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Original Research Article

Evaluation of Different Techniques for Breaking Seed Dormancy of *Heliotropium europaeum* L. (Boraginaceae)

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ABSTRACT

Prediction of germination and emergence of weeds plays a key role in weed management strategies. For this reason, the seed dormancy status of *Heliotropium europaeum* (Boraginaceae) was investigated in North West of Iran. The dormancy breaking treatments were after-ripening, gibberellic acid, hot water, KNO_3, cold stratification, and washing. Seeds were strongly dormant at maturity and maintained high levels of dormancy for long periods of time. However, after 12 months storage at dry conditions, germination percentage increased to 37%. Washing and hot water treatments did not alter seed dormancy behavior, while the influence of gibberellic acid, KNO_3 and cold stratification was significant. The highest value for germination percentage obtained by cold stratification increased the value up to 90%. Results of seedling growth showed that compared to other treatments, the seeds treated by KNO_3 produced longer shoots and vigorous seedlings. The high vigor index was also recorded for cold stratification. It is concluded that the seeds of Heliotrope had a high primary dormancy and among treatments, the cold stratification was a more effective method in breaking seed dormancy .

Key Words: Dormancy, heliotrope, germination, seedling growth

INTRODUCTION

Heliotropium europaeum L., belonging to the Boraginaceae family, is a summer annual weed, which successfully grows on road margins, bare and cultivated fields in North West of Iran (Azerbaijan). Seeds germinate in the warm, moist conditions after late spring or early summer. In rotation crop systems, the fallow year provides a condition in which the population of this plant increases at a high rate because Heliotrope has a massive seeding potential and the seeds are viable for many years in the soil seed bank. The plant contains toxins (Pyrrolizidine alkaloids) that exist in its all parts (Saeedi and Semnani 2009). It can be used for medicinal purposes to treat warts, stimulate bile secretion, regulate menstruation, lower fever, and soothe insect bites, gouts, and inflammation in joints.

During seed development, the embryo gradually enters a quiescent phase due to desiccation. After dispersal from the mother plant, seeds may germinate under suitable conditions, which are specific for each species. However, in many circumstances the germination of a viable seed will not take place even though all the necessary environmental conditions for embryo growth are satisfied. This phenomenon is called seed dormancy. Seed dormancy brings some advantages to the plant. For instance, it provides enough time for seed dispersal over greater distances. It also maximizes seedling survival by the timing of germination under unfavorable conditions. Several external factors release the seed from dormancy, and dormant seeds typically respond to more than one condition. A common method used to release dormancy is after-ripening which is a period of dry storage at room temperature of freshly harvested, mature seeds (Finch-Savage and Leubner-Metzger 2006). Another useful method to overcome dormancy in hard seeds is the hot water. This treatment cracks the macrosclerid layer or affects the strophiolar plug (Francisco *et al.* 2001). This method is also effectively used to break dormancy in rice seeds (Tung *et al.* 2011) and Orka (Mohammadi *et al.* 2012). A stimulatory effect on germination of *Commelina benghalensis* L. seeds has been also reported by hot water at 70^{o}C (Kim *et al.* 1990). Soaking of *Nicandra physalodes* L. Seeds in preheated water at 50^{o}C for 10 minutes and incubated in the light at 25^{o}C did not affect the germination, but the same treatment followed by incubation in the dark instead of light significantly reduced the germination (Watanabe *et al.* 2002). In some species, the accumulation of germination

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inhibitors causes suppression on germination. If they are water soluble, repeated washing with water or soaking in tap water can remove chemical inhibitors and release their dormancy (Bewley and Black 1994). Abscisic acid (ABA) is a well-known compound in seed dormancy to which the embryo growth is very sensitive (Kermode 1995). Other than the above treatments, exogenous growth regulator treatments (i.e., gibberellins and cytokinins) have been found effective to break dormancy in many seed species (Koyuncu 2005; Bradford *et al.* 2008; Rita *et al.* 2010).

The studies of Hunt (2005) show that the seeds of *Heliotropium europaeum* are not dormant, while our primary study did not confirm his results. So, we checked the tests again with other seed lots and the results did not change. Therefore, these experiments are designed to overcome the possible seed dormancy of this plant.

MATERIALS AND METHODS

The seeds of *Heliotropium europaeum* used in this study are found throughout Maragheh and Bonab and occupy a range of semi-dryland habitats in East Azerbaijan province. Seeds were collected at maturity during the 2012 growing season. All seeds were air dried on the lab table at room temperature for approximately two weeks prior to the treatments. They were sorted by hand and air flowed to remove empty seeds in order to have a higher proportion of viable seeds in the experiments. Seed lot was separated to seven subsamples. One sample of seeds was stored at dry conditions during 12 months and was used for after-ripening tests. However, the other subsamples were immediately subjected to following seed dormancy breaking treatments: gibberellic acid, hot water, KNO_3, cold stratification and washing. Untreated seeds were used as the control.

The hot water treatment was performed through soaking the seeds in hot distilled water at 100^{o}C for one min. After the required soaking period, the seeds were removed and cooled. In cold stratification, seeds were placed between rolled filter paper moistened with distilled water and stored in a refrigerator at 2 ± 1˚C for 30 days. In the gibberellic acid treatments, seeds were immersed in solutions of GA 250 mg L-1 for 12 hours. With KNO_3 treatment, seeds were soaked in 1.5 percent KNO_3 solution for one hour. During the washing treatment control seeds were soaked in tap water for 30min then put under running tap water for 30 min. After treatments seeds surface were dried and considered for the germination and seedling test. Seeds were placed between moistened rolled papers and incubated at 22 ± 2˚C in the light. Both control and treated seeds were counted as germinated when the radicles were ≥ 2mm. Another test was carried out to evaluate seedling properties using the same protocol for germination test but the test finished after 14 days. At the end of seedling test, radicles and shoots were cut from the cotyledons and then length of radicles and shoots were measured and mean seedling lengths were determined. The dry weights of seeding properties were negligible, so these data are not mentioned in the means Table 1. The vigor index was calculated by following equation: Vigor Index = (Germination percentage × Seedling length).

Statistical Analysis

The statistical analyses of data, analyzed by SAS Ver. 9.1 software, included ANOVA and Duncan's multiple range test ($p<0.05$).

RESULTS AND DISCUSSION

There was a significant effect of treatments on seed germination percentage $p<0.05$. The treatments had different effects on seed germination (Table 1 and Figure 1). In comparison with control, washing and hot water treatments did not improve this trait. However, after-ripening, Gibberellic acid, KNO_3 and cold stratification remarkably released seed dormancy. Among after-ripening, Gibberellic acid and KNO_3 treatments differences were not significant, while cold stratification had significant differences with them. The highest germination percentage (93%) was recorded with cold stratification followed by KNO_3, after-ripening and Gibberellic acid, respectively.

Table 1. Means comparison of seed treatments effect on dormancy and seedling traits of *Heliotropium europaeum*.

	Germination percentage	Radicle length (mm)	Shoot length (mm)	Shoot to radicle ratio	Seedling length (mm)	Vigor index
After ripening	37 b	2.11 a	2.05 b	1.02 b	4.22 b	1.58 b
Control	0 c	--------	---------	----------	---------	--------
Gibberellic acid	35 b	1.48 b	3.20 a	2.36 ab	4.69 ab	1.68 b
Hot water	0 c	--------	---------	---------	---------	--------
KNo_3	41 b	1.57 ab	3.69 a	2.71 a	5.14 a	2.16 b
Stratification	93 a	1.11 b	2.44 b	2.23 ab	3.58 c	3.35 a
Washing	0 c	---------	---------	----------	---------	--------

Means with the same letter are not significantly different at $p<0.05$.

The results of seedlings derived from effective treatments revealed that radicals, shoot, and seedling lengths varied among treatments (Table 1). Seedling raised in after-ripening treatment had longer radicals than those of the other treatments. However, this treatment produced shortest shoot lengths among treatments. Therefore the shoot to root ratio decreased (Table 1). Among treatments, potassium nitrate had superior shoot length. The highest seedling length was also recorded for KNO_3 treatments. The calculation of vigor index showed that the cold stratification had the highest index among treatments while it was not statistically significant among other treatments (Table 1).

After a period of 12 months storage, a significant increase in seed germination was observed and after-ripening increased seed germination to 37%. It seems that fresh seeds gradually release from dormancy during storage time or the intensity of their dormancy decreases among the seed lots. This plant has indeterminate growth and, as long as it lives, continues to add new flowers. Consequently, seeds with different ages are formed on an inflorescence. Seed age has shown to be effective in overcoming seed dormancy. Oziegbe *et al.* (2010) reported a high germination percentage in six months old seeds of *Ludwigia adscendens* L. as compared to freshly shed seeds. *Triticum durum* L. (Grilli 1993), *Bromus tectorum* L (Christensen *et al.* 1996; Meyer 2004) and *Avena fatua* (Foley, 1994) caryopses have a relative dormancy, which is released during a period of after-ripening. Dry after-ripening probably changes growth inhibitors content or growth promoter contents in the seed embryo, inducing the seed to germinate (Baskin and Baskin 2001). It has been suggested that during dry after-ripening, gene expression (ß-1,3-glucanase transcription and translation) in *Nicotiana tabacum* L. (Solanaceae) seeds may be altered (Leubner-Metzger 2005). GA_3 broke the seed dormancy and 35% of seeds began to germinate. Gibberellins have been shown to break dormancy in numerous seed types (Bewley and Black 1994; Debeaujon *et al.* 2007). Debeaujon and Koornneef (2000) showed that dormancy and germination are the consequence of a balance between many stimulating and inhibiting factors, including GA and ABA. Exogenous application of GA_3 can alter the balance in favor of the stimulus. So, germination processes have been probably started with a high level of GA_3. The results show that seeds of common heliotrope germinate with 1.5% KNO_3 treatment. This finding is in agreement with Bian *et al.* (2013) findings which showed seed germination of *Sorbus pohuashanensis* improved with KNO_3 treatment. Numerous inorganic compounds are present in the soil matrix, however, only nitrate and nitrite significantly influence dormancy state (Baskin and Baskin 2001). Although, the application of KNO_3 is a simple treatment in many seed-testing laboratories, however a clear explanation for its action has not been studied.

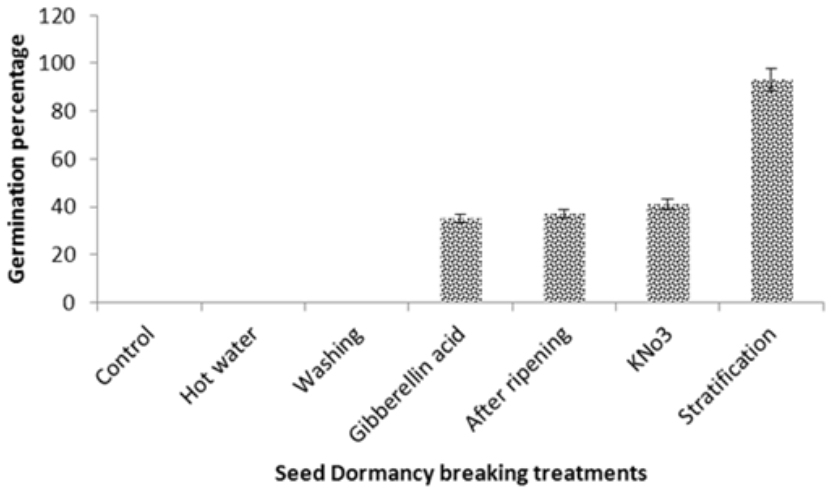

Figure 1. Effects of treatments on seed germination of Heliotrope

Hot water did not overcome seed dormancy in Heliotrope. Though, this treatment has been reported to be effective for removing seed dormancy of wild Vigna species (Wang *et al.* 2011) and *Gymnocladus assamicus* (Choudhury *et al.* 2009). The ability of hot water to release seed germination appears to be depending on species, treatment duration and the availability and requirement of light after treatment. The experiment shows that the water content of Heliotrope seeds raise within first hour after immersing in water therefore the physical (seed coats hardness) dormancy cannot be a limiting factor for this plant (water content of seeds raised up to 50% during 30 min.). Hot water treatment is commonly used to release the seed coat imposing dormancy. Repeated washing of seeds did not improve their germination. In contrast to Hunt (2005) findings in Heliotrope, however, no evidence of leachable germination inhibitors was detected. Probably in our study, the given time for washing was not sufficient to remove the leachable germination inhibitors. The cold stratification vigorously broke dormancy status of Heliotrope and germination percentage increased to 93%. The findings of the current study are consistent with those of Koyuncu (2005) and Pipinis *et al.* (2011). These findings further support the idea of Baskin and Baskin (2001) which suggested dispersed seeds in autumn, commonly require cold stratification to stimulate germination. Dormancy would have been ecologically important for common heliotrope. It would inhibit germination of fresh seeds in late summer and early autumn when environmental conditions are unfavorable for successive plant growth and development.

CONCLUSIONS

We found that seeds of *Heliotropium europae* L. had a high level of dormancy at the fresh harvesting stage. It would be ecologically beneficial for summer annual weeds in North West of Iran, because it prevents fresh seeds from germinating at the end of the growing season when the low or freezing temperature would kill establishing seedlings during winter. Seed treatment with dry after- ripening, Gibberellic acid, KNO_3 and cold stratification can be effective in releasing dormancy and the most effective method to break seed dormancy was cold stratification.

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