



Dormancy breaking and germination of *Prangos ferulaceae* seeds

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Abstract

The seed dormancy of *Prangos ferulaceae* (Apiaceae) has been studied with treatment the seeds by soaking, scarification, cold and warm stratification, alternating temperatures and GA₃. Our results showed that the seeds dormancy can be broken by cold stratification at 5°C and 12°C which induced germination up to 35 and 40%, respectively. Alternating temperatures (15/6°C) promote germination only to 15%. Scarification, soaking, warm stratification and GA₃ had no significant effects on seed germination. It has been found that light inhibits seed germination and the seeds have a negative photoblasticity. It has also been shown that cold temperatures promote the growth of the undeveloped embryo of the plant. After 10 weeks stratification of the seeds at 5°C, the embryo length increased 200%. In conclusion, it is obvious that, the seeds of *P. ferulaceae* have morphophysiological dormancy.

Keywords: Dormancy, *Prangos ferulaceae*, seed germination, stratification.

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INTRODUCTION

Prangos ferulaceae (L.) Lindl (Apiaceae) is a widespread species of *Prangos* genus that is distributed from east Europe to Turkey, Iran, Caucasia and central Asia (Rechinger and Hedge 1987). The plant has been used in Iran as a medicinal plant under the common name of Djashir. In traditional medicine, extracts of the roots and fruits of the plant have been used for the treatment of digestive disorders, healing scars and to stop bleeding in Iran, Turkey, India and different parts of caucasia and central Asia (Shikishima et al. 2001, Tada et al. 2002). On the other hand, the aerial parts of *P. ferulaceae* have high nutrient value and are used in different parts of Iran as an animal fodder. However, the number of wild plants has been declining rapidly due to the changing environmental conditions over the past decades and excessive harvesting of the wild plants from native fields by horticultural traders, herbalists and husbandries. To mitigate this undesirable situation, it is necessary not only to prevent future loss of the native plants, but also to produce them

horticulturally.

Almost all wild species, including *P. ferulaceae*, in the Apiaceae family have seed dormancy which has been a problem in plant production in greenhouses and farming condition. Seed dormancy in different plant families could be endogenous or exogenous in origin. In endogenous dormancies some characteristic of the embryo like endosperm, fruit walls or seed coats covering the embryo or embryo immaturity prevents germination. In exogenous dormancies some physical, chemical or mechanical factors such as impermeable seed coats, germination inhibitors and woody structures inhibit seed germination (Baskin and Baskin 2001). A previous report on germination of *P. ferulaceae* seeds indicates that there is a deep dormancy in the seeds of this plant (Modarres Hashemi 1995). It is obvious that different species of Apiaceae exhibit deep dormancy in

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seeds which is related to their undeveloped embryos (Baskin and Baskin 2001).

In order to domesticate and cultivate, we need information on seed germination and how to break the dormancy of this plant. The objective of this study was to investigate mechanism of seed dormancy, and to find methods to break dormancy for achieving rapid, uniform and high germination.

MATERIAL AND METHODS

Seed materials

Matured seeds of *Prangos ferulaceae* (L.) Lindl. (Apiaceae) were collected in August 2006 from Miyaneh located in the Northwest of Iran on the Eastern side of the Azerbaijan province (37° 40' 57'' N latitude and 47° 40' 54'' E longitude). After drying in open sunlight and removal of unwanted materials, the seeds were put into a paper box in the refrigerator at 4°C until required. The weight of 1000 seeds was 62 g.

In all treatments, quadruplicate samples of 25 seeds were randomly chosen. The seeds were sterilized by soaking in 1% sodium hypochlorite (NaOCl) for 5 min and then rinsed thoroughly with sterilized water prior to applying any treatment. The seeds were placed on Whatman No.1 filter paper moistened with 5 mL of distilled water in sterilized Petri dishes (Nadjafi et al. 2006).

Requirements for dormancy breaking and germination

The following effects were investigated for dormancy breaking and germination: Effects of different temperatures: In a preliminary experiment, after soaking, the seeds were incubated at 5, 10, 15, 20 and 25°C in an incubator for 3 weeks.

Effects of soaking: Seeds were soaked in distilled water for 24 h and they were placed in an incubator at 25°C for 3 weeks.

Effects of scarification: The pericarp around the seeds was removed with a sterilized scalpel without damaging the embryo. Then, the seeds were kept at 25°C for 3 weeks.

Effects of cold stratification and different temperatures: Seeds after being moisturized with distilled water were maintained at a temperature of 5°C in an incubator for 8 weeks. Then, in order to determine optimum temperature for germination, the stratified

seeds were kept at 5, 10, 15, 20 and 25°C in an incubator (Walck and Hidayati 2004).

Effects of duration and temperature of cold stratification: The seeds were separately stratified at 5°C and 12°C in an incubator for 4, 8, 12 and 16 weeks.

Effects of warm stratification: The seeds after being moisturized with distilled water were kept at 15°C in a incubator for 4,8,12 and 16 weeks.

Effects of alternating temperature: After being moisturized with distilled water, the seeds were maintained at 15/6°C in an incubator for 4, 8, 12 and 16 weeks.

Effects of GA₃: The seeds were placed on two sheets of Whatman No.1 filter paper and moistened with distilled water or 100, 200, 500 and 1000 ppm (mg mL⁻¹) of GA₃ dissolved in distilled water.

Effects of light and darkness: The seeds stratified at 5°C for 4,8,12 and 16 weeks, were placed in light (800 Lux) and darkness for 3 weeks.

After each treatment, except treatment 1, the seeds were transferred to an incubator. with continuous darkness, a constant temperature of 15°C, optimum temperature of germination and a relative humidity between 70% and 75%. Germinated seeds were counted and removed every 24 h for 3 weeks. A seed was considered germinated when the tip of the radicle had grown free of the seed coat. Finally, the percentage of germination was subjected to an analysis of variance.

Effects of cold stratification on embryo growth

Seeds were placed in an incubator at 5°C for 2, 4, 6, 8, 10 and 12 weeks. After each time course, ten seed were chosen and their embryos excised and measured. The same procedure was performed for alternating temperatures at 15/5°C. The seeds were also treated with 100, 200, 500 and 1000 mg mL⁻¹ of GA₃ for 2 weeks and their embryos excised and measured (Walck et al. 2002).

Statistical analyses

The means and standard errors were calculated for germination percentages and embryo lengths of seedlings. Data from this study was analysis using SPSS 11.5. After conducting an analysis of variance, the Duncan's multiple range test was used to

detect significant differences among the treatments with a probability of 95% ($p = 0.05$).

RESULTS AND DISCUSSION

Our results showed that in the seeds without any treatment, no germination occurred and different temperatures did not have an effect on seed germination. Thus, it is obvious that there is a deep dormancy in the seeds of *P. ferulaceae*.

Seed soaking and scarification did not have an effect on seed germination and that it appeared no chemical and mechanical factors were involved in the seed dormancy.

Cold stratification has promoted seed germination in *P. ferulaceae*. However, ANOVAs revealed highly significant differences in seed germination percentage at different temperatures after stratification. Maximum percentage of germination occurred at 15°C for 8 weeks as 40% (Fig. 1). Therefore optimum temperature of germination in *P. ferulaceae* was determined to be 15°C.

Seed germination of *P. ferulaceae* was also significantly affected by the duration and temperature of cold stratification. After a 4 week stratification at 5°C and 12°C, germination percentage reached 35% and 8%, respectively. On the other hand, while seed germination after 16 weeks of stratification at 12°C increased to 40%. The germination after stratification at 5°C decreased to 10% in same period (Fig. 2).

Warm stratification and GA₃ could not promote the seeds to germination. Our results also showed that the thermo period or altering temperature (15/6°C) was less effective in overcoming the dormancy than constant low temperatures. Treatment of seeds by altering temperatures (15/6°C) caused dormancy breaking and promoted seed germination up to 14% (Fig. 2).

According to the results given in Table 1, GA₃ has no considerable effects on the seed germination of *P. ferulaceae* and at low concentrations GA₃ exhibited no effects on the seeds. At the concentration of 1000 mg mL⁻¹, GA₃ induced the germination only by 4%.

Warm stratification could not promote the

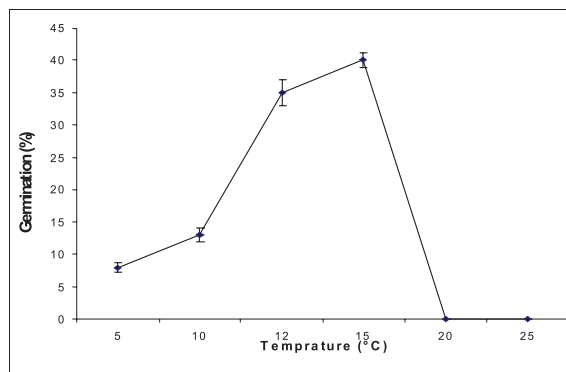


Fig. 1. The cumulative germination percentages (Mean ± SE) of *P. ferulaceae* seeds at different temperatures after 8 weeks of cold stratification (5°C).

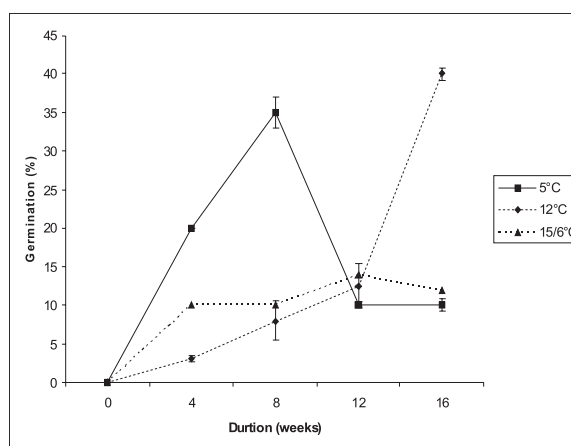


Fig. 2. The cumulative germination percentages (Mean ± SE) of *P. ferulaceae* seeds after cold stratification at 5°C, 12°C and alternating temperatures (15/6°C).

Table 1. The effects of light and darkness after cold stratification (5°C) periods on the germination of *P. ferulaceae* seeds.

Treatments	Stratification period (weeks)			
	4	8	12	16
Light	5 ± 0.4 ^a	8 ± 0.9 ^a	15 ± 1.1 ^a	45 ± 2.3 ^a
Darkness	0 ^b	0 ^b	3 ± 0.7 ^b	11 ± 0.9 ^b

Mean values in the same columns followed by the same letter are not significantly different at the 0.05 level according to the Duncan test.

seeds to germination and germination percentage was zero in warm stratified seeds.

Table 2 shows the effects of light and darkness on seed germination of *P. ferulaceae*. The data showed that after each cold stratification period, there was a significant difference in seed germination percentage in light and darkness. The light

Table 2. The effects of different concentrations of GA₃ on the germination of *P. ferulaceae* seeds.

Treatments	Concentration (g/mL)				
	0	100	200	500	1000
GA ₃	0	0	0	0	4±0.2

considerably reduced the germination of *P. ferulaceae* stratified seeds.

The measurement of embryos excised from the seeds showed that embryo length had increased 200% during the 10 weeks of cold stratification at 5°C. Whereas, the embryo lengths were 3.50 mm in untreated seeds and reached to 7.0 mm in seeds stratified at 5°C for 10 weeks (Table 3). Beside cold stratification, altering temperatures (15/6°C) significantly could induce embryo growth. After 10 weeks of treatment at 15/6°C, the embryo length reached 6.90 mm (Table 3). Based on our results, GA₃ did not promote embryo growth in *P. ferulaceae* seeds. The embryo length of treated seeds with different concentration of GA₃, did not display significant differences with the control seeds embryos. It also appears that *P. ferulaceae* has a linear rudimentary embryo (Fig. 3).

Our results tend to indicate that the effects of cold stratification on promoting germination are temperature and duration dependent. The *P. ferulaceae* seeds stratified at 5°C reach to optimum germination in a shorter time than at 12°C. It was also concluded that the seeds of *P. ferulaceae* have linear, undeveloped embryos (Fig. 3) that must grow from about 3 to 7 mm in length before the radicle emerges

from the seed. As it is shown in Table 3, maximum embryo elongation and optimum seed germination occurred together after approximately 2 months of cold stratification at 5°C. These results suggest that seed germination happens after the entire growth of the immature embryo at low temperatures. Because the embryo of *P. ferulaceae* is surrounded by endosperm, embryo growth and seed germination occurred during chelling and gibberlic acid did not substitute for stratification. The seed dormancy is of a deep complex morphophysiological type that can be broken only by relatively long periods of chilling (Baskin and Baskin 2001). This condition could be supplied readily in its natural habitat in winter where the mean temperatures are less than 10°C. This kind of dormancy has been previously reported from some species of Apiaceae like: *Heracleum*

**Fig. 3.** The embryos of *P. ferulaceae* seeds stratified at 5°C for different time courses.**Table 3.** The effects of duration of cold stratification (5°C) and alternating temperatures (15/6°C) on the embryo length of *P. ferulaceae* seeds.

Treatments	Duration(weeks)					
	0	2	4	6	8	10
Cold stratification	3.50±0.28 ^c	3.35±0.34 ^c	4.00±0.57 ^b	5.40±0.87 ^a	6.31±0.87 ^a	7.00±0.69 ^a
Alternating temperatures	3.50±0.28 ^c	3.12±0.32 ^c	3.90±0.51 ^b	5.1±0.74 ^a	6.25±0.80 ^a	6.90±0.76 ^a

Mean values in the same rows followed by the same letter are not significantly different at the 0.05 level according to the Duncan test

Table 4. The effects of different concentrations of GA₃ on the embryo length of *P. ferulaceae* seeds.

Treatment	Concentration (mg/mL)				
	0	100	200	500	1000
GA ₃	3.28±0.31 ^a	3.32±0.41 ^a	3.37±0.30 ^a	3.29±0.72 ^a	3.17±0.32 ^a

Mean values in the same rows followed by the same letter are not significantly different at the 0.05 level according to the Duncan test.

sphondyllum L. (Stockes 1953), *Osmorhiza aristata* (Thunb.) Rydb. (Walck et al. 2002), *Osmorhiza depauperata* Phil. (Walck and Hidayati 2004) and *Thaspium pinnatifidum* (Buckl.) Gray (Baskin et al. 1992). It also has been found in some species of Liliaceae and Ranunculaceae (Baskin and Baskin 2001).

At low temperature (<15), endosperm is disrupted permitting embryo growth. On the other hand, low temperatures stimulate the breakdown of proteins into soluble nitrogenous compounds and formation of the amino acids glycine and arginine, which are beneficial for embryo growth (Baskin and Baskin 2001).

The data obtained from the present work suggested that the germination of *P. ferulaceae* seeds in its natural habitats in the north west of Iran, Miyaneh must happen in late spring where the mean temperature reaches to 15°C, the optimum temperature for the germination of the species. However, dormancy breaking might occur in the winter where the mean temperatures are below 10°C.

Our finding have also shown that the seeds of *P. ferulaceae* displayed negative photoblasticity and light suppress germination of the seeds and there was previously pointed out in negative photoblastic seeds and phytochrome A control germination (Taiz and Zeiger 2002). Therefore, the seed germination occurs in darkness because continuous white light inhibits germination.

The comparison of our findings with those of Modarres-Hashemi (1995), demonstrated

that different genotypes of the plant that is distributed in various locations exhibit different seed germination percentages at same duration of cold stratification. This is consistent with the fact that seed viability and dormancy are genetic process that may be controlled by environmental factors. The degree of dormancy in a species that is depended to some factors like endosperm thickness could also be considered as a genetic character.

The dispersal of dormant seeds that need time to germination might have evolved as an ancient strategy to disperse germination over time. Seed dormancy could be also defined as a mechanism that causes germination to occur in favorable seasonal conditions to protect seedling from harmful environmental factors like drought and cold (Finch-Savage and Leubne-Metzger 2006). Thus, a diverse range of dormancy mechanisms has evolved in keeping with diversity of climates and habitats in which they operate. The history of temperatures on earth since the Devonian period has been characterized by a repeated increase and decrease. When those temperature shifts caused environmental stress such as low rainfall, seed dormancy may have developed in some species. There is an increasing gradient of seed dormancy from rain forest plants to plants of hot and cold deserts. The percentage of plant species with dormant seeds increased from 39% in tropical rain forests to 84% in hot deserts, and up to 99% of the plant species in the cold deserts (Baskin and Baskin 2001).

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***Prangos ferulaceae* Tohumlarında Dormansinin Kirilmesi ve Çimlenme**

Özet

Prangos ferulaceae (Apiaceae)'da tohum dormansisi; ıslatma, yaralama, soğuk-sıcak derecelendirmesi, değişken sıcaklıklar ve GA₃ uygulamaları ile çalışılmıştır. Sonuçlarımız tohum dormansisinin, sırasıyla %35 ve 40'a kadar çimlenmeye yol açan 5°C ve 12°C'de soğuk derecelendirmesi yoluyla kirilebileceğini göstermiştir. Değişken sıcaklıklar (15/6°C) sadece %15'e kadar çimlenmeye neden olmaktadır. Yaralama, ıslatma, sıcak derecelendirmesi, ve GA₃ uygulamalarının tohum çimlenmesi üzerine herhangi önemli bir etkisi yoktur. Işığın çimlenmeyi inhibe ettiği ve tohumların negatif fotoblastisitesinin olduğu bulunmuştur. Ayrıca, düşük sıcaklıkların bitkide gelişmemiş embriyo büyümesini teşvik ettiği gösterilmiştir. Tohumların 5°C'de 10 haftalık derecelendirilmesinden sonra embriyo uzunluğu %200 artmıştır. Sonuç olarak, *P. ferulaceae* tohumlarının morfofizyolojik dormansisinin olduğu açıktır.

Anahtar Kelimeler: Dormansi, *Prangos ferulaceae*, tohum çimlenmesi, derecelendirme.