

Study of Dormancy Type and Effect of Different Pre-Sowing Treatments on Seed Germination of Bladder-Senna (*Colutea bohsei* Boiss.) in Laboratory Conditions

Ahmad BEIKMOHAMMADI*, Ali TEHRANIFAR, Hossein NEMATİ, Mahmoud SHOOR

*Ferdowsi University of Mashhad, Agriculture Faculty, Horticultural Science Department, Azadi Square, Mashhad, Iran; ah.beik@yahoo.com (*corresponding author)*

Abstract

The present study was conducted in order to evaluate the existence and type of the dormancy and effects of different dormancy breaking treatments on seed germination of *Colutea bohsei* Boiss. (*Fabaceae*) as one of the Iran native plants, arid-land and ornamental shrub with ability of usage in the urban landscape design. The experiment was performed with 15 treatments and 3 replications in a completely randomized design. Seeds were subjected to different treatments including various levels of GA₃, concentrated (98%) H₂SO₄, cold stratification (CS), soaking with tap water, floating in hot water (100°C) followed by continual cooling for 24 hr in the same water and combined treatments. Afterwards seeds were sown in laboratory conditions to determine the factors' effects on germination percentage (GP), germination rate (GR), root and shoot length of *Colutea bohsei* seeds. All of these treatments, except for GA₃ (250 and 500 ppm), increased the percentage and rate of the seed germination. Maximum germination percentage (66.25%) and rate (14.9 seeds per day) in 7 days was obtained at concentrated (98%) H₂SO₄ (15 min). Maximum root and shoot length was observed at concentrated (98%) H₂SO₄ (15 min) plus GA₃ (100 ppm, 24 hr). Use of GA₃ (100 ppm) in 24 hr after H₂SO₄ increased the germination rate and shoot length but this additive effect was not significant.

Keywords: arid-land, drought tolerant, landscape design, native plant, ornamental shrub

Introduction

The *Colutea* L. (bladder-sennas) genus includes about 26 species of deciduous shrubs and small trees with a distribution ranging from the Mediterranean region and South-Eastern Europe and North-West Africa (Browicz, 1963). *Colutea bohsei* Boiss. (*Fabaceae*) is a fast-growing shrub up to 3 m height, with flexible and gray stems and often grows at the altitudes of Alborz Mountains. The global distribution of this shrub is Iran and Turkmenistan; and its distribution in the Iran provinces is Golestan, Mazandaran, Azarbaijan, Khorasan, Semnan and Tehran. From botanical point of view, it bears branchy leaves composed of small and bright green leaflets, yellow flowers and fruit is a pod-shaped balloon (Mozaffarian, 2005). It is a drought-tolerant plant occurring in rocky and steep landscapes and is known as an important species in preventing soil erosion and functional for landscape design. *Colutea* species are cultivated in temperate climates primarily for ornamental purposes (Krüsmann, 1984; Rudolf, 1974). *Colutea* L. species domestication is limited by the low germination rates and high susceptibility of the seeds to fungal attack (Aguinagalde *et al.*, 1990).

Seeds of many woody plant species cannot germinate even if they are sown under optimal moisture, oxygen and soil conditions (Olmez *et al.*, 2008). This problem is called dormancy and its causes are a hard and impermeable

seed coat, immature or dormant embryo, absence of endosperm, or thick, fleshy seed cover (ISTA, 1993). There is great deal of variation in germination ability of seeds even within the same species. Poulsen (1996) reported that dormancy among and within seed lots, of the same species, varied with provenance, crop year, and individual trees.

There are various germination obstacles in *Colutea* seeds (Dirr and Heuser, 1987; Dirr, 1990; Olmez *et al.*, 2007, 2008) resulting in propagation difficulties (Heit, 1967; Piotto *et al.*, 2003). There have been few studies to determine different methods and techniques to overcome seed dormancy in *Colutea* species. Generally, pre-treatments such as submersion in hot water, mechanical or chemical scarification, and hot aeration are used for seed coat dormancy while the cold and warm stratifications are usually applied to dormancy caused by restrictions at the embryo level (Landis *et al.*, 1996). Among these methods and techniques, especially cold stratification, submersion in concentrated H₂SO₄, and steeping seeds in hot water (88-100°C), followed by 24 hr chilling, are well-known and used to increase germination percentage of *Colutea* seeds (Dirr and Heuser, 1987; Piotto *et al.*, 2003; Olmez *et al.*, 2007).

The aim of this study was to investigate the dormancy type and influence of various dormancy-breaking treatments (submersion in tap water, submersion in hot water, submersion in H₂SO₄, GA₃, cold stratification and com-

bined this treatments) on germination of *Colutea bohsei* seeds, sown in laboratory conditions.

Materials and methods

Ripe fruits were collected in mid-summer, 2010 from altitudes (1500 m) of Shahrud (Kalpush) provenance with geographical co-ordinates: latitude 37°13' North and longitude 55°48' East with relatively warm summers and cold winters located in the North part of Iran. The seeds were separated from the fruit and stored at 5±1°C in plastic bags in the refrigerator and then the following pre-sowing treatments were applied to determine their effects on germination percentage (GP), germination rate (GR), root and shoot length of *Colutea bohsei* seeds:

- 1-Floating in tap water (20±1°C) for 24 h;
- 2-Floating in hot water (100°C) followed by continual cooling for 24 h, in the same water;
- 3-Cold stratification (CS) for 15 and 30 days in (5±1°C) into refrigerator;
- 4-Submersion in concentrated (98%) H₂SO₄ for 5, 15 and 30 min;
- 5-Floating in GA₃ (100, 250, and 500 ppm) for 24 h;
- 6-Submersion in concentrated (98%) H₂SO₄ for 15 min, and then floating in GA₃ (100, 250, and 500 ppm) for 24 h;
- 7-Submersion in concentrated (98%) H₂SO₄ for 30 min, and then floating in GA₃ (100 ppm) for 24 h;
- 8-Control (no treatment).

Seeds were stratified by putting layers of moistened sand and seeds on top of each other into refrigerator with mean temperature 5±1°C. Since there was a risk for some of the seeds to be mixed with the sand, because of their small size, linen cloth was placed between the sand and the seeds. The moisture of the sand and the seeds were checked continuously to avoid drying, heating and poor aeration.

Twenty seeds were sown on wet filter paper in each glass petri-dish, under laboratory conditions (mean relative humidity 50% and mean ambient day temperature of 23±2°C and 17±2°C night temperature) in Ferdowsi University of Mashhad in the winter of 2011. Visible radicle growth was used to define germination. Seeds germination recorded daily; continued until no further germination occurred for 5 days; and recording duration of germination was 15 days. Germination percentage and germination rate was calculated using Maguire's equations (1962):

$$GP = 100(N_G/N_T)$$

where N_G is the number of germinated seeds and N_T is the total number of seeds.

$$GR = n_1/t_1 + n_2/t_2 + \dots + n_{15}/t_{15};$$

where n₁, n₂,..., n₁₅ are the number of germinated seeds at times t₁, t₂,..., t₁₅(in days).

For the study of the treatments' effect on root and shoot length, 15 normal seedlings were measured from each replication and expressed in centimeter in the final day of experiment. The experiment was conducted in randomized completely design with 15 treatments and three replications (60 seeds per replication) for each treatment. Data from the treatments were analyzed using SAS statistical software. The LSD test was used for treatment mean comparison ($\alpha = 0.05$).

Results and discussion

Experiment analyse of variance indicates that effects of various treatments on germination percentage (GP), germination rate (GR), root and shoot length of *Colutea bohsei* seeds were significant ($\alpha = 0.01$) (Tab. 1).

Statistical analyses showed that the highest germination percentages (66.2%) were obtained in seeds submersed in H₂SO₄ for 15 min in laboratory conditions (Tab. 2). These results may indicate that the seeds of this shrub have a kind of dormancy that makes necessary their germination's stimulation by removing the hard cover of seeds. Because the H₂SO₄ treatments are used in abrasion and softening the seed coat, it can be stated that the dormancy type of *C. bohsei* seeds is a physical dormancy and is pertaining to the seed coat. Similar findings were reported by Dirr (1990), who found that soaking *Colutea* seeds in H₂SO₄ for 30-60 min resulted in good germination. According to Dirr (1990), some *Colutea* seeds did not germinate easily unless the impermeable seed coat was ruptured by mechanical or chemical scarification. Olmez *et al.* (2008) stated that soaking *C. armena* seeds in H₂SO₄ for 30 min and sown in the greenhouse resulted in good GP value (77.2%). In addition, Olmez *et al.* (2007) reported that scarification of *C. armena* seeds by soaking in H₂SO₄ for 30 min and sown under laboratory conditions gave high GP (82.8%) and a short time to maximum GR (7 days). Ren and Tao (2004) stated that speed and percent germination of the *Calligonum* species can be greatly increased by mechanical scarification or acid treatments.

The lowest average GP (7.5%) was determined at control seeds and those floating in GA₃ (250 and 500 ppm) for 24 hr, which were sown under laboratory conditions (Tab. 2). Dirr and Heuser (1987) reported that untreated *Colutea* seeds could be sown in autumn, but scarification was required for spring-sowing. Among combined treatments,

Tab. 1. Summary of analysis of variance for measured parameters in *Colutea bohsei* Boiss. seeds

Source of variation	df	Mean square			
		Germination rate (GR)	Germination percentage (GP)	Root length	Shoot length
Factor	14	129**	1850**	1.73**	9**
Error	45	4	58	0.05	0.06

** Significant at $p < 0.01$; df: degrees of freedom

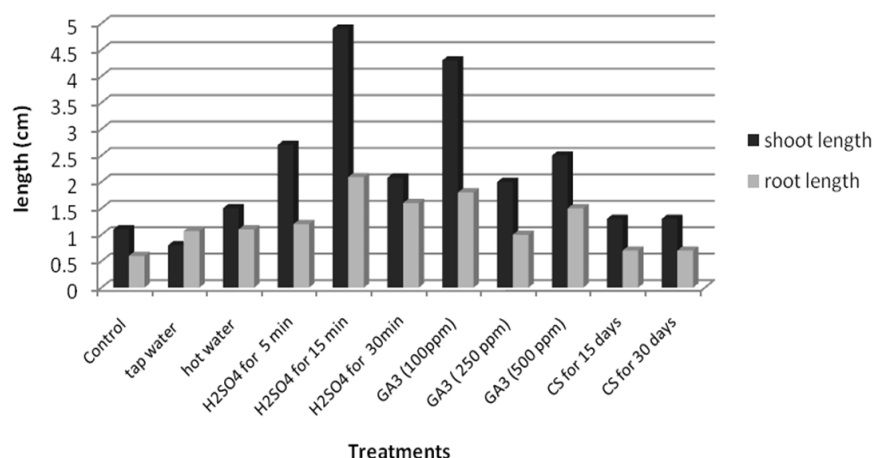


Fig. 1. Effects of various treatments on root and shoot length of *Colutea bohsei* seeds in laboratory conditions in the end of experiment

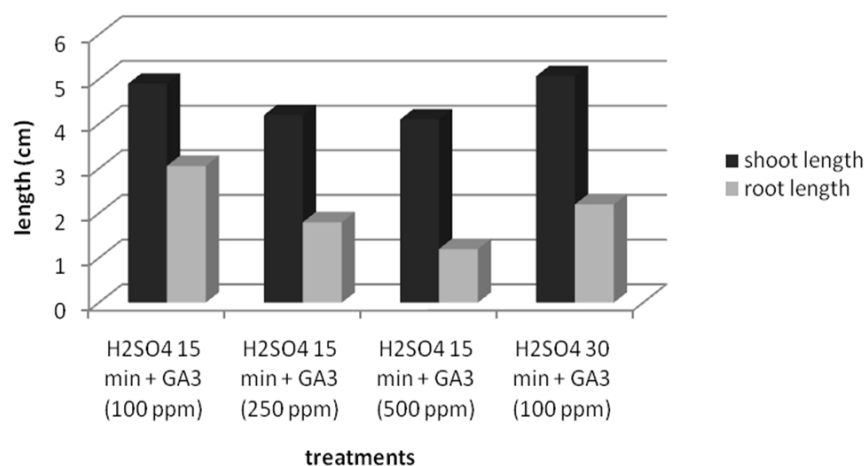


Fig. 2. Effect of combined dormancy breaking treatments on shoot and root length of *Colutea bohsei* seeds in laboratory conditions in the end of experiment

Tab. 2. Effect of various dormancy breaking treatments on germination percentage and germination rate of *Colutea bohsei* Boiss. seeds in laboratory conditions

Treatments	Germination percentage (%)	Germination rate (in day)
Control (no treatment)	7.5 h	1.5 gh
Floating in tap water (20 ±1°C) for 24 h,	22.5 f	4.1 fg
Floating in hot water (100°C) followed by continual cooling for 24 h, in the same water,	18.7 fg	2.3 gh
Submersion in concentrated (98%) H ₂ SO ₄ (5min)	38.7 dc	6 ef
Submersion in concentrated (98%) H ₂ SO ₄ (15 min)	66.2 a	14.9 ab
Submersion in concentrated (98%) H ₂ SO ₄ (30 min)	53.7 b	10.2 d
Floating in GA ₃ (100 ppm) for 24 h,	30 de	8.7 de
Floating in GA ₃ (250 ppm) for 24 h,	7.5 h	0.8 h
Floating in GA ₃ (500 ppm) for 24 h,	7.5 h	0.4 h
Cold stratification (CS) for 15 days in (5±1°C)	11.2 gh	2.8 gh
Cold stratification (CS) for 30 days in (5±1°C)	21.2 fg	2.6 gh
Submersion in concentrated (98%) H ₂ SO ₄ for 15 min + (GA 100 ppm) for 24 h	57.5 ab	13.3 b
Submersion in concentrated (98%) H ₂ SO ₄ for 15 min + (GA 250 ppm) for 24 h	66.2 a	13.2 bc
Submersion in concentrated (98%) H ₂ SO ₄ for 15 min + (GA 500 ppm) for 24 h	48.7 bc	10.4 cd
Submersion in concentrated (98%) H ₂ SO ₄ for 30 min + (GA 100 ppm) for 24 h	66.2 a	17.4 a

Note: Means in column with the same letter are not significantly different (LSD test, $p < 0.05$)



Fig. 3. Seeds germination in laboratory conditions (A) and mature shrub of *Colutea bohsei* Boiss. in habitat (B)

submersion in concentrated (98%) H_2SO_4 for 30 min and then (GA_3 100 ppm 24 hr) resulted the best germination percentage (66.2%) under laboratory conditions (Tab. 2). Similar results by Nadjafi *et al.* (2006), showed that acid scarification by H_2SO_4 (75% v/v), for 5 and 10 min with GA_3 (1500 ppm, 48 hr), broke dormancy and induced 31.9% and 34.1% germination of *T. polium*, respectively. The best germination rates for laboratory conditions were 17.4 seeds in days which were determined in seeds soaked in concentrated (98%) H_2SO_4 for 30 min plus floating in GA_3 (100 ppm) for 24 hr (Tab. 2).

The highest root and shoot length was 3.6 and 4.9 cm respectively, which were observed in seed soaked (98%) H_2SO_4 for 15 min plus floating in GA_3 (100 ppm) for 24 hr. Using of GA_3 (100 ppm, 24 hr) after H_2SO_4 , increased germination rate and shoot length but this additive effect was not significant. In combined treatments ($H_2SO_4 + GA_3$) with increasing GA_3 concentration, shoot and root length decreased (Fig. 2).

Conclusions

Among all the pre-sowing treatments applied, soaking in H_2SO_4 for 15 min resulted the highest GP (66.2%) and GR (14.9%) in the shortest time to maximum GR (8 days) with good root and shoot length that was 2.09 and 4.9 cm respectively. These results may indicate that the seeds of this shrub have a kind of dormancy that indicates their germination's stimulation by removing the hard cover of seeds. Therefore, according to these results pre-treatment by submersion in H_2SO_4 for 15 min can be used to overcome dormancy of the *C. bohsei* Boiss. seeds.

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