Methods to promote germination of *Kelussia odoratissima* Mozaff., an Iranian endemic medicinal plant

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Summary

The response of seeds germination from Apiaceae, *Kelussia odoratissima,* which have interesting horticultural and medicinal properties, was established. In order to optimize germination factors for the first time, seeds were exposed to different types of treatment. In the first experiment, different times of stratification (0, 15, 30 and 45 days at 4°C) were tested, and the best stratification time was 45 days. In the second experiment, the results of the first experiment were followed by various leaching durations (0, 12 and 24 hours with running tap water), Gibberellic Acid (GA_3) (0, 500 and 1000 ppm concentrations for 12 hours) and germination temperatures (5, 10 and 15°C). The treatments, arranged as a factorial experiment, involved three replications per treatment in a completely randomized design. Results revealed that long stratification (45 days) stimulated germination percentage (GP) and mean germination time (MGT) compared to lower times of stratification. It seems that applying external GA_3 has no effect on *K. odoratissima* seed germination. Different temperatures during seed germination affect MGT and GP significantly as the highest MGT and GP were at 5°C and 10°C, respectively. The duration of leaching diminished GP and the best MGT was observed in treatments with no application of leaching. In conclusion, the best germination of *K. odoratissima* was achieved at 10°C after 45 days stratification in 4°C.

Key words: *Kelussia odoratissima, Apiaceae, leaching, stratification, gibberellic acid*

INTRODUCTION

Kelussia odoratissima Mozaff (Apiaceae) locally called "Karafs-koohi", is a wild rebus, erect, glabrous, perennial aromatic herb,which grows to a height of 120 to 200 cm. The flowers are 1–2 mm in diameter, all hermaphrodites. Petals are yellow and produced in compound umbels. It is native to the central region of the Zagros Mountains, Iran and has a great biological diversity. Topographic factors have created a very diverse microclimate and edaphic conditions in this region (fig. 1) [10]. *Kelussia odoratissima* is dispersed around some parts of the Zagros Mountains at heights of 2500 m, including Isfahan, Chahrmahal Bakhtiari and the Lorestan provinces (fig. 1). The temperature in the areas of native dispersion of *Kelussia odoratissima* is usually lower than 20˚C and includes 127 days of frost, with the temperature reaching below zero in the autumn and winter (fig. 2). *Kelussia odoratissima* is a sweet-smelling plant which has anti-inflammatory, sedative, and anti-tussive properties, and is used to garnish food [1]. In Iran, this plant is traditionally consumed as a medicinal plant to treat hypertension, inflammation, ulcers, and cardiovascular diseases [2].

Recent work by Omidbeigi *et al.* [1] showed that the essential oil from the aerial part of the plant contains 23 kinds of different valuable components, of which the major compound is Z-ligustilid. This means *K. odoratissima* can be used as a potential source of Z-ligustilid. Moreover, some investigators reported an antioxidant property of *K. odoratissima* [2].

In natural conditions, the plant is propagated through seeds that are in general produced once a year in late summer. *K. odoratissima* is an overthrowing plant [3, 4]. The flowers start scattering seeds in late summer and in the next spring, seeds will germinate at the average temperature of 5.7^{o}C (fig. 2). As there is no information on the factors affecting the seed germination of *K. odoratissima,* the present study attempted to understand the germination behavior of the seeds exposed to varying durations of stratification (moist chilling) and leaching. At the same time, the effects of different Gibberelic Acid (GA_3) and temperature on germination were also investigated.

MATERIAL AND METHODS

Seed collection

For the first time, different aspects of *Kelussia odoratissima* Mozaff germination dynamics were conducted in controlled laboratory conditions, aimed at optimizing its germination. For this reason, mature seeds of *Kelussia odoratissima* Mozaff were collected from the Milli mountain during late summer from a habitat of natural growth in the Shahrekord province (latitude 32˚ 17´ N longitude 50˚ 51´ E, elevation 2500 m above mean sea level (fig. 1).

For each study conducted, seeds were placed equidistantly apart in 9 cm Petri-dishes lined with two layers of Whatman number 1 filter paper, moistened with 3.0 ml of distilled water. Germination percentage was evaluated using one of the methods of AOSA [5]. Seed germination was considered successful when the root had emerged and reached out by one mm in length. The evaluations were conducted every 24 hours until seeds had either germinated or presented signs of fungal contamination. Before any tests were carried out, seeds were sterilized by keeping them in a commercial 10% (v/v) sodium hypochlorite solution for 10 minutes. After this, the seeds (it is divided schizocarp fruits of *Kelussia odoratissima* to two seeds) were rinsed several times in distilled water. The first experiment was conducted in order to determine the effect of stratification duration on germination. Second experiment treatments included different soaking times, Gibberellic Acid (GA_3) concentrations and germination temperatures, and were conducted respectively. For these reasons, a factorial experiment was established involving three replications per treatment in a completely randomized design. Each experimental plot contained 25 seeds. The results were analyzed with variance analysis, and the means were compared with the Least Significant Difference (LSD) multiple test, using the Statistic Analysis System (SAS, version 9).

First experiment: effect of stratification duration on germination

Treatments included 0, 15, 30 and 45 days of stratification (at 4°C). The seeds allocated to stratification treatments were transferred to moist perlit (1:5, v/v) in 1kg plastic bags and were placed in a refrigerator at 4°C for stratification. The perlit was thoroughly washed and soaked for 3 hours in distilled water before application. Finishing each period of stratification, seeds were transferred to an incubator (at 10˚C).

Second experiment: effect of leaching duration, GA3 concentration and incubating temperature on germination

To determine the optimal leaching duration, seeds were kept in mull cloth in running tap water for 0, 12 and 24 hours. After each period, seeds were imbibed in 0, 500 and 1000 ppm GA_3 for 12 hours. All dishes were maintained at constant temperatures (5, 10 and 15°C) in the B.O.D. incubator for a ten-hour photoperiod of 20 μmol $m^{-2}s^{-2}$, 400–700 nm white fluorescent light (10 hours of light:14 hours of darkness) [5]. The evaluations of germination were conducted every 24 hours until seeds had either germinated, or presented signs of fungal contamination.

The mean germination time (MGT) measured for seed germination for a certain time period after applying each treatment, was calculated by the formula:

$$MGT = {\sum g_i n_i}/{G},$$

where g_i is the number of seeds germinating on the n_i^{th} day of germination testing, and G is the total number of seeds germinating during the test (Hurtman *et al.,* 1997).

Germination percentage (GP) and MGT were scored as a mean of 3 replicates with standard deviation.

RESULTS

Effect of stratification on germination

An increased stratification time improved GP and MGT. The mean for GP in 0, 15, 30 and 45 days of stratification were 0, 31.7, 36.7 and 93% respectively. Forty five days stratification in 4°C gave the best GP and MGT. Therefore, this treatment was chosen for the subsequent experiments (fig. 3).

Effect of incubating temperature on germination

Maximum GP reached the highest amount (95.48%) at 10°C (fig. 6). The MGT was measured as a function of temperature. The temperature which allowed the lowest MGT was 10°C, meaning the greatest germination rate (fig. 4).

At 10°C the minimum time needed for seeds to reach optimum germination (90% or higher) was 15 days. At this length of time, treatments of 5 and 15˚C only showed germination between 30 and 45% (fig. 6).

The highly significant regression ($p<0.000$) observed between GP and all three tested temperatures, and the highest liner relation ($R^2=0.97$, $Y=3.231X+9.076$) was at 10°C (fig. 5).

Effect of leaching duration and GA_3 concentration on germination

The duration of seed leaching did not affect the GP (L_0=81.07%, L_{12}= 85.59% and L_{24}=83.33%). A significant increase in MGT was observed in treatments where leaching was not applied. The MGT was 11.38 per day without leaching, 9.96 per day with 12 hours of leaching, and 9.82 per day with 24 hours of leaching. Applying leaching treatment resulted in the later onset of germination with seed germination starting from the fourth day when 12 and 24 hours of leaching were applied, but from the second day when no leaching was applied.

The mean GP was mainly the same for the three GA_3 concentrations (i.e., $GA_{3(100)}$=83.55, $GA_{3(500)}$=80.07 and $GA_{3(1000)}$=86.37). The mean MGT (i.e., $GA_{3(100)}$=9.90 day, $GA_{3(500)}$=10.69 day and $GA_{3(1000)}$=10.57 day) did not differ significantly among GA_3 treatments.

DISCUSSION

In order to optimize germination, attempts were made to eliminate possible dormancy inhibitors by taking patterns from the native habitat, using several pretreatments. *K. odoratissima* grow in the cold winter climate of its native mountains, where there are almost 127 days of frost per year. Therefore, it clearly appears that stratification during the winter improves germination in mountains native to *K. odoratissima*. The growth may involve breaking seed dormancy. The results from the laboratory conditions confirm the effect of stratification on seed germination of *K. odoratissima*, as the MGT and GP is greatly increased by extending the stratification time to 45 days, whereas, at 0 and 30 days, stratification was not able to stimulate the germination of seeds. During the stratification some biochemicals and phytohormone were changed to be able to germinate dormant seeds. By decreasing abscisic acid (ABA) and increasing GAs, the seeds, with embryo dormancy residing in the embryo, can germinate [6]. Prechiling increases GA biosynthesis, which decreases the accumulation of proteins that control dormancy [7, 8]. For an effective ratio of GA:ABA in *K. odoratissima* germination with 45 days of stratification was effective enough, since applying exogenous GA seems ineffectual.

The best conditions for the germination of *K. odoratissima* have not previously been documented, so the effect of temperature on seed germination was patterned from its habitat. The results from this study indicated that the best temperature for highest GP and lowest MGT is 10°C. Maximum germination was reached at more than half (50% GP), at 18, 5 and 12 days in 5, 10 and 15°C, respectively. The fastest and highest germination (GP) was achieved at 10°C. In its native location, most of the dispersion of *Kelussia odoratissima* is in high altitudes, in areas covered with ice, or on the northern side of the mountains. Therefore, it is justifiable that the optimum temperature in these areas is less than 10°C (fig. 3, 4). In accordance with the abovementioned results, the climate closest to the conditions is presented in figure 2. This suggests that the climate, for the planting of this overthrowing plant, needs to be tested.

However, observations during the experiment showed that leaching had a good effect in decreasing the amount of contamination by fungi and bacteria, as has also been reported for *Cunoniaceae* species by Fogliani *et al*. [9]. Conversely, germination is decreased by increasing the time of leaching.

Application of GA_3 did not significantly affect germination, at the concentrations that were used; the same effect of GA_3 has been shown for seeds of *Fagus sylvatica* and *Picea sitchensis* [10]. However, our results cannot weaken the role of GAs in germination. Evidence showed an absolute dependence on GA_3 for germination of some seeds like Arabidopsis [11, 12] and in cereal [13, 14]. Within some species, germination cannot proceed without gibberellins biosynthesis because of their secondary and physical constraints [15].

Gibberellins biosynthesis increased when the seeds were transferred to a higher temperature after undergoing moist chilling in *Corylus avellana*. Therefore, it was hypothesized that sufficient GAs required for radical protrusion, are produced during moist chilling [16]. Along with what was discovered, sufficient GAs might have developed during stratification of *K. odoratissima*, so the addition of exogenous GA did not have a greater effect on germination. Mortensen and Eriksen's [10] results on *F. sylvatica* revealed that GA has no effect on non-dormant seeds. Therefore, it appears that no dormancy existed after stratification for *K. odoratissima*. The same hypothesis can be developed for *Pseudotsuga menziesi* and *Pinus lambertiona.* Taylor and Wareing [17] and Fernandez [18] showed increase of GA_3 in the embryo axis of *Fagus sylvatica* under 3 and 7 day stratification [18]. Second possibility revealed that GA concentration is too high for *K. odoratissima* germination. Applying lower concentration of GA on dormant *Fagus sylvatica* and *Picea sitchensis* seeds were effective on germination [10]. Accordingly, testing different and lower concentration of GAs in following study is suggested.

CONCLUSION

The best germination temperature for *K. odoratissima* is the condition near to its habitat, i.e., 10°C. it is observed that for probable seed dormancy 45 days stratification in 4°C stimulate seed germination but seed leaching has no effective role.

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METODY PRZYSPIESZENIA KIEŁKOWANIA NASION *KELUSSIA ODORATISSIMA* MOZOFF, IRAŃSKIEJ ENDEMICZNEJ ROŚLIN LECZNICZEJ

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Streszczenie

Badano kiełkowanie nasion *Kelussia odoratissima* (Apiaceae), interesującej rośliny ogrodowej i leczniczej. Po raz pierwszy zajęto się problemem optymalizacji warunków kiełkowania, poddając nasiona różnym zabiegom. W eksperymencie pierwszym zastosowano różny czas stratyfikacji (0, 15, 30 i 45 dni w temp. 4°C). Najlepsza okazała się stratyfikacja trwająca

45 dni. W eksperymencie drugim wyniki poprzedniego badania były pogłębione o badanie różnego czasu wymywania (0, 12 i 24 godziny za pomocą bieżącej wody), działanie kwasu giberelinowego GA_3 (stężenie 0, 500 i 1000 ppm/12 godzin) i badanie różnej temperatury kiełkowania (5, 10, 15ºC). Badania z randomizacją obejmowały trzy powtórzenia każdego badanego czynnika. Wyniki wykazały, że długa stratyfikacja (45 dni) stymuluje procent kiełkowania (GP) i średni czas kiełkowania w porównaniu z krótszym czasem stratyfikacji. Wydaje się, że oddziaływanie zewnętrznym GA_3 nie ma wpływu na kiełkowanie nasion *K. odorastissima*. Różna temperatura podczas kiełkowania nasion wpływa znacznie na MGT i GP; najwyższe wartości MGT i GP wystąpiły odpowiednio przy temp. 5 i 10ºC. Czas trwania wymywania zmniejszał GP i najwyższe wartości MGT obserwowano dla wariantów bez stosowania wymywania. Podsumowując, należy stwierdzić, że *K. odoratissima* najlepiej kiełkowała w 10ºC po stratyfikacji trwającej 45 dni w temp. 4ºC.

Słowa kluczowe: *Kelussia odoratissima, Apiaceae, wymywanie, stratyfikacja, kwas giberelinowy*