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Does physicochemical pre-treatments can alleviate germination and dormancy of *Sophora alopecuroides* seeds?

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ABSTRACT

A factorial experiment in completely randomized design was conducted with three replications in 2012 at the University of Maragheh, Iran, to evaluate the different pre-treatments effects on germination of Sophora seeds. To release the physical dormancy, seeds were exposed to: mechanical scarification; sulfuric acid (65%) for 15 min; sulfuric acid (65%) for 30 min. To examine the existence of physiological dormancy, Seeds were tested with following solutions in the seed bed; distilled water; saturated soil extract solution ; KNO_3 (34 mg\L); Gibberllic acid (20 ppm) and KNO3 (34 mg\L)+ GA3 (20 ppm). The results showed that Germination percentage (GP), Seedling fresh weight (SFW), Seedling dry weight (SDW), Grain fresh weight (GFW), Grain dry weight (GDW) and Resource remobilization (PM) were significantly affected by scarification treatments whereas the influence of media solutions on mentioned traits were not statistically significant. Among the scarification treatments, sulfuric acid (65%) for 30 min improved seed germination percentage from 2.22% to 81.66%. However, the rest treatments had a negligible effect on the germination percentage in comparison with control. Sulfuric acid scarification enhanced seedling properties and the highest values were obtained from sulfuric acid (65%) for 30 min. In conclusion, it seems that the primary control of germination in sophora seeds is located in the seed coats and scarification with sulfuric acid (65%) for 30 min can easily eliminate these limitations and seed dormancy.

INTRODUCTION

Sophora (*Sophora alopecuroides* L.) is perennial legume (Fabaceae) drought-resistant species that is widely distributed in south-west and East Asia, Greece, and south Russia, and it was used as a Chinese traditional medicine and biotic pesticide being rich in alkaloids, flavonoids, and triterpenoides especially in the seeds (Wen Hu et al. 2009). Among the Sophora speciese roots of S. flavescens, S. tonkinensis, and S. alopecuroidesare widely used for the treatment of some skin and gynaecological diseases such as eczema, dermatitis, and colpitis, as well as fever, sore throat, and inflammation (Küçükboyaci et al. 2011). Sophora species are known to contain quinolizidine alkaloids (QAs) as their principal bioactive constituents, which have been shown to exhibit sedative, analgesic, antipyretic, anti-inflammatory, anti-tumour and notableantiviral activities (Kinghorn and Balandrin 1984; Choudhary et al. 2000). QAs are characteristic secondary metabolites of the family Leguminosae and are especially abundant in the tribes Genisteae ,Sophoreae, and Thermopsideae (Wink 1993). QAs also play a chemical defensive role against herbivores and pathogen micro-organisms (Wink 1988; 1992).

Seeds possess remarkably complex and effective protective mechanisms to insure their long survival. Such prolonged survival of the embryo within the seed ensure dispersal, distribution, or spread species over long distance (Nikolaeva 2004). Seed germination may be delayed, for example, by environmental conditions or seed characteristics. The term of seed dormancy generally has been used to describe inactive conditions that resulting from internally imposed germination blocks such as seed coat, dormancy and embryo dormancy (Finch-Savage and Leubener-Metzger 2006).

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flavonoids have important functions in the induction of seed coat-imposed dormancy, as well as in seed longevity and quality (Debeaujon et al. 2000) and legume seed coats possess a wide assortment of flavonoids (Baskin and Baskin, 2004). Most species of Fabaceae produce seeds with physical dormancy which was broken by some environmental factors in the field. The impermeable seed coat is considered the main problem in establishing legume species (Smith et al. 2003). Seed coat-imposed dormancy, known as "hard seededness", is an ecological mechanism that allows the seed to germinate only when conditions are favorable for supporting seedling growth; however, it represents a limitation when prompt and high-level germination is required (Argel and Paton 1999). Therefore, seeds require pre-treatments before sowing to obtain rapid, uniform and high germination rates (Teketay 1996; Huang and Gutterman 2000).

Several pre-sowing treatments have been used to overcome hard seed coat–imposed dormancy (Goslan and Gutterman 1999; Rachel and Galatowitsch 1999) and to increase the permeability of the seed coat to water. Different pre-sowing treatments such as cold stratification, mechanical disruption, or acid and hot water treatments are widely used because they can improve germination within a relatively short period. Hot water treatment can enhance germination of dormant hard-coated seeds by elevating the water and O_2 permeability of the seed coat (Hermansen et al. 1999; Aydın and Uzun, 2001). Some treatments have been proposed for overcoming hard seededness and improving germination rate in legume species (Ibanez and Passera 1997; Sy et al. 2001). Acid treatments are often used to break down especially thick impermeable seed coats. The temperature of the acid and the length of the time the seeds are soaked are very important. In addition to the hard seedness (mechanical resistance), the seed coat or embryo could have some chemical inhibitors that play a key role at germination. For instance, abscisic acid has been found to be the most predominant inhibitor in several species (Baskin and Baskin 2004). Gibberellins and potassium nitrate have been found to release dormancy of several plant species, both as seed coat, dormancy and embryo dormancy (Bewley 1997).

At the germination stage, gibberellin caused activation of some enzymes that involved in germination. KNO3 was reported as an effective agent for reducing light requirement and enhancing germination (pupala and fowler 2003). Potassium nitrate may be helpful for reactivation of metabolic process of seeds. This compound may cause biosynthesis of auxin, which ultimately triggers the growth of embryo (khan et al. 1999).

In the present study we investigated the germination response of Sophora seeds to different seed treatments. Understanding these factors is crucial for successful prevention and control of this specie in weed management at cropping systems.

MATERIAL AND METHODS

Seeds of sophora were collected from research farm of University of Maragheh in October 2012. Seeds dried for two days in open sunlight and moisture content were decreased to eight percentage in wet basis then immature seeds (discolored, wrinkled and cracked seed coats, and broken seeds) or unwanted materials was removed. The experiment was arranged as factorial based on completely randomized design (CRD) design in three replications. To release the physical dormancy (factor A), seeds were exposed to one of the following treatments: (1) untreated (control); (2) mechanical scarification (massive abrasion of the seeds between two sheets of sandpaper; (3) immersion in a concentrated (65%) sulfuric acid for 15 min; (4) immersion in a concentrated (65%) sulfuric acid for 30 min. After treatments, seeds were rinsed three times with tap water for two min. then seeds were air-dried to reach initial moisture levels. To investigating the presence of endogenous dormancy in seeds (factor B), treated Seed were tested with following solutions in seed bed; (1) distilled water; (2) saturated soil extract solution that collected from growing location of sophora (3) KNO3 (34 mg\L); (4) Gibberellic Acid GA3 (20 ppm); (5) KNO3 (34 mg\L)+ GA3 (20 ppm). The germination test was carried out by placing 30 seeds between rolled papers and then incubating in a dark germination chamber at 20˚C for a total of 14 days.

At the end of experiment germination percentage (GP), Seedling fresh weight (SFW), Seedling dry weight (SDW), Grain fresh weight (GFW) , Grain dry weight (GDW) and PM (resource remobilization percentage) were recorded.

For the statistical analysis, the data of germination percentage was transformed to $\arcsin\sqrt{x + 0.5}$. Appropriate analysis of variance to the experimental design was conducted, using SAS software. Means of each trait were compared according to Duncan multiple range test at $p \leq 0.05$. Excel software was used to draw figures.

RESULT AND DISCUSSION

The germination percentage of sophora seeds was affected by pre-germination treatments that were applied for physical dormancy releasing, but treatments for endogenous dormancy had not significantly effect on germination (table 1). Therefore, it was resulted that sophora seed dormancy was attributed to hard seed coat. Among the scarification treatments, soaking the seeds in H_2SO_4 (65%) for 30 min resulted in the highest germination percentage (81.66%) that had a significantly difference in comparison with the rest treatments, whereas there were not significantly different among other treatments (table 2). Treated seeds with sulfuric acid in 30 min had more germination percentage than treated seeds with sulfuric acid in 15 min. It is possible that sulfuric acid application for 15 min cannot enough time to penetrate in hard coat of treated seeds. Acid scarification (H_2SO_4) increased germination and this has been reported for many other species with impermeable seeds. For example, Treating Sesbania rostrata seeds with H_2SO_4 for 30 min increased germination from 12 to 94 % (Sarker et al. 2000). The positive response of seeds to the scarification treatments (H_2SO_4) suggested that the hard testa is responsible for the low percentage germination of untreated seeds by preventing imbibition of water (Balouchi and Modarres Sanavy 2006). Kelly and Van Staden (1986) reported that sulfuric acid scarification of Aspalaths linearis seed for 120 min altered the seed coat structure, extensively damaging cuticle, macrosclereid, osteosclereid, hilar, strophiolar and cotyledon layers, finally reduced their impermeability and increased their germination by 100 percentage.

Mechanical scarification had not significantly different with control on germination. Although a negligible increases was observed in comparison with control (table 2). These finding in contrast with Patane and Gresta (2006) that showed, hand scarification with sandpaper was the best treatment for dormancy releasing. Travlos et al. (2007) also reported that abrasion of the seed with sandpaper increased significantly the germination and emergence percentage and was the most rapid scarification treatment. The beneficial effect of mechanical scarification on seed germination is common at many hard-coated perennial legumes widespread in arid and semi-arid zones (Sy et al. 2001).

Mobilization of carbohydrate reserves under H2SO4 for 30 min was the highest among the scarification treatments and control. This treatment showed a high carbon supply for new developing seedling which may impact seedling vigor and led to better seedling growth.

The interaction of scarification treatments × media solutions only for germination percentage was significant ($p \leq 0.05$) (table 1). Means comparison of

Table 1. Analysis of variance for studied traits

	DF	GP	SFW	SDW	GFW	GDW	PM
Scarification(S)	3	22937.8**	0.128**	0.0004**	0.0007**	0.0003**	0.012**
Media(M)	4	56.76	0.03	0.0001	0.00001	0.0000026	0.00021
S*M	12	53.79*	0.024	0.00003	0.000008	0.0000021	0.00014
E	40	24.107	0.0199	0.00007	0.000009	0.000003	0.0001
CV (%)		21.32	25.3	1.2	10.44	9.09	1.44

* Significant at the 0.05 level. ** Significant at the 0.01 level. Different letters indicating significant difference at $p< 0.05$; Germination percentage (GP), Seedling fresh weight (SFW), Seedling dry weight (SDW), Grain fresh weight (GFW) , Grain dry weight (GDW) and resource remobilization percentage (PM).

Table 2. Mean comparisons of germination characteristics under different seed pre-treatments

		GP	SWF	SWD	WGF	WGD	PM
Scarification treatments	Control	2.22 b	0.044b	0.003bc	0.024c	0.022b	11b
	H_2SO_4-15	4.66 b	0.14a	0.006ab	0.025bc	0.023b	11b
	H_2SO_4-30	81.66a	0.12a	0.008a	0.039a	0.014c	17a
	Mechanical scarification	3.55 b	0.02b	0.002c	0.027b	0.024a	12b
Solutions	Control	22.49	0.062	0.0041	0.029	0.0214	13
	Soil extract	25.41	0.15	0.006	0.029	0.0212	13
	KNO3	24.44	0.08	0.006	0.029	0.0210	13
	GA3	23.05	0.08	0.007	0.027	0.020	12
	KNO3+GA3	19.72	0.04	0.003	0.029	0.0218	13

Different letters indicating significant difference at $p< 0.05$; Germination percentage (GP), Seedling fresh weight (SFW), Seedling dry weight (SDW), Grain fresh weight (GFW) , Grain dry weight (GDW) and resource remobilization percentage (PM).

interaction between scarification treatment and media solutions indicated that the highest and the lowest germination percentage were belong to compound treatment of (H2SO4 for 30 min + soil extract solution) and control, respectively (figure 1). There was not significantly difference for the rest interaction of scarification treatments × media solutions observed for germination percentage. Therefore, it seems likely that the primary control of germination in sophora seeds resides in the seed coats and saturated soil extract
action is prompted by a previous seed coat disruption.

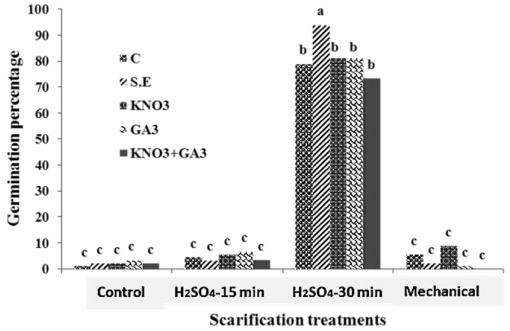

Figure 1. Interaction between (scarification treatments × media solutions) on seed germination of *Sophora alopecuroides*

Seedling fresh weight (SFW), Seedling dry weight (SDW), Grain fresh weight (GFW), Grain dry weight (GDW) and PM were significantly affected by scarification treatments (table 1). SFW, SDW, GFW and PM under H_2SO_4 for 30 min were higher than that of the other treatments, but SFW and SWD under different timing immersion in $H_2SO_4$was not significantly different (table 2). It seems H_2SO_4 for 30 min treatment has effectively affected seed coat structure so water imbibition has promoted. Consequently, the germination processes have done in early stages and it is reasonable that an enough time is caused better growth for seedlings.

Although all solution treatment increased studied traits values in compare to the control but these effects were not statically significant (table 1, table 2). The general low response to GA3 and nitrate may indicate that this species are unresponsive to these treatments or that the concentration tested was not optimal which was confirmed by the present study. Also, the lack of GA3 effectiveness in stimulating seed germination might be referred to the following possibilities: a negative effect of GA3, on the level of some enzymes activity (glutamate-oxaloacetate transaminase, pyruvate kinase and malate dehydrogenase) and consumption of nucleotides in the synthesis of nucleic acid (EL-Dengawy 1997) and/or the production of a proteinaceous germination inhibitor. Further, GA3 is effective in breaking the non-deep physiological dormancy, but it does not overcome the deep physiological dormancy (Baskin and Baskin 1990).

CONCLUSION

In conclusion, the present work has established an effective strategy for breaking seed dormancy and enhancing seed germination of sophora through acid scarification. The success of acid scarification showed that the seed possessed physical dormancy and the physiological dormancy is negligible in this plant.

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