Breaking of Seed Dormancy in *Prangos pabularia* and *Prangos uloptera* Growing in Iran

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Abstract: Background: The *Prangos* genus (Apiaceae) comprised of 30 species distributed from East Europe to China. In this study we study the seed dormancy breaking and germination of *P. pabularia* and *P. uloptera* that are most widespread species of the genus. In order to break the seed dormancy the following treatments, after seeds sterilizing, were performed: different temperatures, scarification, cold stratification, warm stratification, alternating low temperatures, GA₃ and light/darkness. After any treatment, germination percentage and rate were measured. On the other hand, the seeds embryos were excised and its length and morphology were studied. **Results and Conclusion:** The results of this study showed that seeds of studied plants have a deep dormancy that can be broken only by cold stratification. Optimal germination temperature was 5°C for *P. pabularia* and 10°C for *P. uloptera*. GA₃ could not substitute for cold stratification in dormancy breaking. Our finding also revealed that the seeds have undeveloped embryos (3-3.5 mm in length) at seed dispersal time growing approximately to 8 mm during cold stratification. Thus, it could be concluded that seed dormancy in the both *Prangos* species is deep, complex and morphophysiological which should be eliminated by a long period of chilling.

Key words: *Prangos pabularia*, *Prangos uloptera*, seed germination, cold stratification, morphophysiological dormancy

INTRODUCTION

Prangos Lindl is a perennial genus of the Apiaceae represented by 30 species in the world. *Prangos* species are widely used in folk medicine as tonic, anti flatulent, anti hemorrhoids and for treatment of intestinal worms and digestive disorders (Dokoric *et al.*, 2004) and treatment of leukoplakia (Shikishima *et al.*, 2001). *Prangos pabularia* Lindl and *Prangos uloptera* DC. are the most widespread species of the genus that are distributed from East Europe to central and Eastern Asia (Rechinger and Hedge, 1987).

Prangos pabularia has been used in Iran as medicinal plant under common names of Djashir ulofei and Badiane-Kohi. In traditional medicine, extracts of the roots and fruits of the plant has been used for treatment of digestive disorders, healing scars and stopping bleeding in Iran, India and different parts of Caucasia and central Asia (Tada *et al.*, 2002). On the other hand, the aerial parts of both *P. pabularia* and *P. uloptera* have high nutritive 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 changing environmental conditions over past decades and excessive harvesting of the wild plants from native fields by horticultural traders, herbalists and husbandries (Razavi and Hajiboland, 2009).

Earlier we studied seed germination and dormancy of *P. ferulaceae* indicate that there is a deep dormancy in the seeds of this plant (Razavi and Hajiboland, 2009). It is obvious that different species of Apiaceae exhibit deep dormancy in seeds that is related to their undeveloped embryos (Baskin and Baskin, 2001).

In order to domesticate and cultivate, we need information on seed germination, dormancy breaking and embryo growth of this plant that are investigated in the present study.

MATERIALS AND METHODS

Seed materials: Matured seeds of *P. pabularia* and *P. uloptera* were collected in August 2006 from Siahrood and Mishov dagh in East Azarbaijan province, Iran, respectively. After removal of unwanted materials, the seeds were put into a paper box in the refrigerator at 4°C until required. Weight of 1000 seeds was 21 and 28 g for *P. pabularia* and *P. uloptera*, respectively.

In all treatment, quadruplicate sample of 25 seeds were randomly chosen from seeds. The seeds were sterilized by soaking in 1% sodium hypochlorite (NaOCL) for 5 min and subsequently 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 break 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 week.

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 2, 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 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 30/15°C in a incubator for 4, 8 and 12 weeks.

Effects of alternating low temperatures: 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 8 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 5°C for *P. pabularia* and 10°C for *P. uloptera*, optimum temperature of germination in each species 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. The germination rate was calculated according to usual method (Zheng *et al.*, 2004).

Embryo morphology and effects of cold stratification on embryo growth: After any treatment, the seeds embryos were excised and were studied morphologically by binuclear. Then, 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 and observed. The seeds were also treated with 100, 200, 500 and 1000 mg mL⁻¹ of GA3 for 2 and 6 weeks and their embryos excised and measured (Walck *et al.*, 2002).

Statistical analyses: The means and standard errors were calculated for germination percentages, germination rate 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

It was described that after stratification, there is a significantly differences ($p \leq 0.05$) in seed germination of Prangos species in darkness and light in as much as in *P. pabularia* seed, the germination entirely was inhibited by light (Table 1).

Preliminary tests showed that without any treatment, seed germination was not occurred revealing a kind of deep seed dormancy might exist in the tested species. According to the experiments, in both species, constant temperatures, scarification and soaking of seeds had no effects on seed germination indicating there is no exogenous dormancy in the seeds and the seed dormancy might has endogenous origin.

The obtained results revealed that only stratification at low temperatures promote the germination of *P. pabularia* and *P. uloptera* seeds. The results also indicated that after stratification, the germination percentage and rate were significantly differed at various incubation temperatures ($p \leq 0.05$). Whereas, in *P. pabularia* seed, maximum germination occurred at 5°C, the optimum germination in *P. uloptera* was found to be at 10°C (Table 1). It is interesting to note that seed germination in both species was significantly affected ($p \leq 0.05$) by stratification temperature and duration. While, maximum seed germination in the both species occurred after 4 weeks of cold stratification at 5°C, in stratification at 12°C, maximum percentage and rate of germination occurred after 16 and 12 weeks for *P. pabularia* and *P. uloptera* seeds, respectively (Table 1).

Table 1: Treatments applied for dormancy breaking of tested seeds

Treatments	<i>P. pabularia</i>		<i>P. uloptera</i>	
	G.R.	G.P	G.R.	G.P.
Temperature (5°C)	0	0	0	0
Temperature (10°C)	0	0	0	0
Temperature (12°C)	0	0	0	0
Temperature (15°C)	0	0	0	0
Temperature (20°C)	0	0	0	0
Temperature (25°C)	0	0	0	0
Scarification	0	0	0	0
Soaking cool water (72 h)	0	0	0	0
Stratification (5°C,8 weeks)+2°C	4.2±0.80 ^a	30±1.7 ^a	1.8±0.62 ^{ab}	10±1.1 ^b
Stratification (5°C,8 weeks)+5°C	4.8±0.95 ^a	35±4.1 ^a	2.2±0.2 ^{ab}	13±2.5 ^b
Stratification (5°C,8 weeks)+10°C	3.3±0.54 ^{ab}	19±3.1 ^b	3±0.30 ^b	21±0.4 _c
Stratification (5°C,8 weeks)+12°C	2.3±0.51 ^{bc}	17±4.7 ^b	0.6±0.28 ^{ab}	5±2.8 _{ab}
Stratification (5°C,8 weeks)+15°C	3.2±0.61 ^{ab}	17±2.5 ^b	1.1±0.71 ^{ab}	5±2.7 _{ab}
Stratification (5°C,8 weeks)+20°C	0.3±0.13 ^d	3±0.6 ^c	0 ^a	0 ^a
Stratification (5°C,8 weeks)+25°C	1.2±0.52 ^{bc}	8±2.5 ^{bc}	0 ^a	0 ^a
Stratification (5°C,4 weeks)	10.2±0.59 ^a	51±2.5 ^a	5.4±1.11 ^a	42±3.4 ^a
Stratification (5°C,8 weeks)	4.8±0.90 ^b	35±4.1 ^b	5.4±1.20 ^a	41±3.4 ^a
Stratification (5°C,12 weeks)	3.3±0.71 ^b	30±4.0 ^b	4.6±0.81 ^b	26±4.2 ^b
Stratification (5°C,16 weeks)	4.1±0.78 ^b	38±3.8 ^{ab}	4.6±1.01 ^b	12±1.6 ^c
Stratification (12°C,4 weeks)	2.6±0.71 ^a	43±1.1 ^a	0.6±0.09 ^c	10±0.9 ^a
Stratification (12°C,8 weeks)	4.2±0.19 ^{ab}	35±1.2 ^b	1.8±0.72 ^{bc}	12.5±2.7 ^a
Stratification (12°C,12 weeks)	4.8±0.80 ^b	40±2.2 ^a	4.3±0.17 ^a	40±0.7 ^b
Stratification (12°C,16 weeks)	8.9±1.28 ^{ab}	65±3.5 ^c	3.2±0.62 ^{ab}	30±0.7 ^{ab}
Alternating temperatures (15/6°C,4 weeks)	1.7±0.61 ^a	30±1.1 ^a	0.7±0.03 ^a	12±0.5 ^a
Alternating temperatures (15/6°C,8 weeks)	2.4±.59 ^a	20±4.1 ^b	0.2±0.01 ^b	2.5±0.3 ^b
Alternating temperatures (15/6°C,12 weeks)	1.2±0.54 ^b	20±0.9 ^b	0.1±0.01 ^b	2.5±0.3 ^b
Alternating temperatures (30/15°C,4 weeks)	0	0	0	0
Alternating temperatures (30/15°C,8 weeks)	0	0	0	0
Alternating temperatures (30/15°C,12 weeks)	0	0	0	0
Stratification (5°C,8 weeks)+light	3.7±0.04 ^a	15±1.3 ^a	0 ^a	0 ^a
Stratification (5°C,8 weeks)+darkness	121.9±0.20	65±0.9 ^b	6.6±0.31 ^b	40±3.8 ^b
GA3 (100 ppm, 1 week)	0	0	0 ^a	0 ^a
GA3 (500 ppm, 1 week)	0	0	0 ^a	00 ^a
GA3 (1000 ppm, 1 week)	0	0	0.12±0.03 ^b	2±0.12 ^b

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

Table 2: Effects of duration of cold stratification (5°C) on embryo growth of *P. pabularia* and *P. uloptera*

Embryo length (mm)	Duration (Weeks)						
	0	2	4	6	8	10	12
<i>P. pabularia</i>	3.10±0.3 ^a	3.35±0.2 ^a	5.25±0.62 ^b	6.84±0.4 ^c	7.60±0.4 ^d	7.84±0.1 ^d	7.98±0.1 ^d
<i>P. uloptera</i>	3.16±0.1 ^a	5.05±.05 ^b	5.9±0.37 ^b	7.60±0.73 ^c	7.80±0.34 ^c	7.90±0.34 ^c	8.70±0.40 ^c

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

Table 3: Effects of different concentration of GA3 on embryo growth of *P. pabularia* and *P. uloptera*

Embryo length (mm)	Concentration (ppm)						
	0	100	500	1000	100	500	1000
<i>P. pabularia</i>	3.10±0.31 ^a	4.33±0.20 ^b	4.33±0.21 ^b	4.60±0.30 ^b	6.00±0.80 ^c	6.66±0.60 ^c	9.30±0.70 ^d
<i>P. uloptera</i>	3.16±0.16 ^a	3.93±.06 ^a	4.53±0.42 ^b	5.60±0.37 ^c	6.50±0.42 ^c	7.20±0.34 ^d	8.70±0.40 ^d

Treatment duration 2-6 weeks. Mean values in the same row followed by the same letter are not significantly different at the 0.05 level according to the Duncan test

The present findings suggested that altering temperatures (15/6°C) had less effective than cold stratifications in breaking the seed dormancy.

It was also described that GA3 and warm stratification could not overcome seed dormancy. However, at high concentration (1000 ppm) GA3 stimulated the radicle emergence of *P. uloptera* seeds only to 2% of control (Table 1).

The results of embryo studies showed that the species of *Prangos* have linear undeveloped embryos

with approximately 3 mm in length at seed dispersal period that reach to 7-8 mm during germination (Table 2). The present work showed that embryos length in *P. pabularia* reach from 3.10 to 7.98 mm during 12 weeks of cold stratification (5°C). This finding also revealed that in *P. uloptera*, embryos grew from 3.16 to 8.70 mm in length during same condition of cold stratification (Fig. 1a,b). This results indicated that GA3 can not substitute the cold stratification for breaking seed dormancy, although it stimulated embryos growth (Table 3).

Fig. 1 (a-b): The embryos of *P. pabularia* and *P. uloptera* seeds stratified at 5°C for different time courses

DISCUSSION

The results of present work revealed that seeds of *P. pabularia* and *P. uloptera* were dormant at the time of dispersal. The comparison of obtained data with classification of Baskin and Baskin (2001) indicated that the kind of seed dormancy in this plants was Morphophysiological (MPD). This kind of dormancy is related to presence of a undeveloped embryos surrounding by a thick endosperm that prevent from radicle emergence. At low temperatures (<15°C), endosperm is disrupted to yield nutrients such as glycine and argentine promoting embryos growth. When embryos grow enough to start splitting the endosperm and seed coats, the germination can be occurred (Baskin and Baskin, 2001).

Classification of the type of MPD requires an understanding of conditions necessary for embryos growth. The eight types of MPD are initially divided into two categories, simple and complex, on the basis of temperature at the time of embryo growth. Seeds with

simple MPD need relatively high temperatures (>15°C) for embryo growth whereas those with complex need low temperatures. Further classification of the complex type of MPD into non deep, intermediate or deep depends on the temperature regime required for dormancy break and whether GA3 substitutes for warm or cold stratification. Non deep complex MPD is broken by warm stratification followed by cold stratification, whereas intermediate complex MPD and deep complex MPD are broken only by cold stratification. GA3 substitutes for warm but not cold stratification in seeds with complex MPD and it overcomes dormancy in seeds with intermediate complex MPD but not in seeds with deep complex MPD (Baskin and Baskin, 2001; Walck *et al.*, 2002).

This study demonstrated that seeds of *P. pabularia* and *P. uloptera* have deep complex MPD because cold stratification at 5°C broke physiological dormancy and GA3 did not. In both species embryos growth are happen in low temperatures (<12°C) but germination occur at various temperatures. Optimal germination temperature is determined as 5°C for *P. pabularia* and 10°C for *P. uloptera* seeds. Although, GA3 stimulated embryos growth in both species, it could not entirely overcome seed dormancy.

It is also known that seeds of the both species of *Prangos* exhibit negative photoblasticity and light inhibit seed germination.

MPD has been previously reported from some species of Apiaceae like: *Heracleum sphondylium* (Stokes, 1953), *Osmorhiza aristata* (Walck *et al.*, 2002), *Osmorhiza depauperata* (Walck and Hidayati, 2004) and *Thaspium pinnatifidum* (Baskin *et al.*, 1992). It also has been found in some species of Liliaceae and Ranunculaceae (Baskin and Baskin, 2001). In a previous study we also reported that seed dormancy in *P. ferulaceae* was deep complex MPD as well as. We demonstrated that optimal temperature for seed germination of this plant was 15°C (Razavi and Hajiboland, 2009). It is well-known that different *Prangos* species are Iran-Turan elements distributing in semi-arid temperate regions mostly in Turkey, Iran, Caucasia and central Asia (Rechinger and Hedge, 1987). It is thus concluded that seeds of this species are prevented from germination after dispersal in late summer and autumn because they are dormant. Dormancy break in nature take place under the low temperatures of winter and germination occur in late winter and early winter in related to optimal temperature of seed germination in the species. In fact, MPD in some species of temperate regions is a evolutionary mechanism preventing germination when seedlings would be unlikely to survive. In the other words, it protect seedlings from summer drought and winter cold in semi-arid temperate climate.

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