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Evaluation of seed dormancy breaking methods in *Astragalus parrowianus*

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ABSTRACT: Seed dormancy is the most important limiting factor in domesticating plants.Therefore, a main research priority is to break seed dormancy for the aforementioned purpose. An experiment was performed to study the effects of scarification, gibberellic acid and cold stratification on seed germination of *Astragalus parrowianus* in Seed Science and Technology Laboratory, Faculty of Agriculture, IAUM, Iran, during 2012. A factorial experiment with a complete randomized design carried out with three replications. Factors included two levels of scarification (with and without scarification), four levels of cold stratification at 4 °C (0, 7, 14 and 21 days), and three levels of gibberellic acid (0, 500 and 1000 ppm). The highest germination percentage was obtained by the interaction between scarification and cold stratification at 7 or 14 days. Although cold stratification can intensify the effect of scarification, it was not effective alone. Applying gibberellic acid had no effect. Based on our results, the seeds were dormant via a water-impermeable seed coat, requiring mechanical scarification to imbibe water and to germinate.

***Keywords*:** Cold stratification, Germination, Gibberellic acid, Scarification, Seed dormancy

INTRODUCTION

Astragalus species are one of the largest genuses of plant belonging to the legume family, Fabaceae, subfamily Faboideae. More than 1000 species of *Astragalus* are found in Iran (Gharreman, 1988). They are used in the pharmaceutical industry as an anti-HIV and anti-cancer compound and medical industry as a natural gum and an herbal medicine (Du, 2003; Rios and Waterman, 1997). A proprietary extract of the dried root of A. membranaceus, called TA-65, was associated with a significant age-reversal effect in the immune system (Harley, 2011). Moreover, a major active constituent of A. membranaceus, astragaloside, were extracted and are being investigated to prevent the development of hypertension (Zhang, 2011). *A. parrowianus* is one of the important species of this genus, growing in Iran. *A. parrowianus* is a long-lived perennial spreading by roots and seed. The roots are woody, coarse and deeply penetrating. The stems are semi-short, 10-100 cm long and grow in dense clumps close to the ground. The stems and leaves are covered with dense, long, compressed whitish, grayish or silvery hairs which give a woolly appearance (Maassoumi, 2000). In domestication of a wild plant, one of the problems is seed dormancy. Many factors can influence the seed dormancy. It is well established by experimental evidence that there is a water- or gas-impermeable seed coat or there is a chemical inhibitor into seed coat in Fabaceae, creating a major obstacle to germinate (Foley,2001; Finch and Leubner, 2006). This impermeable coat prevents the seed from taking up water or gases. Therefore, the seed is prevented from germinating until dormancy is broken (Baskin and Baskin, 1998).

Therefore, previous studies have shown that germination in the legume family, called hard seed, is accelerated by de-hulling via sulfuric acid or scarifying via sand paper. This type of dormancy is known as physical dormancy. Previous studies have shown that scarification with sand paper was the best treatment for breaking physical

dormancy of this genus; for instance, *A. fridae* (Arbabian, 2009), *A. tribuloides* (Fateh, 2006), and *A. hamosus* (Patanè and Gresta, 2006). In the other hands, many studies have shown the cold stratification and gibberellic acid are effective for overcoming seed dormancy of this genus; for instance, *A. membranaceus* (Wang, 2009) and *A. cyclophyllos* (Keshtkar, 2008). So far, not enough data are available to break seed dormancy of *A. parrowianus*; therefore, the objective of this research was to determine the best treatment for overcoming its dormancy.

MATERIALS AND METHODS

This research was carried out as a factorial experiment in a completely randomized design with three replications in Seed Science and Technology Laboratory, Faculty of Agriculture, Islamic Azad University of Mashhad in Iran, during 2012. The experimental factors included scarification at two levels (with and without scarification), cold stratification at 4 °C with four levels (0, 7, 14 and 21 days), and gibberellic acid (Sigma-Aldrich company of German) at three levels (0, 500 and 1000 ppm). The seeds of *A. parrowianus* were collected from plants in the fields of Toroq village in Esfahan, Iran at 2011. The seeds were disinfected with sodium hypo chloride (5%) for a period of 2 min, and then dried after washing with distilled water. Scarification treatment was done by a sand paper. To treat cold stratification, the seeds were soaked in distilled water for a period of 30 min and then placed in moist paper towels at 4 °C. They were kept in a designated time period. To treat gibberellic acid, the seeds were kept in gibberellic acid solution at designated concentrations for a period of 8 h. After treatment, 20 seeds were placed in placed in 10 cm diameter Petri dishes on top of two layers of Whatman filter paper and were added ten ml of distilled water to them. Then, the Petri dishes incubated for 48 h at 25±1 °C. Daily observations were made on the number of seeds germinated in each dish until the number of germinated seeds stabilized. Seeds were considered to have germinated when the emerging radicles were over 2 mm long**.** Then, germination percentage was evaluated. The data were subjected to analyze of variance using in SAS software. Mean comparison was performed with Duncan's test at the 0.05 probability level. Figures were drawn by Microsoft Office Excel software.

RESULTS AND DISCUSSION

The results of analysis of variance are presented in Table 1.

Table 1. Different traits analysis of variance of Astragalus parrowianus seeds

Source of variation	Degree of freedom	Germination%
Scarification	1	91378/12**
Stratification	3	2503/93**
GA	2	166/72**
Stratification×Scarification	3	2826/90**
Scarification×GA	2	122**
Stratification×GA	6	74/20**
Stratification×Scarification×GA	6	25/77^{ns}
Error	48	19/72
CV(%)		9/88

* and **significant at 5% and 1% level respectively ns not significant

The germination percentage was significantly influenced by scarification, cold stratification and scarification × cold stratification (Table 2). The highest percentage of germination was recorded in scarification × cold stratification at 7 days (95%) and 14 days (97%). There is no difference between these two treatments, while they were significantly difference to compare with other treatments. The lowest percentage of germination was obtained by 0 and 21 days of stratification and no scarification (10%).

Table 2. Interaction between scarification and stratification on seed germination of *Astragalus Parrowianus*

Scarification	Cold stratification time (day)			
	0	7	14	21
Non scarification	10 d	9 d	7 d	10 d
Scarification	45 c	95 a	97 a	85 b

Means by same letters showed not significant differences

Results showed that scarification, improved seed germination of *A. parrowianus.* This finding was in agreement with the results of Fateh (2006) who found that scarification by sand paper led to an increase in germination of *A. tribuloides*. This result can be related to altering the seed coat to make it permeable to water and gases, especially oxygen and carbon dioxide, resulting in improvement at germination percentage (Baskin and Baskin, 1998). Simple effect of stratification was not significant on germination in this species, whereas the previous studies have reported that cold stratification was effective to germinate the seeds of other genus in this plant family such as *Medicago rigidula* and *Medicago polymorpha* (Balouchi, 2008). Therefore, our study has clearly shown that the best treatment for improving germination of this species was scarification; thus, cold stratification had merely a booster effect on it. This result was consistent with findings reported by (Eisvand, 2006) working with *A. siliquosus*. They stated that up to 95% of seed were germinated by scarification, whereas up to 98% of seed were germinated by a combination of scarification and cold stratification.

Based on the results, applying gibberellic acid at each two concentrations with or without scarification had no effect on germination in this species, showing a physical dormancy. Nonetheless, gibberellic acid was effective for overcoming seed *A. cyclophyllos* (Keshtkar, 2008).

The interaction of scarification and gibberellic acid showed that the highest germination percentage was obtained by scarification with no GA (Table 3).

Table 3. Interaction between scarification and GA on seed germination of *Astragalus Parrowianus*

Scarification	Gibberellic acid (ppm)		
	0	500	1000
Non Scarification	9 c	9 c	8 c
Scarification	85 a	76 b	79 ab

Means by same letters showed not significant differences

The interaction of stratification and gibberellic acid showed that the highest germination percentage was obtained by 21 days cold stratification with no GA (Table 4). In these conditions, gibberellic acid had not any positive or negative effect on germination. Moreover, the lowest germination percentage was obtained by 0 days cold stratification × 500 ppm gibberellic acid, showing a negative effect due to gibberellic acid.

Table 4. Interaction between stratification and GA on seed germination of *Astragalus Parrowianus*

Cold time (day)	Stratification	Gibberellic acid (ppm)		
		0	500	1000
0		32 d	22 e	27 de
7		51 ab	52 ab	52 ab
14		52 ab	51 ab	52 ab
21		55 a	45 bc	43 c

Means by same letters showed not significant differences

Conclusion

The results of this study showed that scarification was the best treatment to improve germination of *A. parrowianus* seeds. Although cold stratification can intensify the effect of scarification, it was not effective alone. Gibberellic acid with or without scarification had not any impact on germination of *A. parrowianus* seeds. Therefore, based on Baskin and Baskin (1998), dormancy in seeds of *A. parrowianus* is undoubtedly of kind of physical dormancy due to an impermeable seed coat.

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