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Germination response of endangered medicinal plant, *Levisticum officinale*, to stratification and some plant growth regulators

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Abstract

Levisticum officinale is an important and endangered wild medicinal plant belongs to Apiaceae family. Pre experiment showed that seeds of this plant have some degree of dormancy and it's germination is very low. Therefore, a factorial experiment laid out in completely randomized design with 3 replications to evaluate the effect of different plant growth regulators including: gibberellic acid (GA_3), methyl jasmonate (MeJA), salicylic acid (SA) and stratification (4˚ C for 30 days) to alleviate seed dormancy. Seed germination percentage, germination rate, means germination time, seed vigor index and seedling growth traits were recorded during experiment. The findings indicated that stratification or application of plant growth regulators alone could improve germination partially, but their effect was not considerable, while application of plant growth regulators combined to stratification was more effective. The maximum germination percentages (77.66%), the maximum of shoot dry weight (4.2 mg) and root dry weight (1.4 mg) achieved when 10 µM of MeJA was applied. However, for the other studied traits 2000 mg/l GA_3 was the best treatment. The results suggest that L.officinale has dormancy which can help the plant for addaptation in cold climate. Application GA 2000 mg/l in combination to stratification is recommended in order to improve germination characters.

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Introduction

The genus *Levisticum officinale* (Lovage) is a valuable medicinal herb belonging to Apiaceae family. All parts of plant are aromatic and have variety of uses from landscaping and culinary to cosmetic and medicinal. In folk medicine it is used for treatment of stomach upset, reduce excess gas and uses as a diuretic (Hog *et al.*, 2001). The only naturally habitat of this species in Iran is Hezar mountain in Kerman Province between 3000 and 3400 m altitudes. The wild populations are distributed in a limited area and closely linked to the winter rain and to snow falls. The rhizomes must survive the hard conditions in winter. As other alpine species and based to primary experiment lovage seeds exhibit some degree of dormancy and germination percentage of the species is very low. Low seed germination, high harvesting by native people, low rain fall and over exploitation of natural population causes the species is getting endangered. One of the challenges in successful germination of wild species is absences of enough information about the conditions which are required for seed germination (Gupta and Bandopadhyay, 2013) .Viable seeds that do not germinate are defined dormant seeds. Seed dormancy is a mechanism by which seeds can inhibit their germination in order to wait for more favorable conditions (Finkelstein *et al.*, 2008). If a seed is not exposed to sufficient moisture, proper temperature, oxygen, and for some species light, the seed will not germinate. Many different techniques are being used to overcome dormancy, increasing germination percentage and reducing germination time. It has been suggested that populations which normally exposing long periods with snow cover and hard winter conditions would require long periods of cold stratification for seed germination (Meyer and Monsen, 1991). Stratification is a method which is customarily used to break seed dormancy and is necessary for increasing germination of Apiaceae family (Kretshmer, 1999). A period of chilling is useful to relieve primary dormancy of many northern hemisphere species (Baskin, 2001). It has been widely used as a pre-sowing treatment for breaking dormancy and enhancing the maximum rate of germination of dormant seeds of many different species (Fang *et al.*, 2006). Also, plant growth regulators are chemicals which in small amounts can regulate different plant activities including seed dormancy. Different plant hormones can regulate different plant activities including seed dormancy and germination, growth and development of plant cells and tissues (Agraeber *et al.*, 2012). Gibberellins are common used to eliminate the chilling requirements of some plant seeds and increasing their germination (El-Dengawy, 2005). It plays a role in promotion of enzymes synthesis that converts stored nutrients carbohydrates which are needed for rapid cell respiration during germination (Bakrim *et al.*, 2007). SA as a natural signal molecule has been shown to play important roles in regulating a number of physiological events in plants such as enhancement of tolerance to biotic and abiotic stress, seed germination and seedling growth parameters (Kumar *et al.*, 2010). Because *L. officinale* populations are rare and represented in a limited area in South East of Iran, there is no information about germination traits of this species. The aim of the present study was to examine the effect of different treatments on seed germination and determine effective method for breaking seed dormancy of *L. officinale*.

Materials and methods

This study was carried out at the Department of Biology, ShahidBahonar University of Iran.

Seed collection and preparation

L. officinale mature seeds were collected from natural habitat located in Hezar Mountain in South East of Iran, Kerman Province. The seeds were surface sterilized by soaking in 70 % ethanol for 1 min, and immediately soaked in 2 % sodium hypochlorite for 10 min. They were rinsed thoroughly with double sterilized water for 30 minutes before applying any treatment.

Seed germination procedure

In this study 8 different plant growth regulators and stratification treatments were applied. To study the

effect of hormone treatments on germination, surface sterilized seeds soaked for 24 h in: distilled water (control), 0.75 and 1.5 mM SA, (iii) 5 and 10 µM MeJA. (IV), 500, 1000 and 2000 mg/l GA_3. Three replicates of 30 seeds were placed in Petri dishes on two layers filter paper (Whatman No. 1) moisture with distill water. The dishes containing treated seeds were divided in two groups; the first group was transferred to a growth chamber at 23±2 °C, and the second group was placed in refrigerator (+4 °C) for 30 days. Every 2 days germinated seeds were counted .during 25 days. Seeds were considered germinated when the radicle was approximately 2 mm long. Parameters measured in this experiment were: Germination percentage germination rate and mean germination time were computed according to the following equations.

1) $GP = \frac{\text{Number of germination seed}}{\text{Total number of seeds}} \times 100$,

2) Where GP is germination percentage

3) $GR=\sum_1^i ni/Di$, Where GR is germination rate (the number of germinated seeds per day), n is number of germinated seed on day i and Di is the number of days after beginning the experiment (Agrawal, 1995)

4) MGT = N1T1 + N2T2 + N3T3 / N

Where MGT is mean germination time, N1, N2... Nn shows number of germinated seeds in the first, second and nth day, respectively. T1, T2, ... Tn show the count of first, second, ... n^{th} day, and N shows the total number of germinated seeds (Ellis and Roberts., 1981). Shoot and root length (mm), shoot and root dry weight was calculated as growth parameters (Nikolaeva, 1997) and seedling vigor index was calculated as the index of growth (Vashisth and Nagarajan, 2010).

5) Seed vigor index = Germination percentage × Seedling length.

Statistical analysis

Experiment was randomized complete design with 4 replications. The statistical analysis was made using the GLM procedure of SAS. The difference between the means was compared using the Duncan's multiple test ($p < 0.05$). An experiment with a Factorial completely randomized design with three replications was done. Analysis of variance was performed using the ANOVA procedure by SAS software version 9.1 (SAS Institute, Cary, North Carolina). The difference between the means was compared using the LSD ($p < 0.05$).

Results

Plant growth regulators were added in the forms of GA_3, SA, MeJA. Also, stratification (at 2 level: 0 days and 30 days) alone or in combination with plant growth regulators were used to find the best treatment to stimulate germination of *L. officinale* seeds. Analysis of variance showed that experimental factors, stratification and hormone treatments, significantly ($P < 0.01$) affected all germination traits. Also the interaction effect of hormone and stratification on studied traits was significant (Table 1).

Table 1. Mean squares for germination percentage and rate, seed vigor index, shoot and root length, shoot and root dry.

S.O.V	df	Germination percentage (%)	Germination rate (seeds /day)	Mean germination time(day)	Shoot length (mm)	Root length (mm)	Shoot dry weight (mg)	Root dry weight (mg)	vigor index (SIV)
Stratification (A)	1	11324.52**	14.09**	1119.50**	3120.19**	1166.66**	9.40**	1.85**	1356230.47**
Hormone (B)	7	443.14**	0.17**	4.93**	58.38**	63.259**	0.48**	0.14**	18465.31**
Interaction (A*B)	7	261.97**	0.11**	3.65**	41.78**	30.82**	0.74**	0.10ns	5095.75**
Error	32	5.27	0.001	0.78	0.36	0.61	0.03	0.007	117.33
Coefficient of variation		5.16	5.53	5.61	1.705	3.45	5.15	10.38	3.68

**, denote significant differences at 0.01 % levels probability

Germination percentage, germination rate and mean germination time

According to the results, applied treatments significantly improved all germination traits including germination percentage, seedling growth including shoot length (SHL), root length, shoot dry weight (SDW), root dry weight (RDW), seed vigor index (SIV) and decreased mean germination time (MGT). There was a significant difference between non-treated seeds (control) and treated seeds and the lowest values were recorded for control (table 2). Compare to control the stratification increased GP from 24.66% to 41% and GR from 0.10 to 1.22 seed per day and resulted in higher values for seedling growth parameters (Table 2).

Table 2. Mean comparisons the effect of various treatments on *Levisticum officinale* seed germination.

Treatment	Germination percentage (%)	Germination rate (seeds per day)	Mean germination time (day)	Shoot length (mm)	Root length (mm)	Shoot dry weight (mg)	Root dry weight(mg)	vigor index(SIV)
Control	24.67±1.52^{h}	0.23±0.02^{i}	19.87±0.96^{bc}	23.41±0.52^{i}	11.06±0.54^{k}	3.48±0.05^{def}	0.56±0.07^{h}	45.02±5.92^{i}
Stratification	41.01±0.73^{f}	1.22±0.03^{d}	13.12±0.73^{d}	36.00±0.88^{e}	23.22±0.83^{e}	3.63±0.32^{cdef}	0.79±0.01^{cde}	345.17±9.27^{e}
GA500 (mg/l)	30.00±0.00^{g}	0.39±0.01^{g}	18.33±0.57^{c}	31.34±0.56^{f}	20.08±0.37^{g}	3.41±0.22^{ef}	0.52±0.02^{h}	154.25±2.78^{g}
GA1000 (mg/l)	28.33±2.88^{gh}	0.35±0.04^{gh}	19.75±1.40^{bc}	26.25±0.43^{h}	17.19±0.83^{i}	2.86±0.12^{g}	0.53±0.01^{h}	170.11±11.39^{g}
GA2000 (mg/l)	40.67±1.15^{f}	0.30±0.00^{hi}	19.89±0.45^{bc}	29.58±0.38^{g}	21.22±0.25^{fg}	3.47±0.15^{def}	0.62±0.04^{fgh}	156.52±5.84^{g}
SA 0.75 (mM)	37.00± 2^{f}	0.40±0.02^{g}	19.49±1.18^{bc}	26.25±0.69^{h}	16.52±049^{i}	2.92±0.07^{g}	0.56±0.03^{h}	171.20±2.82^{g}
SA 1.5 (mM)	38.33±2.88^{f}	0.37±0.05^{gh}	20.56±1.11^{b}	30.68±0.59^{f}	25.41±0.62^{d}	3.50±0.15^{def}	0.93±0.05^{bc}	217.42±14.35^{f}
MJ5 (µM)	27.33±2.31^{gh}	0.34±0.00^{gh}	20.43±1.13^{b}	25.99±0.49^{h}	18.72±0.70^{h}	2.39±0.24^{h}	0.60±0.12^{gh}	156.51±4.21^{g}
MJ10 (µM)	30.66±2.09^{g}	0.22±0.02^{hi}	21.03±0.86^{b}	26.28±0.25^{h}	15.22±0.54^{i}	1.75±0.20^{i}	0.77±0.04^{def}	83.89±4.41^{h}
GA500 (mg/l)+ST	71.66±2.87^{b}	1.66±0.11^{a}	9.76± 0.21^{fg}	39.85±0.16^{d}	28.81±0.99^{c}	3.73±0.15^{cde}	0.95±0.12^{b}	487.27±15.19^{bc}
GA1000 (mg/l) +ST	58.33±2.87^{c}	1.10±0.03^{e}	11.44±0.80^{e}	42.00±0.88^{c}	30.22±1.07^{b}	3.78±0.22^{bcd}	0.75±0.08^{efg}	493.11±13.98^{b}
GA2000 (mg/l) +ST	73.33±2.87^{b}	1.64±0.04^{a}	9.33± 0.41^{g}	52.55±0.69^{a}	33.00±0.66^{a}	4.10±0.10^{ab}	1.36±0.15^{a}	537.28±14.92^{a}
SA 0.75 (mM) +ST	48.67±2.30^{e}	1.25±0.03^{d}	11.86±0.29^{de}	42.00±0.88^{d}	31.89±0.76^{a}	3.66±0.11^{cdef}	1.03±0.05^{b}	467.78±17.66^{d}
SA 1.5 (mM) +ST	60.33±1.52^{c}	1.13±0.08^{e}	11.46±0.53^{e}	46.00±0.33^{b}	26.55±0.50^{d}	3.76±0.23^{cd}	1.06±0.06^{b}	462.73±11.05^{e}
MJ5 (µM) +ST	53.33±1.52^{d}	1.54±0.02^{b}	11.17±1.43^{ef}	44.99±0.33^{b}	22.44±0.69^{ef}	3.93±0.15^{abc}	1.06±0.11^{b}	478.99 ±15.69^{bcd}
MJ10 (µM) +ST	77.67±2.51^{a}	1.45±0.02^{c}	11.18±0.73^{ef}	36.63±0.61^{e}	23.33±1.52^{e}	4.20±0.17^{a}	1.40±0.17^{a}	469.33±7.05^{d}

Different letters indicate significant differences in each treatment as determined by LSD test at P = 0.05, ST= Stratification.

According to the results, it was found that the effect of stratification on germination and seedling growth was more considerable than hormonal treatments. Furthermore, applying hormone treatment along with stratification improved germination and seedling growth considerably.

The highest germination percentage observed in MeJA 10 µM treated seeds following 2000 and 500 mg/l GA_3 (77.66%, 73.33% and 71.66%, respectively). Applying GA_3 together with stratification showed that increasing in GA_3 concentration from 500 to 2000 mg/l caused changes in GP, GR and MGT sinusoidal and in which the highest values were achieved in the concentrations of 2000 mg/l and 500 mg/l, while the lowest values were achieved in 1000 mg/l. Results showed that application of 2000 mg/l and 500mg/l GA_3caused significantly increasing in germination rate. Mean comparison results displayed that the least mean germination time obtained from 2000 and 500 mg/l GA_3 without any significant difference (9.37 and 9.75 days respectively).

Seedling growth parameters

The most effective treatment to increase shoot and root length was 2000 mg/l GA_3.With increasing in GA_3 concentration shoot and root length increased significantly. Evaluation of growth parameters in treated seeds indicated that the highest shoot length (52.55mm) and root length (33.00 mm) were obtained in 2000 mg/l GA_3 along with stratification. When the seeds were treated with GA_3 seed vigor index significantly increased and its highest amount (537.28) was achieved from 2000 mg/l GA_3 (Table2).

Application of 10 µM MeJA caused the highest shoot dry weight, although there was no significant difference between 5 and 10 µM MeJA with 2000 mg/l GA_3. The results showed that the effect of seed treatment by 10 µM MeJA and 2000 mg/l GA_3 on root dry weight was the same, meaning that root dry weight was similar within these two treatments (1.400 and 1.366 mg, respectively).

Discussion

Results showed that all used treatments including hormones combined with stratification, stratification and hormones alone could improve germination traits compare to control, respectively. At all it was revealed that 2000 mg/l GA_3 combined with stratification was the best treatment for improving almost studied traits. According to the results treating seeds only with plant growth regulators or stratification could only remove seed dormancy partially. So the effect of these two factors on removing lovage seed dormancy can be negligible if they use alone. When soaked seeds in plant growth regulators stratified at 4 °C for 30 days all germination traits including final germination percentage, germination rate, shoot and root length, shoot and root dry weight significantly increased and mean germination time decreased. It was found stratification could amplify plant growth regulators effect. Apiacea family species possess physiological dormancy (Baskin, 2001)that is an ecological adaptation way for the plants distributed in cold climate. Regard to *L. officinale* distribution in cold climate stratification is a helpful treatment to break this type of dormancy. Growth of embryo in Apiaceae seeds is not complete at the moment of dispersal. So it has to grow before germination starts, therefore a delay in germination is observed (morphological dormancy). In addition, physiological dormancy (PD), preventing germination in unfavorable conditions (Nikolava, 1977). Seeds with both an under developed embryo and physiological dormancy are referred to as morphophysiologically dormant (MPD).

Chilling might stimulates hydrolytic enzyme activity, enzyme concentrations or enzyme production (Bewley, 1997). It probably activates respiratory systems contain citric acid cycle, glycolysis, and pentose phosphate pathway which provide necessary ATP for germination process (Norastehnia *et al.*, 2007). In other hand moist-chilling condition may induces or activates many genes involved in dormancy breaking. It has been shown that stratification causes an increase in internal GA_3 concentration (Apipinis *et al.*, 2012). In evaluation of final germination percentage methyl jasmonate was the best treatment. It could enhance final germination percentage 53% higher than control. The present results indicate a stimulatory effect of MeJA on germination percentage. Also some reports have been shown that JA is able to stimulate germination of dormant seeds by decrease in sensitivity to abscisic acid (ABA). Priming sweet pepper seed with MeJA improved seed germination and rate (Korkmaz, 2005). Similarly, in dormant seeds of *Acer tataricum* (Berestetzky, 1995), *pseudotsuga menziesii*(Jarvis *et al.*, 1997) and apple embryo (Ranjan and Lewak, 1995), JA strongly increased germination. JA involvement in the control of hydrolytic enzymes and stimulates sucrose hydrolysis has been demonstrated. The products of sucrose hydrolysis are catabolized via glycolysis and following produces required ATP for germination (Bogatek *et al.,* 2002). It has been reported that in some plants jasmonate acts as ABA and inhibited germination of seeds without dormancy, for example Sunflower (Corbineau *et al.*, 1988), Lettuce (Yamane *et al.*, 1980) and Amaranthus (Kepczynski and Bialecka, 1997).

The second effective treatment for germination percentage was GA_3+chilling, in which 500 and 2000 mg/l GA_3 caused increasing germination percentage and rate, shoot length and root length. Evaluation of growth parameters in treated seeds indicated that the highest shoot length and root length were obtained in 2000 mg/l GA_3 combined with stratification. This treatment could supply embryo metabolic activity demands which are necessary for the beginning of germination process.α-amylase is a key enzyme for

seed germination. This enzyme hydrolyzes the insoluble starch to soluble glucose as a substrate respiration and consequently limited energy production. GA_3 and stratification stimulate α-amylase synthesis and its secretion from aleurone layers (Norastehnia *et al.*, 2007). Published reports showed that germination and dormancy breaking can be induced by GA_3 in some Apiaceae species such as *Ducrosia anethifolia* (Ashtari *et al.,* 2013), *Ferulla gummosa (*Rahnama-Ghahfarokhi and Tavakkol-Afshari, 2007), some legumes (De Morais *et al.,* 2014), *Alstromerialigtu* (Nasri *et al.*, 2014), *Allium hirtifolium* (Ebrahimi *et al.*, 2014),*Stachysgermanica* (Güleryüz *et al.,* 2011) and *Acalypha indica*(Gupta and Bandopadhyay, 2013). Seed dormancy is under control of some factors: molecular alteration of proteins, hormones and the balance between ABA and GAs (Finch-Savage and Leubner-Metzger, 2006). Also some research demonstrated that application of GA_3 together with stratification increased seedling growth in *Alostromia hybrid* (Nasri *et al.,* 2014),*Acacia nilotica* and *Prosopis cineraria* (Dhupper, 2013). Both treatments of 5,10 µM MeJA and 2000 mg/l GA_3 together with chilling significantly increased shoot dry weight without any significant difference. The increased herb growth due to PGRs application could be due to the stimulation of cell division and elongation while increasing plasticity of cell wall seed (Mondal, 1975).These treatments could increase seed vigor index and provide subsequent higher growth and reducing chances of their mortality.

Conclusion

L. officinale possess physiological dormancy as an adaptation way for the plants distributed in cold climate. plant growth regulators or stratification could only remove seed dormancy partially, whereas chilling pretreatment could amplify plant growth regulators effect.In evaluation of final germination percentage and shoot dry weight methyl jasmonate along with moist chilling was the best treatment . For other evaluated parameters application of GA 2000 mg/l is suggested.

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