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Full Length Research Paper

Impact of Pretreatment Methods and Environmental Conditions on Bladder-Senna Seed Germination

Zafer Olmez* and Askin Gokturk

Artvin Coruh University, Faculty of Forestry, 08000 Artvin, Turkey.

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This study was carried out to determine which pre-treatments should be preferred to overcome dormancy problems of *Colutea armena* seeds which were collected from three different provenances. Pre-treatments applied to the seeds were submersion in concentrated (98%) sulphuric acid for 30 min, floating in hot water (100°C) followed by continual cooling for 24 h in the same water, soaking in tap water (20 ± 1°C) for 24 h and cold stratification for 20, 40 and 60 days. The seeds were sown in polyethylene pots in the greenhouse and in seedbeds under open field conditions. The statistical approach was a randomized complete block design with three replications. Germinated seeds were observed periodically during 70 days to determine germination percentages and germination rates. The highest germination percentages (52.2, 56.7 and 60.5%) were obtained in seeds which were submersed in sulphuric acid for 30 min and sown in the greenhouse for all three provenances. The best germination rates for provenances of Köprüba ı and Derinköy were 14 and 16 days which were determined in seeds soaked in sulphuric acid for 30 min and sown in the greenhouse. On the other hand, the best germination rate (12 days) of provenance of Salkımlı was determined in seeds which were floated in hot water (100°C) followed by continual cooling for 24 h in the same water and sown under open field conditions.

Key words: *Colutea armena*, germination, seed, greenhouse, open field condition.

INTRODUCTION

Vegetation cover is one of the most important factors in preventing and controlling soil erosion. It gives long-term soil surface protection by providing leaf cover that re-duces rain-drop effect. In addition, it helps better soil structure development through establishing a root sys-tem, thereby increasing infiltration and soil stability (Pritchett and Fisher, 1987; Balcı, 1996). The genus *Colutea* L. (bladder-senna) includes about 26 species of deciduous shrubs and small trees with a distribution rang-ing from the Mediterranean region and South-Eastern Europe and North-West Africa (Browicz, 1963). *Colutea armena* (Boiss. and Huet.) is a drought-tolerant plant occurring in rocky and steep landscapes and is known as an important species in preventing soil erosion (Dirr, 1990; Pijut, 2008). *Colutea* species are cultivated in temperate climates primarily for ornamental purposes (Rudolf, 1974; Krüsmann, 1984). *Colutea* L. is a genus of great rusticity and is valuable as forage. Its domestication is limited by the low germination rates and high suscep-tibility 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 (Ürgenç and Çepel, 2001). This pro-blem is called dormancy and its causes are a hard and

*Corresponding author. E-mail: zaferolmez@yahoo.com. Fax: +90 4662126951.

Table 1. Provenances of the collected *Colutea armena* seeds.

Provenance	Exposure	Altitude (m)	Latitude	Longitude
Artvin-Köprüba ı	NE	212	41°11' 14" N	41°49' 43" E
Artvin-Salkımlı	SE	550	41°11' 42" N	41°52' 13" E
Artvin-Derinköy	SW	860	41°05' 54" N	41°52' 50" E

impermeable seed coat, immature or dormant embryo, absence of endosperm, or thick, fleshy seed cover (ISTA, 1966 and 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 varies 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, Olmez et al., 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 aera-tion 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 H_2SO_4, and steeping seeds in hot water (88 - 100°C) followed by 24 h chilling are well-known and used to increase germi-nation percentage of *Colutea* seeds (Allue Andrade, 1983; Dirr and Heuser, 1987; Piotto et al., 2003; Olmez et al., 2007).

The aim of this study was to examine the influence of four pre- treatments (cold stratification, submersion in sulphuric acid, submersion in hot water followed by 24 h chilling and submersion in tap water for 24 h) on dor-mancy of *C. armena* seeds which were sown both in the greenhouse and under open field conditions.

MATERIALS AND METHODS

Ripe fruits were collected in August, 2004 from 15 *C. armena* individuals, representing three different provenances (Derinköy, Salkımlı and Köprüba ı) in Artvin located in the North-Eastern part of Turkey (Table 1). The seeds were separated from the fruit material, rinsed with tap water, dried in the shade, and stored at 5 ± 1°C in plastic bags after the ratios of filled seeds to all the collected seeds were determined.

The following pre-treatments were applied to determine their effects on germination percentage (GP) and germination rate (GR) of *C. armena* seeds;

Cold stratification (CS) for 20, 40 and 60 days,
Floating in tap water (20 ± 1°C) for 24 h,
Floating in hot water (100°C) followed by continual cooling for 24 h in the same water,
Submersion in concentrated (98%) sulphuric acid for 30 min,
Control (no treatment).

The seeds were stratified by putting layers of moistened sand and seeds on top of each other. 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 mean temperature of the room where cold stratification was applied on the seeds was 5 ± 1°C. The moisture of the sand and the seeds were checked continuously against drying, heating and poor aera-tion.

The seeds were sown in polyethylene pots in the greenhouse and in seedbeds under open field conditions in the spring (March) of 2005. Polyethylene pots were filled with growing medium com-posed of forest soil, creek sand, and manure (1:1:1). The experi-mental design was a randomized complete block with three repli-cations (30 seeds per replication) for each treatment.

The number of germinated seeds (evaluation done according to ISTA Rules (1993)) was recorded for 7^{th}, 10^{th}, 14^{th} and 21^{st} days and in every week (7 days) after the the 21^{st} day counting. Germination percentage (GP) and germination rate (GR) values were determined for each pre-treatment and filled seed ratios were used to determine GP. The following formula was used for deter-mining GR (Pieper, 1952);

$$GR = \frac{(n1 \times t1) + (n2 \times t2) + (n3 \times t3) + \ldots + (ni \times ti)}{T}$$

Where *GR:* Germination rate; *n:* Number of days for each counting of germinated seeds; *t:* Number of germinated seeds at each counting day; and T: Total number of germinated seeds

The experiment lasted for about 70 (80) days when it was observed that the seeds stopped germinating. Data from the treatments were analyzed using the SPSS statistical software after arc-sinus transformation was applied to GP values to meet ANOVA assum-ptions. The multifactor ANOVA and Duncan tests were used to compare treatment groups to find out whether they showed any statistically significant differences with significance level ( ) set at 0.05.

RESULTS AND DISCUSSION

Statistical analyses showed that the highest germination percentages (52.2, 56.7 and 60.5%) for all three provenances (Derinköy, Köprüba ı and Salkımlı, respectively) were obtained in seeds which were submersed in sulphuric acid for 30 min and sown in the greenhouse (Table 2). Similar findings were reported by Dirr (1990) who found that soaking *Colutea* seeds in sulphuric acid 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 sulphuric acid for 30 min and sown in the greenhouse resulted in good GP value (77.2%). In addition, Olmez et al. (2007) reported

Table 2. Relationship of the germination percentage and rate of *C. armena* seeds with different pre-treatments for three provenances in the greenhouse (G) and under open field (OF) conditions.

Pre-treatments	F-Ratio	GP (%)	F-Ratio	GR (day)
Provenance of Derinköy				
Cold stratification for 60 days (OF)	4.07*	11.11a	17.62*	39de
Floating in tap water for 24 h (OF)	1.17**	12.22a	1.11**	20abc
Control (OF)	3.59***	14.44a	3.21***	25abcd
Cold stratification for 40 days (OF)		16.67a		43de
Control (G)		20.00a		44de
Cold stratification for 20 days (OF)		20.00a		29abcde
Floating in tap water for 24 h (G)		21.11a		42de
Cold stratification for 40 days (G)		22.22ab		48e
Submersion in sulphuric acid for 30 min (OF)		24.44ab		28abcd
Cold stratification for 60 days (G)		28.89ab		39cde
Floating in hot water (100°C) followed by continual cooling for 24 h in the same water (OF)		28.89ab		27abcd
Cold stratification for 20 days (G)		31.11ab		37bcde
Floating in hot water (100°C) followed by continual cooling for 24 h in the same water (G)		41.11bc		20ab
Submersion in sulphuric acid for 30 min (G)		52.22c		16a
Provenance of Köprüba ı				
Cold stratification for 60 days (OF)		13.89a		35abcd
Control (OF)		15.05a		23abc
Cold stratification for 40 days (OF)	3.88***	16.20a	6.23*	29abcd
Floating in hot water (100°C) followed by continual cooling for 24 h in the same water (OF)		21.99ab	2.08***	19abc
Floating in tap water for 24 h (OF)		24.31ab		27abcd
Cold stratification for 20 days (OF)		28.94abc		33abcd
Submersion in sulphuric acid for 30 min (OF)		28.94abc		16ab
Cold stratification for 20 days (G)		33.56abcd		39cd
Cold stratification for 40 days (G)		42.82bd		36abcd
Floating in hot water (100°C) followed by continual cooling for 24 h in the same water(G)		45.14bd		23abc
Control (G)		50.92cd		37bcd
Cold stratification for 60 days (G)		52.08cd		47d
Submersion in sulphuric acid for 30 min (G)		56.71d		14a
Floating in tap water for 24 h (G)		56.71d		28abcd
Provenance of Salkımlı				
Cold stratification for 20 days (OF)		4.94a		22abcd
Floating in tap water for 24 h (OF)		8.64a		28abcd
Control (OF)		9.88a		30abcd
Cold stratification for 60 days (OF)	3.18*	17.28ab	5.58*	39abcd
Cold stratification for 40 days (OF)	12.21**	17.28ab	2.02***	40bcd
Control (G)	8.47***	28.39bc		45cd
Floating in tap water for 24 h (G)		29.63bc		44cd
Cold stratification for 60 days (G)		32.09bcd		50d
Cold stratification for 40 days (G)		33.33bcd		37abcd
Floating in hot water (100°C) followed by continual cooling for 24 h in the same water (OF)		35.80cd		12a
Cold stratification for 20 days (G)		37.04cd		42bcd
Submersion in sulphuric acid for 30 min (OF)		40.74cd		20abc
Floating in hot water (100°C) followed by continual cooling for 24 h in the same water (G)		49.38de		29abcd
Submersion in sulphuric acid for 30 min (G)		60.49e		15ab

*VS: Greenhouse (Treatment), significantly different at α = 0.05, **VS: Open Field (Treatment), significantly different at α = 0.05, ***VS: Greenhouse*Open Field (Treatment), significantly different at α= 0.05.
Means in column with the same letter are not significantly different at = 0.05.

Table 3. Relationship of the germination percentage and rate with different pre-treatments and provenances in the greenhouse (G) and under open field (OF) conditions.

Pre-treatments	F-Ratio	GP (%)	F-Ratio	GR (days)
Control (OF)	4.418*	13.26a	5.484*	26abc
Cold stratification for 60 days (OF)		14.04a		38cde
Floating in tap water for 24 h (OF)		15.20a		25ab
Cold stratification for 40 days (OF)		16.76a		28bcd
Cold stratification for 20 days (OF)		18.32ab		38cde
Floating in hot water (100°C) followed by continual cooling for 24 h in the same water (OF)		28.85bc		19ab
Submersion in sulphuric acid for 30 min (OF)		31.19c		40de
Cold stratification for 20 days (G)		32.75c		38cde
Control (G)		33.14c		39de
Cold stratification for 40 days (G)		33.92cd		42e
Floating in tap water for 24 h (G)		35.87cd		45e
Cold stratification for 60 days (G)		37.82cd		22ab
Floating in hot water (100°C) followed by continual cooling for 24 h in the same water (G)		45.22d		24ab
Submersion in sulphuric acid for 30 min (G)		56.53e		15a
Provenances				
Derinköy	7.898**	25.89a	1.214	33
Salkımlı		27.40a		32
Köprüba ı		35.17b		29

* VS: Pre-treatments, significantly different at α= 0.05, ** VS: Provenances, significantly different at α = 0.05.
Means in column with the same letter are not significantly different at = 0.05.

that scarification of *C. armena* seeds by soaking in sulphuric acid for 30 min and sown in the laboratory conditions gave high GP (82.8%) and a short time to maximum GR (7 days).

The lowest average GP (13.3%) was determined in control seeds which were sown under open field conditions (Table 3). Dirr and Heuser (1987) reported that untreated *Colutea* seeds could be sown in the autumn, but scarification was required for spring- sowing. It can be also stated that there is an affirmative effect of green-house condition on GP (Table 3).

The best germination rates for provenances of Köprüba ı and Derinköy were 14 and 16 days which were determined in seeds soaked in sulphuric acid for 30 min and sown in the greenhouse. On the other hand, the best germination rate (12 days) of provenance of Salkımlı was determined in seeds which were floated in hot water (100°C) followed by continual cooling for 24 h in the same water and sown under open field conditions (Table 2). While sulphuric acid treatment gave the best average germination rate at 15 days in the greenhouse, it took 45 days for seeds cold stratified for 60 days to reach the slowest germination rate (Table 3).

The hot water pre-treatment resulted in higher GP and shorter time to maximum GR (45.2% and 24 days in the greenhouse and 28.9% and 19 days under open field conditions, respectively) compared to the tap water pre-treatment (35.9% and 38 days in the greenhouse and 15.2% and 25 days under open field conditions, respectively) (Table 3). These findings are supported by similar studies done on *Colutea* seeds (Allue Andrade, 1983; Dirr and Heuser, 1987; Olmez et al., 2008). Dirr (1990) stated that soaking *Colutea* seeds initially in 88°C-water and allowing them to remain in that water for 24 h result-ed in good GP values. In addition, Piotto et al. (2003) reported that scarification of *Colutea* seeds follow- ed by floating in hot water (80°C) gave high GP and a short time to maximum GR.

The maximum average GP value among the CS pre-treatments was 37.8% for seeds that were cold stratified for 60 days and sown in the greenhouse. The analyses also revealed that the seeds collected from Köprüba ı resulted in the highest GP value (35.2%) among the provenances (Table 3).

Consequently, among all the pre-treatments applied to the *C. armena* seeds, soaking in sulphuric acid for 30 min resulted in the highest GP (56.5%) and the shortest time before maximum GR (15 days). It is followed by the pre-treatment of floating in hot water (100°C) followed by continual cooling for 24 h in the same water with 45.2% of GP. Therefore, these results indicate that the pre-treatment by submersion in sulphuric acid for 30 min and

sowing in greenhouse condition should be used to overcome dormancy of the *C. armena* seeds.

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