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Effect of Different Treatments on Improving Seed Germination Characteristics of *Astragalus adscendens* and *Astragalus podolobus*

Ali Tavili[A], Mahsa Mirdashtvan[B], Rezvan Alijani[C], Masoud Yousefi[D], Salman Zare[E]

[A]Associate Prof., Natural Resources Faculty, University of Tehran, Iran (Corresponding Author),
Email: atavili@ut.ac.ir
[B]Ph.D. Student of Watershed Management, Gorgan Agricultural and Natural Resources Sciences University, Gorgan, Iran
[C]M.Sc. in Watershed Management
[D]Senior Expert of Research, Education and Extensions Organization of Jihade Agriculture Ministry, Tehran, Iran
[E]Ph.D. Student of Combating Desertification, Natural Resources Faculty, University of Tehran, Iran

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Abstract. Nowadays, in many rangelands, due to over grazing, and problems that some plants have in germination, the rate of forage production is greatly reduced. So to take advantages of the benefits of such plants, it is necessary to identify and remove barriers of germination and establishment of suitable plants. The present study was designed to investigate different treatments of breaking dormancy and stimulating seed germination of *Astragalus adscendens* and *Astragalus podolobus* and offer the most effective treatment to enhance germination and growth rate. The experiment was conducted using Completely Randomized Design (CRD) with four replications. Treatments included hot water for 5 minute, 30% hydrogen peroxide for 5 minute, 98% sulfuric acid for 5 minute and chilling at 4°C for 10 days. Data were collected for seed germination percentage, Germination Rate (GR) and Mean Germination Time (MGT). Based on the results, treatment with 98% sulfuric acid for 5 min for *A. adscendens* and hot water for 5 min for *A. podolobus* were the best treatments to break dormancy and stimulate seed germination, so that the germination percentage increased from 44% to 82% in *A. adscendens* and from 28% to 62% in *A. podolobus*. In *A. adscendens* 98% sulfuric acid reduced the amount of MGT from 8 days for control treatment to 5 days. In *A. podolobus* hot water treatment was more effective on MGT reduction and reduced it from 7 to 5 days. Although sulfuric acid had the highest effect in breaking dormancy, but its application in a vast scale is not easy, therefore the hot water was suggested as the substitution treatment.

Key words: Seed dormancy breaking, Seed germination, Germination rate, *Astragalus adscendens*, *Astragalus podolobus*

Introduction

Iran with the wide diversity of climate and abundant plant genetic resources is known as one of the richest country in terms of national resources and natural talents. Iran also has the world's most important source of Astragals growth and over 804 species exist in Iran flora (Masumi and Ghahramani, 2006).

Forests and rangelands are often considered by producing timber and forage, but the by-product of this part of natural resources has a significant economic role (Ebrahimi-e- Rastaghi, 1981). Due to the diversity in species, Astragals are valuable from various aspects of medicine, industry, sand dune fixation and forage production, so that some of its barb species like *Astragalus adscendens* has industrial aspects and manna is obtained from it (Masumi and Ghahraman, 2006).

Astragalus is known in English language as Milk vetch, Astragal and Tragacanta and has Greek root of Astrone (means star) and Coala (means latex) that was used for the first time by Diuscurid. As it was referred, numerous species of Astragal have been used for a variety of purposes which two of them are *A. adscendens* and *A. podolobus*. The species of *A. adscendens* is as a shrub, perennial plant has woody stems to a height of 0.4 to 1.5 m, which is the best wood that is extremely favorite tribe and why it exists in the habitat that migrate along or adjacent to the bungalow, Astragal tribes have been faced with a serious threat (Jouri and Mahdavi, 2009).

A. podolobus is a half-shrub plant, perennial with a height of 20 to 40 cm, sometimes 70 cm. This is the most valuable species without thorns that are observed in the rangelands. Its wild hay has a high palatability and is frequently grazed by sheep and goats (Jouri and Mahdavi, 2009).

Despite all the features mentioned above, for plant regeneration from seed, its hard shell is one of the main obstacles in plant germination and reproduction. The main reason of being the hard seed relates to the physical and chemical characteristics of the coat of the seed (Tran and Cavanagh, 1984); so it seems that the force of water uptake and growth is not sufficient for the splitting of the seed coat and germination (Ghaderi *et al*., 2008). Previous studies have shown that remove the thick crust wall is caused rapid germination (Baskin and Baskin, 1998).

Levitt (1974) have explained that the seed coat scarification with concentrated sulfuric acid in seed dormancy, due to lack of water penetration into the skin, causes accelerated germination. Aliero (2004) also reported that scarification of seed to break seed dormancy in seeds of *Parkia biglobosa* was effective. Tavili *et al.* (2010) expressed that chilling at 4°C for 10 days has had a positive impact on increasing and improving germination of two species of *Descurainia sophia* and *Plantago ovata*.

Zare *et al.* (2011) stated that scarification with sandy paper and 98% sulfuric acid treatments are the most effective treatments to break dormancy and improve germination in two species of *Prosopis koelziana* and *Prosopis juliflora* and the treatment of 98% sulfuric acid at 15 min gave the best results.

Jabarzare *et al.* (2010) evaluated pre-chilling effect at 5°C on *Artemisia sieberi* seeds germination and seedling establishment. Their result showed positive effect of pre-chilling.

Ansari *et al.* (2013) tested heat shock treatment on improvement of seed germination characteristics in *Secale montanum* under aging conditions. The results were different based on the used temperature.

According to the results of above researches, in the most cases, seed scarification by sulfuric acid rated concentrated due to destruction of the seed coat and the sclerotic cells, allow

water to penetrate for the process of dewatering and remove the dormancy that is caused by the lack of water penetration to the testa.

Due to problems in the natural germination of *A. podolobus* and *A. adscendens* seeds, in this study the effects of different treatments on the germination characteristics of these plants and finding the most effective germination treatment, have been investigated.

Materials and Methods

Seeds were provided from "Pakan Bazr" company in Isfahan, Iran. After ensuring the presence of seed dormancy due to the thick crust, a Completely Randomized experimental Design (CRD) with four replications was done in order to break dormancy and increase germination percentage. Treatments were included scarification with 98% sulfuric acid for 5 min, scarification with hot water for 5 min, chilling at 4 °C for 10 days and scarification with 30% hydrogen peroxide for 1 hour.

After treatment of the seeds and disinfection Petri dishes with 98% neutral spirits and distilled water, 10 seeds were placed on a layer of filter paper with distilled water humidifier and then Petri dishes were transferred to the germinator with 20±1°C temperature and humidity of 70%. The germinated seeds counting started from the second day and continued until the end of experiment on a daily basis. Finally the attributes seed germination percentage, speed and the time of germination were calculated.

The mean germination time and the speed of germination (germination rate) were calculated using (Equations 1 and 2), respectively (Ellis & Roberts, 1981).

$$MGT = \frac{\sum D.N}{n} \qquad \text{(Equation 1)}$$

Where

MGT= Mean germination time

N= The number of seeds that have germinated in the D-Day

n= The total number of germinated seeds

D= The number of days from beginning the germination

$$GR = \frac{1}{MGT} \qquad \text{(Equation 2)}$$

Where

GR= Germination Rate

MSTAT-C Software, ANOVA and Duncan's multiple range test at 5% level were used for data analysis and comparing the means of data.

Results

Analysis of variance of understudy characteristics in *A. podolobus* and *A. adscendens* seeds are given in Table 1.

The influence of treatments on germination showed that chilling, 98% sulfuric acid and hot water have increased germination percentage in *A. adscendens* species, while the 30% hydrogen peroxide treatment has no significant effect. 98% sulfuric acid for 5 min was the best treatment, which increased the germination percentage from 44% to 82%; also among chilling, hot water and hydrogen peroxide treatments, no significant difference was observed.

Table 1. Analysis of variance of measured properties in seeds of *Astragalus adscendens* and *A. podolobus*

Sources of Variation	DF	MS		
		Germination %	Mean Germination Time	Germination Rate
Species	1	0.068^{ns}	0.087^{ns}	0.001^{**}
Treatments	4	0.162^{*}	2.551^{ns}	0.002^{**}
Species × Treatments	4	0.119^{*}	4.786^{ns}	0.005^{**}
Error	30	0.041	1.002	0.001

*, **, ns=significance at 1%, 5% levels, and non significant, respectively

from 28% to 62%. Among the chilling, sulfuric acid and hot water treatments, there is no significant difference; hydrogen peroxide treatment also has no significant effect on germination percentage (Fig. 1).

The results *A. podolobus* showed that chilling, 98% sulfuric acid, hot water and hydrogen peroxide treatments had positive effects on increasing germination percentage however; the hot water treatment was the most effective, so that the germination percentage increased

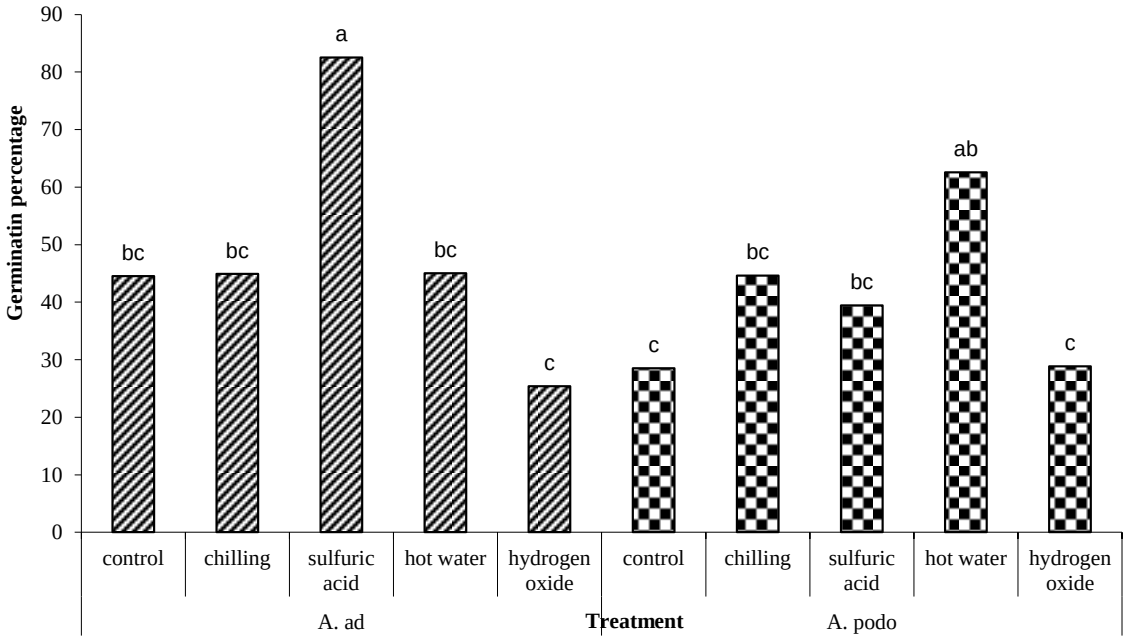

Fig. 1. Comparison of the effect of different treatments on seed germination percentage of *A. adscendens* and *A. podolobus*
The means of the treatments with same letters were not significantly different based on DMRT P< 0.05

98% acid sulfuric and hot water treatments reduced MGT in *A. podolobus* species; however hot water treatment was more effective on MGT reduction and reduced it from 7 to 5 days. Meanwhile, hydrogen peroxide treatment had no significant effect on MGT (Fig. 2).

In *A. adscendens*, all treatments caused reduction of the mean germinating time. The best treatment was 98% sulfuric acid which reduced the amount of MGT from 8 days for control treatment to 5 days. No significant difference was observed among chilling, hot water and 30% hydrogen peroxide treatments. Chilling,

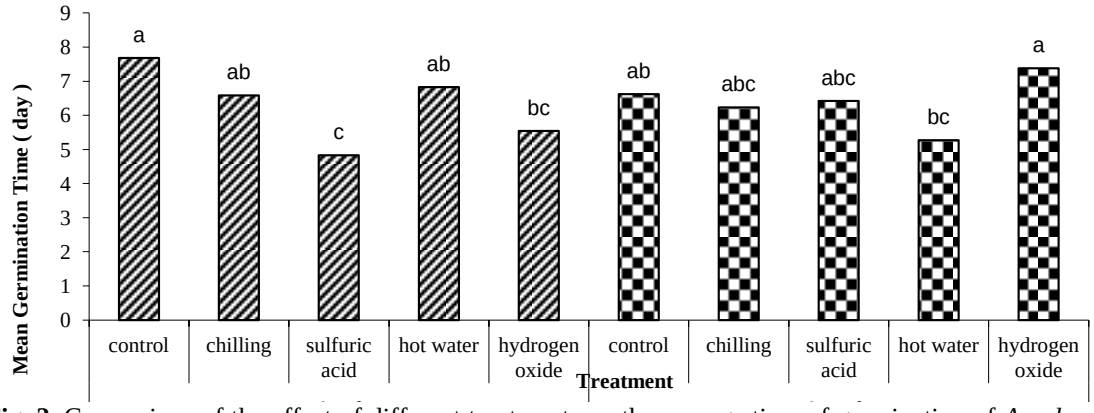

Fig. 2. Comparison of the effect of different treatments on the average time of germination of *A. adscendens* and *A. podolobus*
The means of the treatments with same letters were not significantly different based on DMRT P< 0.05

In *A. podolobus*, all treatments except to hydrogen peroxide were effective on increasing germination rate; although hot water for 5 min was the most effective one; however no significant difference was observed among hot water, sulfuric acid and chilling treatments (Fig. 3).

The last parameter of investigation was Germination Rate (GR). The results of study on *A. adscendens* species showed that all treatments increased germination rate. The sulfuric acid treatment was the most effective treatment; also between hydrogen peroxide and sulfuric acid treatments, and between hot water and chilling, no significant difference was observed.

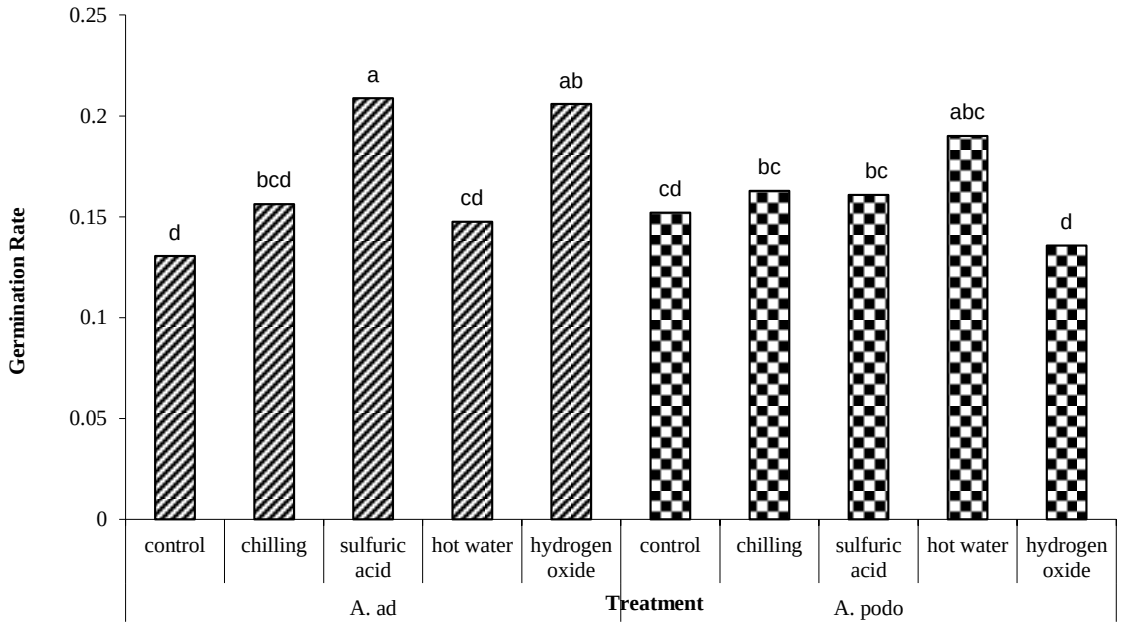

Fig. 3. Comparison of the effect of different treatments on seed germination rate of *A. adscendens* and *A. podolobus*
The means of the treatments with same letters were not significantly different based on DMRT P< 0.05

Discussion

Plant propagation using seeds is the easiest and cheapest way for cultivation; however; physical dormancy due to hard seed coat acts as a barrier to the uptake of water and exchange of gases from its surface.

The results of determined treatments showed that the best treatment for breaking dormancy in two species of *A. adscendens* and *A. podolobus* are were 98% sulfuric acid for 5 min and hot water for 5 min, respectively. These findings were in agreement with the investigation results of Maddah Arefi *et al*. (2004) on breaking dormancy of some tropical plants and species of the Leguminosae family which had physical dormancy. To break dormancy in these species respectively hot water and sulfuric acid treatments were appropriate. Another study by Isvand *et al*. (2005) showed that the application of sandy paper scarification and chilling treatments, resolved more than 98% of seed dormancy in *Astragalus siliquosus*. As it is observed in Fig. 1, in the two studied species especially *A. adscendens*, sulfuric acid increased the germination percentage in comparison with control treatment. In a research done by Isvand *et al*. (2005) the role of sulfuric acid in breaking

dormancy of *Dendrostellera lessertii* had been mentioned. They announced that among the used treatments, the maximum germination obtained when 95% sulfuric acid was applied for 5 min. It seems that due to existence of hard and thick testa in *A. adscendens* seeds, imbibition is done rarely and seeds are not able to germinate, but using sulfuric acid results the reduction of seed coat thickness and leads to entrance of water to the seed which in turn causes initiation of germination process (Tavili, 2012). Sulfuric acid due to destruction of seed coat and scheloroid cells allows water to penetrate for the dewatering process and removes the seed dormancy due to lack of water penetration into the shell. Such a mechanism could be considered for hot water effect on *A. podolobus* seeds. Treating with hot water causes the plant cord to open and the seeds germinate.

The results obtained from current study demonstrate that understudy species dormancy could be categorized as mechanically and is related to exogenous dormancy. Despite this, Fateh (2005) found that dormancy of *A. tribuloides* seeds is endogenous and stratification is the best treatment for dormancy breaking. This shows that different species in *Astragalus* genus show different behavior for dormancy and dormancy breaking.

In general, treatment with acid or hot water resulted increase of seed permeability with elimination of cord seed valve in understudy species, but it should be noted that scratching is not always beneficial because medium hard shell seeds of forage plants in the natural environment is useful for the survival (Jouri & Mahdavi, 2009).

It was concluded , sulfuric acid had the higher effect in breaking dormancy, but its application in a vast scale is not easy, therefore the hot water could be considered as the substitution treatment.

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بررسی اثر تیمارهای مختلف بر بهبود ویژگی‌های جوانه زنی بذر گونه‌های *Astragalus adscendens* و *Astragalus podolobus*

علی طویلی[الف]، مهسا میردشتوان[ب]، رضوان علیجانی[ج]، مسعود یوسفی[د]، سلمان زارع[ن]

[الف]دانشیار دانشکده منابع طبیعی دانشگاه تهران (نگارنده مسئول)، پست الکترونیک: atavili@ut.ac.ir
[ب]دانشجوی دکترای آبخیزداری دانشگاه علوم کشاورزی و منابع طبیعی گرگان
[ج]کارشناس ارشد آبخیزداری
[د]کارشناس ارشد سازمان تحقیقات، آموزش و ترویج کشاورزی
[ن]دانشجوی دکترای بیابان زدایی دانشکده منابع طبیعی دانشگاه تهران

چکیده. با توجه به وجود فشار چرای دام و مشکلات ناشی از جوانه‌زنی برخی گیاهان مهم مرتعی، تولید این گیاهان در عرصه‌ای مرتعی کاهش یافته است. به منظور تقویت حضور این دسته از گیاهان در مراتع، شناسایی موانع جوانه‌زنی و رفع آنها جهت استقرار موفقیت آمیز حایز اهمیت است. تحقیق حاضر نیز بر همین اساس و به منظور یافتن موثرترین راه‌های شکست خواب و بهبود جوانه‌زنی دو گونه مهم دارویی-صنعتی یعنی ***Astragalus adscendens*** و ***Astragalus podolobus*** انجام شد. ویژگی‌های جوانه زنی مورد مطالعه شامل درصد جوانه‌زنی، سرعت و زمان جوانه‌زنی بود. تحقیق بر اساس طرح آزمایشی کاملا تصادفی و در چهار تکرار انجام شد. تیمارهای مورد استفاده در این تحقیق عبارت است از: آب جوش (۵ دقیقه)، پرکسید هیدروژن ۳۰٪ (۵ دقیقه)، اسید سولفوریک ۹۸٪ (۵ دقیقه) و سرمادهی در دمای ۴ درجه سانتیگراد به مدت ۱۰ روز. بذور دو گونه مورد مطالعه نسبت به تیمارهای مورد استفاده واکنش متفاوتی نشان دادند بطوری که بذور گونه ***A. adscendens*** با کاربرد اسید سولفوریک ۹۸٪ و بذور گونه ***A. podolobus*** با کاربرد آب جوش بهترین حالت ویژگی‌های جوانه زنی مورد مطالعه را نشان دادند. درصد جوانه زنی در نتیجه تاثیر اسید سولفوریک در بذور گونه ***A. adscendens*** از ۴۴ به ۸۲ درصد و در بذور گونه ***A. podolobus*** در نتیجه تاثیر آب جوش از ۲۸ به ۶۲ درصد افزایش یافت. نتایج بدست آمده نشان داد که اگرچه اعمال تیمار اسید سولفوریک موجب افزایش جوانه‌زنی شد ولی بعلت مشکلات ایمنی در کاربرد آن بنابراین آب داغ بعنوان روش جایگزین توصیه می‌شود.

کلمات کلیدی: شکست خواب بذر، درصد جوانه‌زنی، سرعت جوانه‌زنی، ***Astragalus adscendens***، ***Astragalus podolobus***