iran and the world and applies in Tranquilizers/Sedatives. Valerian (*Valeriana officinalis* L., Valerianceae) is a perennial herbaceous plant and native to Eastern and Central Europe. Rootlet branches exit from plant rhizome with a length ranged 15-20 cm and a diameter of 2-5 mm. The most important active agent is valerenic acid extracted from plant root and rhizomes. Three groups of chemical compounds include monosesquiterpenes, iridoid triesters, and pyridine alkaloids in this plant. Also, the flower of this species is white or pink with inflorescence of corymb and the fruit is the ovoid shaped akene. *Valeriana officinalis* L. grows in moderate and humid climates of Europe and Asia. In our country, it is found as a wild plant in northwestern regions of Iran and also surrounding of Isfahan. Proliferation of this plant is performed by two general methods including bush division and seeds but applying seeds is more important (Gruenwald, 2000).

Seed germination is one complex and dynamic step in plant growth and it can influence on function through effects on seedling establishment. In fact, germination is one of the important and sensitive steps in plant life cycle and also a key process in developing seedling (Ashraf & Foolad, 2005). Also, factors including inappropriate plantation ground, weak quality of seed, environmental stresses like high and low temperature, saltiness and drought, germination and establishment of seedlings. Most pharmaceutical species have seeds showing weak and inconsistent germination because of ecological adaptation with special environmental conditions. For this reason, different treatments are suggested to achieve optimum germination in these plants and one of them is seed priming (Hashemi Manesh, et al., 2008).

Several kinds of treatment for priming include osmopriming, hydropriming, matrix priming and hormone priming that apply for consistency and acceleration of germination, seedling growth, and increased function in most corps under normal condition and stress. Among priming methods, osmopriming is especially important and involves a procedure for delivery of the controlled water to seeds using molecules like polyethylene glycol, KNO_3, etc. Polyethylene glycol is one of the most applicable molecules for inducing drought stress in germination phase. The mentioned material dissolved in water induces a decrease of released water energy and subsequently, the water potential is decreased. The decreased water potential due to dissolved materials is called the decreased osmotic potential. In fennel (*Foeniculum vulgare* Mill), dill (*Anethum graveolens* L.) and ajowan (*Carum copticum* L.), it was found that increased drought stress due to polyethylene glycol influenced on germination percentage and rate, as well as rootlet and shootlet lengths to be decreased (Broumandzadeh Z, Kouchaki, A. 2005). In another experiment, it was shown that osmopriming of fennel (*Foeniculum vulgare*) seeds with NaCl, PEG and K_2SO_4 Causes germination percentage and rootlet length and shootlet length to be increased compared to control treatment (Neamatollahi, et al., 2009**).** Makkizadeh Tafti, et al. (2006) suggested that osmopriming of European borage (*Borago officinalis* L.) seeds with polyethylene glycol at concentration of -8 bar made germination percentage and rate of seeds to be increased. Also, osmopriming of Borage (*Borago officinalis* L.) seeds made germination percentage, germination rate, and establishment to be increased under drought stress (Tavakol Afshari, et al, 2007). Therefore, regarding the importance of seed germination in pharmaceutical herbs in special condition, the aim of this investigation is determine the effect of osmopriming on germination characteristics in *Valeriana officinalis* L. seeds under drought stress.

Materials and Methods:

This study was performed as a factorial experiment in completely randomized design carried out with four replications at the Research Laboratory of Shahrekord University, 2013. The experiment factors included osmopriming levels (0, -4, -8, -12 bar) as main factor and drought stress levels (0, -2, -4, -6 bar) as secondary factor. First, seed vigour tests were performed and the obtained results showed that seed vigour for *Valeriana officinalis* L. was 70-80%. Therefore, seeds were placed in osmotic solutions for 12 hours. For drought stress, polyethylene glycol 6000 was used. In each petri dish, 25 seeds were placed after disinfection and then 3 ml of osmotic solution was added into each treatment depending on their kinds. After this step, the seeds related into each osmotic levels and duration of preparation were placed on new petri dishes with filter paper individually.

For preventing humidity interchanges, petri dishes were fastened with Selefon sheets and transferred into germinator. The conditions of germinator were as follows: 16 hours in illumination and 8 hours in darkness with temperature of 25°C and humidity percentage of 45. Seeds were counted daily with a germinations criterion as the growth of coleoptiles of 1 mm. After experiments, traits like germination percentage, germination rate (GR), mean germination time (MGT), rootlet length and shootlet length were measured. Mean germination time is related to duration (day) taken for rootlet to exit. The smaller this number is, the higher speed germination gets as a characteristic for germination rate and velocity (Afzal, 2005).

$$\mathrm{MGT} = \frac{\varepsilon(n_i t_i)}{N} \qquad \mathrm{GR} = \Sigma \frac{\mathrm{n_i}}{\mathrm{t_i}}$$

Where, n is the number of germinated seeds during t days, t is the number of days from the beginning of germination and ∑n is the total number of germinated seeds. Finally, the statistical analysis was performed by SAS software and the comparison of mean studied traits was done with LSD test at $p<0.05$.

Results

The results of analysis of variance showed that the effects of osmopriming, drought stress and their interaction were statistically significance on germination percentage, germination rate, mean germination time, rootlet length and shootlet length (Table 1).

Table 1-The results obtained from analysis of variance among germination characteristics in *Valeriana officinalis* L. seeds affected under the studies treatments

Source of variation	Degrees of freedom	Mean Squares Germination percentage	Germination rate	Mean germination time	Rootlet length	Shootlet length
Block	3	29.60^{ns}	0.02^{ns}	0.24^{ns}	1.42^{ns}	0.18^{ns}
Osmopriming	3	163.91**	0.17**	4.56**	3.97*	1.47**
Drought stress	3	19810.52**	16.42**	143.42**	65.16**	123.48**
Osmopriming ×drougth stress	9	396.18**	0.09**	5.78**	119.08**	3.01**
Error	45	18.50	0.33	0.20	1.41	0.17
CV (%)	-	12.52	18.05	13.92	17.76	9.31

Note: ^{ns},* and ** are non-significant and significant at p<0.05 and p<0.01, respectively.

Germination percentage

The comparison of different levels of osmopriming showed that the highest level of this trait was obtained in treatment as -8 bar and also control treatment and the lowest level of this trait was achieved in treatment as -4 bar (Table 2). The comparison of different levels of drought stress showed that the highest levels of this trait were associated to control treatment and the lowest levels of this trait were found with treatment as -6 bar (Table 2).

Also, the comparison of mean interaction for different levels of osmopriming and drought stress showed that the highest levels of this trait were associated to control treatment with drought level of treatment as -2 bar and the lowest levels were found with treatment as -8 bar with drought level of control treatment (Figure 1).

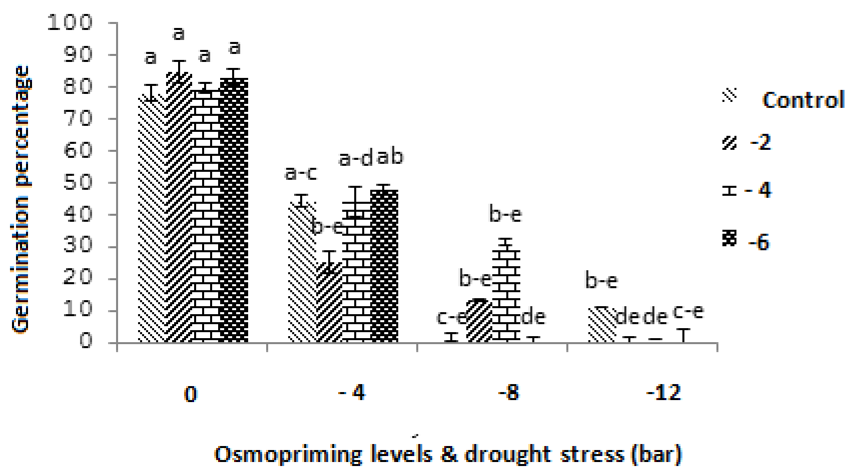

Figure 1- Mean interaction for different levels of osmopriming and drought stress on germination percentage (Similar letters indicate no significant statistical difference at P value <0.05)

Germination rate

The comparison of different levels of osmopriming showed that the highest level of this trait was obtained in treatment as -8 bar and also control treatment and the lowest level of this trait was achieved in treatment as -4 bar (Table 2). The comparison of different levels of drought stress showed that the highest levels of this trait were associated to control treatment and the lowest levels of this trait were found with treatment as -6 bar (Table 2).

Also, the comparison of mean interaction for different levels of osmopriming and drought stress showed that the highest levels of this trait were associated to control treatment with drought level of treatment as -4 bar that did not show any significant statistical differences between control treatment of osmopriming with control treatment of drought, control treatment of osmopriming with treatment of drought as -2 bar, and control treatment of osmopriming with treatment of drought as -6 bar and the lowest levels were found with treatment as -12 bar with drought level of control treatment (Figure 2).

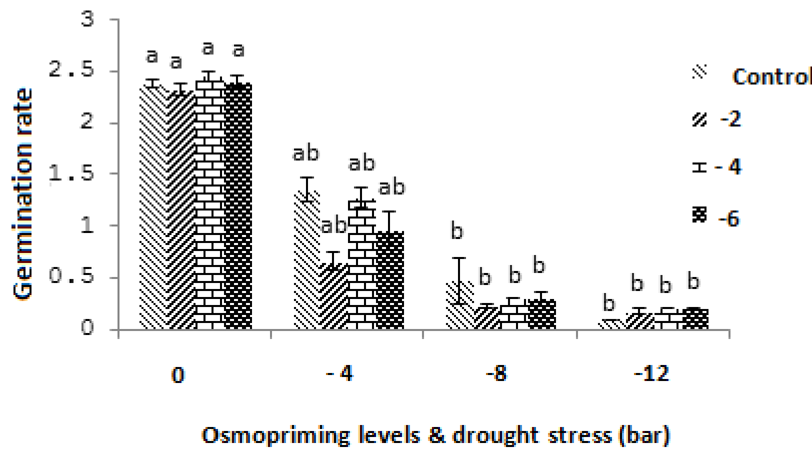

Figure 2- Mean interaction for different levels of osmopriming and drought stress on germination rate (Similar letters indicate no significant statistical difference at P value <0.05)

Table 2-The comparison of mean main effects on germination characteristics in *Valeriana officinalis* L. seed affected under the studies treatments

Treatment	Germination percentage	Germination rate	Mean germination time (day)	Rootlet length (cm)	Shootlet length (cm)
Osmopriming					
Control	33.87^{b}	1.07^{a}	3.24^{b}	6.89^{ab}	4.41^{b}
-4 bar	31.20^{b}	0.84^{b}	2.58^{c}	7.28^{a}	4.87^{a}
-8 bar	38.81^{a}	1.05^{a}	3.89^{a}	6.12^{b}	4.15^{b}
-12 bar	33.5^{b}	0.96^{ab}	3.18^{b}	6.51^{ab}	4.37^{b}
Drought stress					
Control	81.5^{a}	2.38^{a}	6.92^{a}	8.37^{a}	8.25^{a}
-2 bar	40.45^{b}	1.06^{b}	4.27^{b}	3.97^{c}	4.47^{b}
-4 bar	11.93^{c}	0.32^{c}	1.49^{c}	8.07^{a}	3.38^{c}
-6 bar	3.5^{d}	0.16^{d}	0.20^{d}	6.40^{b}	1.70^{d}

Note: Similar letters indicate no significant statistical difference at P value <0.05) based on LSD test.

Mean germination time

The comparison of different levels of osmopriming showed that the highest level of this trait was obtained in treatment as -8 bar and the lowest level of this trait was achieved in treatment as -4 bar (Table 2). The comparison of different levels of drought stress showed that the highest levels of this trait were associated to control treatment and the lowest levels of this trait were found with treatment as -6 bar (Table 2).

Also, the comparison of mean interaction for different levels of osmopriming and drought stress showed that the highest levels of this trait were associated to control treatment with drought level of treatment as -6 bar and the lowest levels were found with treatment as -12 bar with drought level of -4 bar (Figure 3).

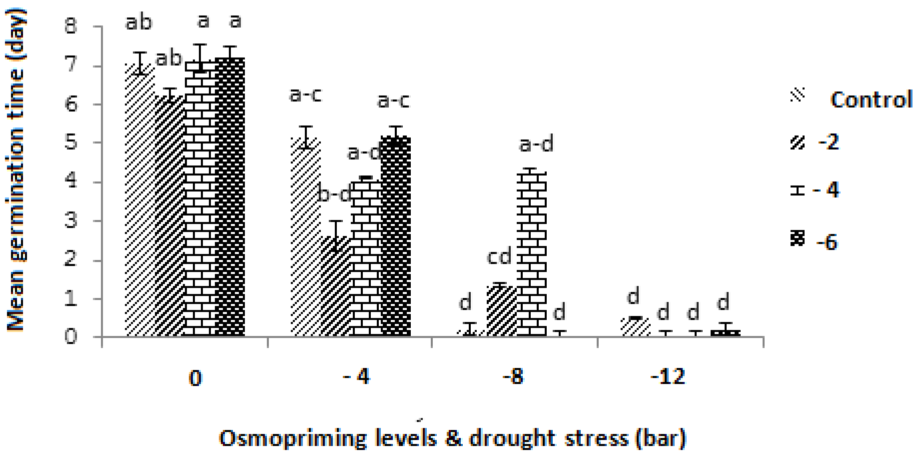

Figure 3- Mean interaction for different levels of osmopriming and drought stress on mean germination time (Similar letters indicate no significant statistical difference at P value <0.05)

Rootlet length

The comparison of different levels of osmopriming showed that the highest level of this trait was obtained in treatment as -4 bar and the lowest level of this trait was achieved in treatment as -8 bar (Table 2). The comparison of different levels of drought stress showed that the highest levels of this trait were associated to control treatment and the lowest levels of this trait were found with treatment as -2 bar (Table 2).

Also, the comparison of mean interaction for different levels of osmopriming and drought stress showed that the highest levels of this trait were associated to treatment as -12 bar with drought level of -6 bar and the lowest levels were found with treatment as -4 bar with drought level of -6 bar (Figure 4).

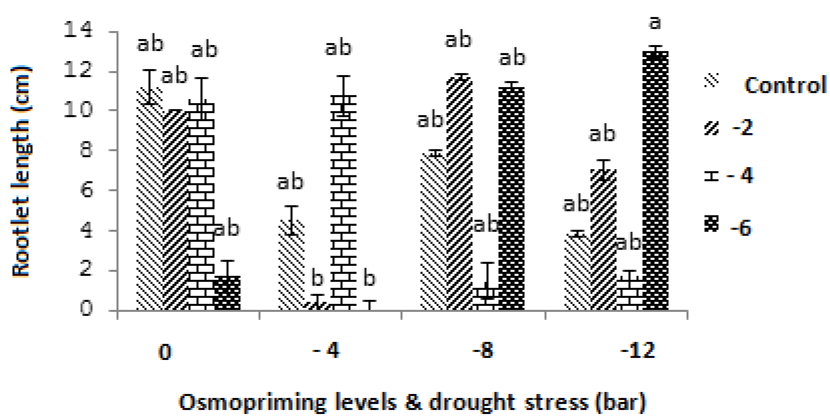

Figure 4- Mean interaction for different levels of osmopriming and drought stress on shootlet length (Similar letters indicate no significant statistical difference at P value <0.05)

Shootlet length

The comparison of different levels of osmopriming showed that the highest level of this trait was obtained in treatment as -4 bar and the lowest level of this trait was achieved in treatment as -8 bar that did not show any significant statistical differences with control treatment and treatment as -12 bar (Table 2). The comparison of different levels of drought stress showed that the highest levels of this trait were associated to control treatment and the lowest levels of this trait were found with treatment as -6 bar (Table 2).

Also, the comparison of mean interaction for different levels of osmopriming and drought stress showed that the highest levels of this trait were associated to control treatment with drought level of control treatment and the lowest levels were found with treatment as -12 bar with drought level of -4 bar (Figure 5).

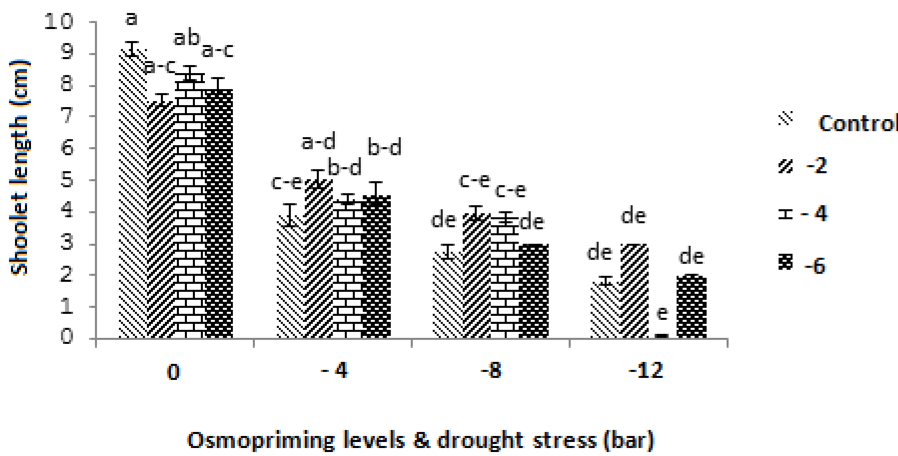

Figure 5- Mean interaction for different levels of osmopriming and drought stress on shootlet length (Similar letters indicate no significant statistical difference at P value <0.05)

Discussion:

The drought stress made all traits in relation with seed germination to be decreased. The decrease of these traits mainly was due to lack of sufficient water potential inside seeds and seedlings. In drought stress, seeds are not able to absorb water sufficiently due to negative water potential in drought stress condition compared with a normal condition, therefore, water potential inside seed and seedling is decreased. This factor makes traits associated with germination to be decreased in two ways, one is direct effect of water shortage resulted in slowing down metabolic activities involving in germination process (all need to aqueous medium for their activities) and another is indirect effect of water that is required for plant cell growth like Turger. For plant cell to be grown, definite threshold levels of Turger are needed with a different threshold for root and shoot cells (Kafi, et al., 2000).

Also, germination percentage, germination rate, mean germination time, rootlet length and shootlet length were decreased in Cumin (*Cuminum ciminum* L., Rahimi A. 2012), *Satureja khuzistanica Jamzad*. (Eisavand, et al., 2013) and Isabgol (*Plantago ovate*, Hosseini & Rezvani Moghadam, 2006) after polyethylene glycol induced an increase of drought stress. The study on a pharmaceutical herb like Achillea showed that increased drought stress led into decreased germination percentage, germination rate and rootlet length in all Achillea species, as treatment of distilled water induced the highest level (Ghani, et al., 2009). In addition, Farzaneh, et al. (2009) studied on sweet basil (*Ocimum basilicum* L.) and presented that increased drought stress led into decreased rootlet length and shootlet length. Fateh & Alimohammadi (2010) assessed a pharmaceutical herb like thyme (*Thymus Vulgaris*) and noticed that the increased drought stress resulted in decreased germination percentage, germination rate, rootlet length and shootlet length. Therefore, it was seemed that increased drought stress made many activities like cell division and growth to be stopped and subsequently, all traits associated with germination were decreased (Eisavand & Ashuri, 2010).

In this study, priming causes the improvement of germination percentage, germination rate, and mean germination time. However, rootlet length and shootlet length were decreased with increasing priming. A reason for these phenomena is that seeds cause an improvement in germination by water absorption during priming. The optimum conditions needed for priming are different for variant species. In suitable conditions, the processes have been started during osmopriming like transportation of food reservoirs, activation and re-synthesis of some enzymes synthesis of DNA and RNA, synthesis of ATP, as well as repairing damages to membrane system (Bray, 1995). The priming induces enzymatic activity to be increased and neutralize the effects of peroxidation (McDonald, 1999). Therefore, the improvement of some studied traits was occurred in this investigation during priming process. Also during osmopriming, the improvement of germination percentage, germination rate and mean germination time were observed in some plants like gladiolus (*Gladiojus alatus,* Ramazan, et al., 2010), rangpur lime *(Citrus limonia* Osbeck, Dantas, et al., 2010), and canola *(Artemisia sp*., Heshmat, et al., 2005), but decreased rootlet and shootlet lengths were found in wheat (*Triticum aestivum*, Sharifizadeh, et al., 2006) and thyme (*Thymus Vulgaris*, Fateh & Alimohammadi, 2010).

As a result of seed priming, several molecular and biochemical changes occur including the increased enzymatic and metabolic activities, protein synthesis, respiratory activities and production of Adenosin triphosphate that is essential for the synthesis of macromolecules, membranes and necessary materials for cell wall (Khan, 1992). During priming, embryo develops and makes endosperm to be compressed led into this result that the produced force for embryo, hydroletic activities of cell walls of endosperm and the room made inside of primed seed can accelerate the rootlet to be exited and germination rate through the facilitation of water absorption (Liu, et al., 1993). Hus and Sung (1997) reported that priming can make an increase in antioxidant enzymes including Glutathione and Ascorbate in seeds and these enzymes make the activity of lipid peroxidation to be decreased during germination and cause germination rate to be increased. Priming develops second phase from three germination phases and accelerate germination through shortening time of metabolism time. In osmopriming, the synthesis of protein and DNA is increased and also it influences on phospholipids of cell membrane (Bradford, 1995).

In the other words, germination percentage, germination rate and mean germination time of treated seeds were started earlier compared to control seeds and subsequently, seeds were established earlier under environment stress but change trends of roolet length and shootlet length decreased through increasing different levels of osmopriming. Generally, it can be concluded that germination and developing seedling completely are keys for controlling resistance and establishment of plant. Plant establishment can result in increased resistance to drought, saltiness and increased function of pharmaceutical and agronomic herbs.

Conclusion:

Overall, the results showed that the effects of osmopriming treatments, drought stress and their interactions on all studied germination traits were significant, as the levels of -4 and -8 bar indicated the highest effects on all mentioned trait among all different osmopriming levels.

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