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Seed dormancy breaking *of Heracleum persicum* L. a medicinal plant

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ABSTRACT

Heracleum persicum Desf. (Apiaceae) is one of most widespread species of the genus which is native in Iran, Iraq and Turkey. The whole plant is characterized by anise odor, and its fruits are used for the preparation of pickles as spices in Iran, where named as golpar. Seeds of *H. persicum* were sterilized by NaOCL1% for 5 min. Treatments as cold stratification (4°C), altering temperature (15/4°C), light/darkness and various concentration of GA_3 were subjected to seeds. After each treatment seeds were transferred to germinators with constant temperature of 25°C, and relative humidity between 75%. Germinated seeds were counted after 21 days. On the other hand, after each time course, ten seed were chosen and their embryos excised and measured. Our results showed that cold stratification at 4°C has induced seed germination in *H. persicum*. Maximum germination was occurred after 16 weeks of stratification up to 12.5%. Treatment of seeds by altering temperatures (15/4°C), caused dormancy breaking and promoted seed germination up to 5%. The results of embryos morphology showed that embryo length of *H. persicum* had increased approximately 500% during 16 weeks of cold stratification at 4°C. It was concluded that the seed dormancy of *H. persicum* is of deep complex morphophysiological type that can be broken only by relatively long period of chilling.

KEYWORDS

Heracleum persicum;
Seed dormancy;
Morphophysiological dormancy.

INTRODUCTION

In the flora of Iran, genus *Heracleum* (Apiaceae) consists of 10 perennial species which 4 of them are endemic and others are indigenous to Anatolia, Caucasus, Iraq and Iran[1].

Heracleum species are widely used in folk medicine as anticonvulsant[2] antipyretic, analgesic, diaphoretic[3], antiseptic and carminative[4], for rheumatic disease, lumbago, gastralgia[5] and in the treatment of hypertension[6], epilepsy[7], dysentery and diarrhea[8].

Heracleum persicum Desf. Is one of most widespread species of the genus which is native in Iran, Iraq and Turkey. The plant is most often 1-2 m in height. Each plant often has more than one steam, each sream is purple, 1.5 to 2 cm thick at the base with large even areas of purple to purple-red color at the base. Leaves are up to 2m in length and deeply incised with very sharp points, with 2-3 pairs of lateral leaf segments and less deeply serrate[1]. The whole plant is characterized by anise odor, and its fruits

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are used for the preparation of pickles as spices in Iran, where named as golpar[4].

A survey of literature revealed that roots of *H. persicum* contain useful natural products as isobergapten, bergapten, isopimpenellin, pimpinellin and sphonding[9]. In the last decades increased harvesting of the plant from natural habitats caused serious damage on its populations. The present study focused on seed germination and dormancy breaking of *H. persicum*

EXPERIMENTAL

Seed materials

The seeds of *H. persicum* were collected from 20 Km of Ardabil (Hir village), North west of Iran in September 2011. The plant was identified by the department of biology, faculty of sciences, University of Mohaghegh Ardabili. A voucher specimen has been deposited at the herbarium of the faculty of sciences, University of Mohaghegh Ardabili. 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. 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 Watman No.1 filter paper moistened with 5 ml of distilled water in sterilized Petri dishes[10].

Requirements for dormancy breaking and germination

Treatment of cold stratification: Seeds of *H. persicum* after moisturized with distilled water were maintained at a temperature of 4°C for 4, 8, 12, 16 and 20 weeks, separately[11].

Effects of warm stratification: Seeds after moisturized with distilled water were placed at 15°C for 4,8,12 and 16 weeks.

Effects of alternative temperature: after moisturized, seeds were maintained at 15/4°C for 4°C for 4, 8, 12, 16 and 20 weeks.

Effects of GA_3: Seeds were placed on two sheets of whatman No.1 filter paper moistured with distilled water or 100, 500 and 1000 ppm (mg/ml) of GA_3 dissolved in distilled water.

Effects of light and darkness: Seeds stratified at 5°C for 8 weeks, were placed in light (800Lux) and darkness.

After each treatment seeds were transferred to germinators with continuous darkness, constant temperature of 25°C, optimum temperature of germination and relative humidity between 70% and 75%. Germinated seeds were counted and removed every 24 h for 21 days. 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 at 4°C for 4°C for 4, 8, 12, 16 and 20 weeks. After each time course, ten seed were chosen and their embryos excised and measured[10].

Statistical analyses

Means and standard errors were calculated for germination percentages and embryo lengths of seedlings. Data from this study were analysis using SPSS 11.5. After conducting an analysis of variance, the Duncan test was used to detect significant differences among the treatments with a probability of 95% ($p= 0.05$).

RESULTS

The results of present work indicated that no germination occurred in the seeds of *H. persicum* without any treatment. Therefore, it is obvious that there is a certain dormancy in the seeds of *H. persicum.*

Our results showed that cold stratification at 4°C has induced seed germination in *H. persicum.* Increasing of cold stratification course caused significant effects on seed germination percentage at *p*d"0.05. After 12 weeks of the stratification, 7.5% of seed germination was recorded. Maximum germination was occurred after 16 weeks of stratification. No more germination was occurred with increasing of stratification period after 16 weeks TABLE 1.

Our results also showed that altering temperature

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TABLE 1 : Effects of duration of cold stratification and altering temperature on seed germination of *H. persicum*

Treatment	Course(weeks)					
	0	4	8	12	16	20
4°C	0 [e]	0 [c]	0 [c]	7.5± 0.20 [b]	12.5± 0.45 [a]	13± 0.48 [a]
15/4°C	0 [c]	0 [c]	0 [c]	2.5± 0.34 [b]	5± 0.25[a]	5± 0.16[a]

fied at 4°C for 16 weeks TABLE 2. It was also appeared that *H. persicum* has a linear rudimentary embryo Figure 1.

The alternated altering temperatures (15/4°C) promote embryo growth as well as cold stratification TABLE 2.

TABLE 2: Effects of duration of cold stratification and altering temperature on embryo growth of *H. persicum*

Treatment	Course(weeks)					
	0	4	8	12	16	20
4°C	1.13± 0.21 [e]	2.13± 0.25 [d]	3.38± 0.12 [c]	5.75± 0.25 [b]	6.63± 0.40 [a]	6.66± 0.58 [a]
15/4°C	1.13± 0.16 [e]	2.31± 0.05 [d]	3.90± 0.37 [c]	5.25± 0.73 [b]	6.13± 0.34[a]	6.10± 0.14[a]

*** 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**

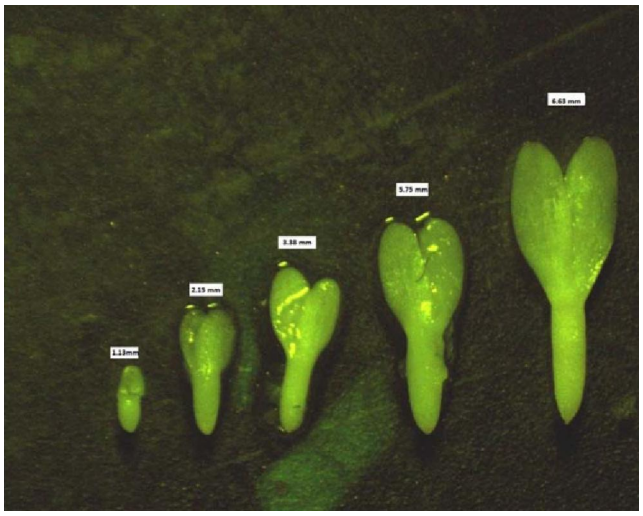

Figure 1 : Embryo growth of *H. persicum* after 0, 4, 8, 12 and 16 weeks of cold stratification in 4°C

(15/4°C) had less effective to overcome the dormancy than constant low temperatures. Treatment of seeds by altering temperatures (15/4°C), caused dormancy breaking and promoted seed germination up to 5% TABLE 1.

Warm stratification and GA_3 could not promote the seeds to germination.

There was a high significant differences in germination percentage between stratified seeds incubated in light and darkness as well as. Light stunted seed germination in *H. persicum.*

The results of embryos morphology showed that embryo length of *H. persicum* had increased approximately 500% during 16 weeks of cold stratification at 4°C. Whereas, embryo lengths was 1.13 mm in untreated seeds, it reached to 6.63 mm in seeds strati-

DISCUSSION

Dormancy is defined as a state in which the seed does not germinate although external conditions are suitable. Seeds of many plant species can remain buried in the soil for long periods of time with little damage, provided the conditions are favourable and these seeds are known as the seed bank. This allows the seeds to wait for favourable conditions and reduces the mortality of newly emerging seedlings[12].

It can be concluded from our results that seeds of *H. persicum* when released from the umbel, contain an underdeveloped embryo and almost all seeds are dormant and do not germinate in autumn. Before germination, a period of embryo growth and breaking of dormancy by cold and wet conditions is required. A period of two months at 2-4°C can be sufficient to break dormancy under experimental conditions; in the field, dormancy is broken during autumn and winter. Seed germination happen after entire growth of immature embryo at low temperatures.

Because embryo of *H. persicum* is surrounding by endosperm, embryo growth and seed germination occurred during cold and gibberllic acid did not substitute for stratification, The seed dormancy is of deep complex morphophysiological type that can be broken only by relatively long period of chilling[13]. This kind of dormancy has been previously reported from some other species of the *Heracleum* genus like: *H. sphondyllum*[14].

At low temperature (<15), endosperm is disrupted

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permitting embryo growth. On the other hand, low temperature stimulate the breakdown of proteins into soluble nitrogenous compounds and of formation of the amino acids glycine and argentine, which are beneficial for embryo growth[13].

Our finding have also shown that the seeds of *H. persicum* displayed negative photoblasticity and light suppress germination of the seeds. It has previously pointed out that in negative photoblastic seeds, phytochrome A control germination Therefore, the seed germination occur in darkness because continuous white light inhibits germination[11].

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