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Full Length Research Paper

Methods to break seed dormancy of *Astragalus cyclophyllon*

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The aim of this study was to enhance the germination rate of *Astragalus cyclophyllon* seeds which have a very low germination rate under normal conditions. The seeds were soaked for 72 h in 100, 200, 300, 400 and 500 ppm gibberellic acid (GA_3) solution, H_2SO_4 concentrations (50 and 98%) at two treatment times (5 and 10 min) and 60, 80 and 100ºC hot water at two treatment times (5 and 10 min) before placing in Petri dishes. The fresh seeds (non-stratified) of *A. cyclophyllon* had 55% germination. Analysis of variance indicated that both GA3 and H_2SO_4 concentrations had significant effects on seed germination and final germination percentage. The highest germination percentage (81%) was obtained when the seeds were treated with 500 ppm GA_3. The results showed that hot water treatments are not useful methods for breaking the seeds dormancy.

Key words: Chemical stratification, gibberellins, mechanical scarification.

INTRODUCTION

Astragalus is one of the biggest Angiospermous genera with about 1600 species widely distributed in the Old and the New World. More than 550 species of the genus *Astragalus* grow in Iran (Mozaffarian, 1996). *Astragalus cyclophyllon*, is native to Iran, and is the sole species in its genus, which is mainly found at 1700 - 2600 m elevation in mountainous and plain regions with an average annual precipitation of 200 - 500 mm (Parsa, 1984).

Seed germination studies are key tools in conservation programs because they can be used for management programs and species reintroduction (Ortega-Base and Rojas-Arechiga, 2007). Over the past 30 years, dormancy has been widely studied but the regulatory principles behind changes in several types of dormancies remain unclear. To accelerate breaking seed dormancy, hormones have been applied in several studies (Zigas and Coombe, 1977; Mehanna et al., 1985; Chang and Sung, 2000; Keshtkar et al., 2008). Gibberellic acid (GA_3) is one of the hormones proposed to control primary dormancy by inducing germination (Iglesias and Babiano, 1997). Plant growth regulators such as GA, chemicals such as sulfuric acid (Nadjafi et al., 2006; Rahnama-Gahfarokhi and Tavakol-Afshar, 2007) and mechanical scarification such as hot water (Hermansen et al., 1999) have been recommended to break dormancy and enhance germination. The objectives of this study were to determine the effect of exogenous applied GA_3, sulfuric acid and hot water for breaking the dormancy of *A. cyclophyllon*.

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MATERIAL AND METHODS

Seed collecting and processing

The seeds of *A. cyclophyllon* were collected using random sampling technique from 7 local government areas of Isfahan province in Iran. Seeds were separated from the undesired materials and unripe seeds on arrival at the laboratory and dry stored in a sealed plastic box at 5ºC with 10% seed moisture content.

Seed viability and germination testing

Viability of 4 replicates of 25 seeds/species was assessed using the tetrazolium chloride (TZ) staining technique (ISTA, 2003). Seeds were initially hydrated on plain agar for 24 h at room temperature before being scarified (away from the embryo axis) and placed in TZ solution at 30ºC and darkness for 24 h. Seeds were then cut in

half and examined. Only uniformly stained red/dark pink embryos were considered 'viable'.

The seeds were surface sterilized by soaking in 5% sodium hypochlorite (NaOCl), solution for 5 min and subsequently rinsed thoroughly with sterilized water prior to applying any treatment. Germination experiments were conducted using four replications of 25 seeds per treatment. Seeds were placed on double layered Whatman No.1 filter paper moistened with 10 ml of distilled water in sterilized Petri dishes with 15 cm diameter. All dishes were sealed with a trip of parafilm to reduce water loss. Treatments were as follows:

Chemical stratification

In the chemical stratification treatment, a set of seeds were immersed in two sulfuric acid (H_2SO_4) concentrations (50 and 98%) and two treatment times (5 and 10 min) were used. Thereafter, the seeds were rinsed several times in clean distilled water and tested for germination.

Gibberellins treatment

GA_3 (SIGMA, Germany, 90%) was mixed with distil water and made to different concentrations. The seeds were soaked in six GA_3 concentrations (0, 100, 200, 300, 400, 500 PPM) in light and at room temperature for 72 h.

Mechanical scarification

In the Mechanical scarification treatment, seeds were treated in 60, 80 and 100ºC hot water bath and two treatment time (5 and 10 min). Then the seeds were left in the water overnight (for 10 h) while it gradually cooled down to room temperature.

Germination

After each treatment, seeds were transferred to a germinator with alternate light/darkness (12h each) and temperatures of 20 and 8ºC respectively, and relative humidity of 70 to 75%. Germinated seeds were counted and removed every 24 h for 60 days. A seed was considered germinated when the tip of the radicle had grown free of the seed coat (Wiese and Binning, 1987; Auld et al., 1988).

The germination rate was calculated according to Wiese and Binning (1987) as follows:

$Gr = \Sigma$ (number germinating since n-1)/ n

Where: Gr = germination rate; n = the days of incubation.

Statistical analysis

At first, the raw data were tested in SAS software (SAS Institute, Cary NC, USA) for normality and the root square transformation method was employed for data transformation. Then the data were analyzed using analysis of variance (ANOVA) and the Duncan's multiple range test ($P < 0.05$).

RESULTS

Seed viability of *A. cyclophyllon* was 87% according to TZ staining. All treatments had a significant effect on the germination rate of the seeds ($P < 0.01$).

Chemical stratification

Soaking seeds in different H_2SO_4 treatments showed significant differences in seed germination ($P < 0.01$). However, no significant difference in germination observed between seeds treated with H_2SO_4 concentration (50%) at two times (5 and 10 min). The application of H_2SO_4 treatments promoted seed dormancy breaking; H_2SO_4 concentration of 98% for 10 min increased the germination percentage to 71% (Table 1).

GA_3 treatments

The response to GA_3 was depending on the concentration of GA_3 and a significant difference in germination was observed among seeds treated with various concentration of GA_3. As a whole, different GA_3 treatments conspicuously increased germination rate and percentage. At lower concentration (100 ppm) increase in germination were low (about 3%) and at higher concentrations, germination was increased by about 26 to 81% (Table 1).

Mechanical scarification

The results of the ANOVA showed that hot water treatments had a significant impact ($P < 0.01$) on the examined germination. Application of hot water in 100ºC for 10 min gave 41% germination which is lower than non-stratified seeds germination. However, seed treatment with 80ºC water for 5 min gave 64% germination (Table 1).

DISCUSSION

The strong inhibitory effect of the seed coat on seed germination may be caused by several possible mechanisms, including mechanical constraint, prevention of water and oxygen uptake, and retention or production of chemical inhibitors (Taiz and Zeiger, 2002). The integument breaking or softening, for instance, is needed to remove dormancy imposed by seed coat hardness or impermeability. However, it is very difficult to use mechanical scarification to break the hard seed coat of *A. cyclophyllon*. Therefore, chemical scarification (softening the hard seed coat with concentrated H_2SO_4) was used to remove exogenous dormancy. In the present study, it was found that a significant number of *A. cyclophyllon* seeds that had been treated with H_2SO_4 germinated (Table 1). The response to H_2SO_4 as a method for breaking seed dormancy was consistent with other studies (Hermansen et al., 2000; Nadjafi et al., 2006; Rahnama-Gahfarokhi and Tavakol-Afshar., 2007).

Endogenous gibberellins have been widely studied in relation to the breaking of seed dormancy in various species. GA_3 has been exogenously applied as a substi-

Table 1. Mean comparison for different traits in GA, hot water and sulfuric acid treatments for breaking the dormancy of *Astragalus cyclophyllon*.

Treatment	Means	
	Germination (%)	Germination rate
Gibberellic acid		
100 ppm (GA)	58 ± 0.03d*	0.84 ± 0.04 e
200 ppm (GA)	62 ± 0.03 d	1.01 ± 0.04 d
300 ppm (GA)	72 ± 0.03 c	1.17 ± 0.04 c
400 ppm (GA)	76 ± 0.03 b	1.37 ± 0.04 b
500 ppm (GA)	81 ± 0.03 a	1.46 ± 0.04 a
Control	55 ± 0.03 d	0.81 ± 0.04 e
Hot water		
60ºC (5 min)	55 ± 0.02 bc	0.83 ± 0.03 c
60ºC (10 min)	57 ± 0.02 b	0.90 ± 0.03 b
80ºC (5 min)	63 ± 0.02 a	1.09 ± 0.03 a
80ºC (10 min)	52 ± 0.02 c	0.81 ± 0.03 c
100ºC (5 min)	54 ± 0.02 bc	0.83 ± 0.03 c
100ºC (10 min)	41 ± 0.02 d	0.68 ± 0.03 d
Control	55 ± 0.02 bc	0.81 ± 0.03 c
Sulfuric acid		
H_2SO_4 50% (5 min)	59 ± 0.03 c	0.92 ± 0.03 d
H_2SO_4 50% (10 min)	61 ± 0.03 bc	0.97 ± 0.03 c
H_2SO_4 98% (5 min)	63 ± 0.03 b	1.04 ± 0.03 b
H_2SO_4 98% (10 min)	71 ± 0.03 a	1.15 ± 0.03 a
Control	55 ± 0.03 d	0.81 ± 0.03 e

Mean values in the same column followed by the same letter are not significantly different at the 0.05 level according to the Duncan test.

tute for stratification and has increased germination in many plant species, including *Leucospermum* (Brits et al., 1995), *Fagus sylvatica* (Nicolas et al., 1996) and *Helianthus* (Sieler, 1998). In a previous study, it has also been reported that germination of *Echinacea angustifolia* seeds was improved by GA_3 and it was suggested that GA_3 affects physiological as well as metabolic activities of seeds, resulting in early germination (Chuanren et al., 2004). Seeds of *A. cyclophyllon* tested in our study did not show any favorable reaction to low concentrations of GA_3, because of their high rate of dormancy, but germination increased with higher concentrations of GA_3 (≥ 300 ppm).

Hot water treatments have been reported to enhance germination of hard coated seeds by elevating water and O_2 permeability of the testa (Msanga and Maghembe, 1986; Teketay, 1998; Aydın and Uzun, 2001). However, in our case, hot water treatments did not induce germination. With respect to the germination pretreatments, our results demonstrate that hot water with temperature above 80ºC treatments applied to *A. cyclophyllon* is harmful to the seeds because germination is reduced considerably compared to the control.

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