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Full Length Research Paper

Effects of cold stratification and sulphuric acid pre-treatments on germination of three provenances of smoke-tree (*Cotinus coggygria* Scop.) seeds in greenhouse and laboratory conditions

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This study was carried out to determine which pre-treatments should be preferred to overcome dormancy problems of *Cotinus coggygria* seeds which were collected from three different provenances. Pre-treatments applied to the seeds were submersion in concentrated (98%) sulphuric acid (10, 20, 30, 40 and 50 min) and submersion in sulphuric acid (10, 20, 30, 40 and 50 min) followed by cold stratification (15, 30, 45 and 60 days). The seeds were sown in polyethylene pots in the greenhouse and sown at 22 ± 1°C under darkness in laboratory conditions. The statistical approach was a randomized complete block design with 3 replications. Germinated seeds were observed periodically for 50 days to determine germination percentages and germination rates. Submersion in H_2SO_4 for 50 min with cold stratification for 15 days gave the highest germination percentage (88.1%) in the laboratory while, while the highest germination percentage (70.2%) was obtained from submersion in H_2SO_4 for 10 min with cold stratification for 30 days pre-treatment in the greenhouse. The analyses also revealed that the seeds collected from Ardanuç resulted in the highest germination percentage value (44.6%) in the laboratory among the provenances. The highest germination percentage (53.2%) sown in the greenhouse conditions was determined from the seeds collected in Artvin.

Key words: *Cotinus coggygria*, germination, pre-treatments, seed dormancy.

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 reduces rain-drop effects. In addition, it helps better soil structure development through establishing a root system, thereby increasing infiltration and soil stability (Pritchett and Fisher, 1987; Balcı, 1996). *Cotinus coggygria* Scop. growing in steep and rocky landscapes is drought-tolerant plant that is important in preventing soil erosion. This species is also important as an ornamental plants in many countries. Several cultivars produce a long period of midsummer floral and fruit ornamentation, showy plumose inflorescences and vivid autumn foliage color (Rudolf, 1974; Dirr, 1990; Krüssmann, 1984; Koller and Shadow, 1991; Pijut, 2008). The genus *Cotinus* widely distributed through Central and Southern Europe to the Himalayas, Southwest China and the Southeastern United States (Krüssmann, 1984).

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 problem is called dormancy and their causes are a hard and impermeable seed coat, immature or dormant embryo, absence of endosperm, or thick, fleshy seed cover (ISTA, 1966, 1993). There is a 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 *Cotinus* seeds (Rudolf, 1974; Dirr and Heuser, 1987; Takos and

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Table 1. Provenances of the collected *Cotinus coggygria* seeds.

Provenances	Exposure	Altitude (m)	Latitude	Longitude
Ardanuç	S	700	41° 35' 42" N	42° 15' 32" E
Artvin	E	622	41° 11' 37" N	41° 48' 38" E
Derinköy	SW	860	41° 05' 54" N	41° 52' 50" E

Efthimiou, 2002; Piotto et al., 2003; Olmez et al., 2007a; Olmez et al., 2008) resulting in propagation difficulties. A few studies have tried to determine different methods and techniques to overcome seed dormancy in *Cotinus* 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 H_2SO_4 and steeping seeds in hot water (88 - 100°C) followed by 24 h chilling are used to increase germination percentage of *Cotinus* seeds (Rudolf, 1974; Dirr and Heuser, 1987; Stilinovic and Grbic, 1988; Dirr, 1990; Piotto et al., 2003, Olmez et al., 2007a, Olmez et al., 2008).

The aim of this study was to examine the influence of cold stratification and submersion in sulphuric acid pre-treatments on dormancy of *C. coggygria* seeds which were sown both in the laboratory and in the greenhouse conditions.

MATERIALS AND METHODS

The fruits were collected by hand as soon as they were ripe in late August 2007 from *C. coggygria* individuals, representing 3 different provenances (Ardanuç, Artvin and Derinköy) 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.

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

i. Submersion in concentrated (98%) sulphuric acid for 10, 20, 30, 40 and 50 min.
Submersion in concentrated (98%) sulphuric acid for 10, 20, 30, 40 and 50 min + cold stratification (CS) for 15, 30, 45 and 60 days.
Control.

The seeds were stratified by putting layers of moistened sand and seeds on top of each other in closed boxes. 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 and the moisture of the sand and the seeds were checked regularly against drying, heating and poor aeration.

The seeds were sown in polyethylene pots in the greenhouse and into the petri dishes filled using filter paper at 22 ± 1°C under darkness in the spring (April) of 2008. Polyethylene pots were filled with growing medium composed of forest soil, creek sand and manure (3:1:1). The experimental design was a randomized complete block with three replications (30 seeds in each replication) for each treatment.

The number of germinated seeds [evaluation done according to ISTA Rules (1993)] was recorded for 7th, 10th, 14th, 21st, 28th, 35th and 42nd days. The GP and GR values were determined for each pre-treatment. The formula used in determining GR values is as follows (Pieper, 1952);

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

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 50 days when it was observed that the seeds had stopped germinating. Data from the pre-treatments were analyzed using the SPSS statistical software after arcsinus transformation was applied to GP values to meet ANOVA assumptions. 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 (α) set at 0.05.

RESULTS AND DISCUSSION

Statistical analyses showed that the pre-treatments used in this study affected seed GP and GR both in the greenhouse and laboratory conditions significantly. Submersion in H_2SO_4 for 50 min with CS for 15 days gave the highest GP (88.1%) in the laboratory while, the highest GP (70.2%) was obtained from submersion in H_2SO_4 for 10 min with CS for 30 days pre-treatment in the greenhouse. Increasing the duration of CS when it was used with H_2SO_4 resulted in a decrease in GPs both in the greenhouse and laboratory (Table 2). Previous studies that used H_2SO_4 application and the combinations of CS with H_2SO_4 reported successful outcomes to overcome dormancy of *C. coggygria* seeds (Dirr and Heuser, 1987; Takos and Efthimiou, 2002; Piotto et al., 2003; Olmez et al., 2007a; Olmez et al., 2008). Smoke-tree seeds have both a hard seedcoat and an internal dormancy, thus causing slow and irregular germination. Seeds can be stimulated to germinate more uniformly by sulphuric acid scarification followed by cold stratification (Rudolf, 1974; Dirr and Heuser, 1987; Stilinovic and Grbic, 1988).

Takos and Efthimiou (2002) reported a higher germination (73%) in the laboratory than nursery conditions (19%) in *C. coggygria* seeds when submersion in H_2SO_4 for 30 min followed by CS for 60 days pre-treatment was

Table 2. Results of statistical analyses showing the relationship of the germination percentage and rate with different pre-treatments for laboratory and greenhouse conditions.

Pre-treatments in the laboratory	F-Ratio	GP (%)	F-Ratio	GR (days)
10 min H_2SO_4 + 60 day cold stratification		0.00a		0a
20 min H_2SO_4 + 60 day cold stratification		0.00a		0a
30 min H_2SO_4 + 60 day cold stratification	39.983*	0.00a	5.274*	0a
50 min H_2SO_4 + 60 day cold stratification		0.00a		0a
30 min H_2SO_4		0.37a		4abcd
20 min H_2SO_4		0.74a		8bcde
50 min H_2SO_4		0.74a		9cde
10 min H_2SO_4		1.11a		4abcd
Control		2.22a		2abc
40 min H_2SO_4		2.59a		18f
40 min H_2SO_4 + 60 day cold stratification		6.67a		1ab
20 min H_2SO_4 + 45 day cold stratification		56.27b		11def
50 min H_2SO_4 + 45 day cold stratification		56.64b		9de
30 min H_2SO_4 + 30 day cold stratification		57.36b		12ef
10 min H_2SO_4 + 15 day cold stratification		57.5b		15ef
10 min H_2SO_4 + 45 day cold stratification		58.11b		12ef
40 min H_2SO_4 + 45 day cold stratification		62.57bc		10de
20 min H_2SO_4 + 15 day cold stratification		62.59bc		12ef
30 min H_2SO_4 + 45 day cold stratification		68.88bcd		10def
50 min H_2SO_4 + 30 day cold stratification		69.22bcd		12ef
40 min H_2SO_4 + 30 day cold stratification		69.93bcd		11def
30 min H_2SO_4 + 15 day cold stratification		73.29bcde		10de
10 min H_2SO_4 + 30 day cold stratification		73.76bcde		12ef
20 min H_2SO_4 + 30 day cold stratification		78.12cde		11def
40 min H_2SO_4 + 15 day cold stratification		83.68de		11def
50 min H_2SO_4 + 15 day cold stratification		88.11e		11def
Pre-treatments in the greenhouse				
Control		2.80a		28c
10 min H_2SO_4		6.47a		23bc
20 min H_2SO_4	22.989*	11.82a	3.921*	29c
40 min H_2SO_4		12.79a		27c
50 min H_2SO_4		15.27a		17ab
30 min H_2SO_4		16.80 a		30c
20 min H_2SO_4 + 45 day cold stratification		36.98b		14a
30 min H_2SO_4 + 45 day cold stratification		37.67b		14a
40 min H_2SO_4 + 45 day cold stratification		50.29bc		12a
50 min H_2SO_4 + 45 day cold stratification		51.64bcd		15ab
10 min H_2SO_4 + 45 day cold stratification		54.61cde		14a
20 min H_2SO_4 + 30 day cold stratification		63.51cde		14a
10 min H_2SO_4 + 15 day cold stratification		63.80cde		17ab
20 min H_2SO_4 + 15 day cold stratification		64.99cde		16ab
50 min H_2SO_4 + 30 day cold stratification		65.00cde		14a
40 min H_2SO_4 + 30 day cold stratification		65.50cde		12a
30 min H_2SO_4 + 15 day cold stratification		67.30de		13a
40 min H_2SO_4 + 15 day cold stratification		67.98e		14a
50 min H_2SO_4 + 15 day cold stratification		68.86e		13a
30 min H_2SO_4 + 30 day cold stratification		69.79e		13a
10 min H_2SO_4 + 30 day cold stratification		70.18e		14a

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

Table 3. Results of statistical analyses showing the relationship of the germination percentage and rate with different provenances for laboratory and greenhouse conditions.

Provenances in the laboratory	F-Ratio	GP (%)	F-Ratio	GR (days)
Derinköy	6.529*	35.15a	4.273*	7a
Artvin		39.21a		9ab
Ardanuç		44.55b		10b
Provenances in the greenhouse				
Derinköy	8.082 ^{NS}	48.15	0.959^{NS}	13
Ardanuç		48.46		17
Artvin		53.16		18

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

used. In general, the present results for *C. coggygria* seeds were parallel to the above studies' findings since immersing in H_2SO_4 followed by CS resulted in early, uniform and high GP in the laboratory and greenhouse. Olmez et al. (2007b) stated that the highest GP (44.2%) was obtained in *C. coggyria* seeds that were cold stratified for 60 days and sown under greenhouse conditions. In addition, scarification with sulphuric acid (20-80 min) followed by cold stratification for 60-80 days pretreatments were suggested by Piotto et al. (2003) for *C. coggyria* seeds. Olmez et al. (2008) reported that while submersion in sulphuric acid for 20 min with cold stratification for 60 days gave the highest germination (82.77%) in the greenhouse, 80 min sulphuric acid with 60 day CS pretreatment gave the highest germination percentage (12.47%) under open field conditions for *C. coggyria*.

The maximum GP value among the H_2SO_4 pre-treatments was 2.6% for seeds that were submersed for 40 min and sown in the laboratory and was 26.8% from 30 min H_2SO_4 pre-treatment in the greenhouse. CS with submersion in H_2SO_4 pre-treatments gave higher GP values both in the greenhouse and in the laboratory than H_2SO_4 pre-treatment alone (Table 2). According to Dirr (1990), *Cotinus* seeds did not germinate easily unless the hard seed coat was ruptured by mechanical or chemical scarification. The analyses also revealed that the seeds collected from Ardanuç resulted in the highest GP value (44.6%) in the laboratory among the provenances. The highest average GP (53.2%) was determined from the seeds collected in Artvin and sown in the greenhouse conditions (Table 3).

The best germination rate (12 days) was determined in seeds soaked in H_2SO_4 for 40 min followed by 30 and 45 day CS pre-treatments and sown in the greenhouse. The GR was 11 days for the highest GP (88.1%) that were obtained from submersion in H_2SO_4 for 50 min with CS for 15 days pre-treatment in the laboratory condition (Table 2). It was observed that *C. coggygria* seeds were germinating while the stratification treatment was continuing after 45th day. This may explain why we could not obtain any germination from the seeds that were cold stratified for 60 days and sown in the laboratory.

Consequently, among all the pre-treatments applied to the *C. coggygria* seeds, submersion in H_2SO_4 for 50 min with 15 day-CS in the laboratory and submersion in H_2SO_4 for 10 min with 30 day-CS resulted in the highest GPs, respectively 88.1% and 70.2%. The results for indicate that the duration of pre-treatment of submersion in H_2SO_4 between 10 and 50 with CS up to 45 days could be preferably used to overcome germination dormancy of *C. coggygria* seeds.

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