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Full Length Research Paper

Dormancy breaking in *Cotinus coggygria* Scop. seeds of three provenances

S. Guner and F. Tilki*

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

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With the objective of studying the effect of pretreatments involving sulfuric acid scarification, cold moist stratification, and scarification followed by stratification on germination of *Cotinus coggygria* Scop. seeds of different provenances, fruits were collected in three provenances from Artvin, Turkey. Sulfuric acid was not found an effective scarifying agent for breaking seed coat dormancy in *C. coggygria* seeds. Germination percentage and germination rate increased significantly with increasing stratification duration (30 to 120 days), with optimum at 120 days for all seed provenances. When averaged over all provenances, cold stratification for 3 months resulted higher germination percentage (GP = 72.3%) and germination rate (PV = 8.2) than that of scarification. Also, germination response for different scarification and stratification treatments revealed a significant difference on germination of the provenances. Seeds scarified with sulfuric acid followed by cold moist stratification showed significant improvement for germination performance. Highest improvement observed under scarification (20 - 40 min) followed by stratification (30 or 60 days) treatments (GP > 90%; PV > 16), and seeds from three provenances exhibited uniformity in response.

Key words: Common smoketree, scarification, seed dormancy, seed germination, stratification.

INTRODUCTION

Cotinus coggygria Scop. (common smoketree) is an upright, spreading, multi-stemmed shrub that is grown because of its many ornamental landscape qualities and its adaptability to widely divergent soils (Dirr, 1990). Several cultivars produce a long period of midsummer floral and fruit ornamentation, showy plumose inflorescences, and vivid autumn foliage color (Krussmann, 1984; Dirr, 1990; Koller and Shadow, 1991). The small, usually infertile, yellowish flowers bloom in June to July and seed crops are produced annually but are often poor (Krussmann, 1984).

Regeneration from seeds is the most often used and cheapest method of propagation in many ornamental and forestry species. But, several germination inhibitors are present in the seed coats or embryos of dormant seeds (Bradbeer, 1988; Bewley and Black, 1994). Dormancy is the inability of a seed to germinate, even under conditions that are normally considered favorable for germination.

*Corresponding author. E-mail: fahrettintilki@yahoo.com. Tel.: +90-466-212-6948, Fax: +90-466-212-6951.

Stratification, scarification and other chemicals can over-come seed dormancy and promote seed germination on many species of angiosperms and gymnosperms (Bradbeer, 1988; Bonner et al., 1994; Leadem, 1996; Tilki, 2004, 2007; Cicek and Tilki, 2008).

Seeds of *Cotinus* species have both a hard seed coat and an internal dormancy, thus restricting seed germination, and pretreatments with cold stratification, sulfuric acid or sulfuric acid scarification followed by cold stratification have a promotive effect on the germination of *Cotinus* species (Heit, 1967; Rudolf, 1974; Dirr and Heuser, 1987; Stilinovic and Grbic, 1988; Dirr, 1990; Kebesoglu, 2008).

The degree of dormancy may be expected to show some variation related to climate of origin, and seed dormancy can vary considerably among different clones, from seed lot to seed lot and among seeds within one seed lot (Edwards, 1980; Leadem, 1996; Tilki and Guner, 2007). Since determining the optimal pretreatments is paramount, the aim of this research was to establish an effective method for improving germination of three provenances of *C. coggygria* seeds.

Table 1. Effect of sulfuric acid pretreatment on germination percentage of *Cotinus coggygria* seed.

Sulfuric acid (Conc.)	Provenance			Treatment mean
	Artvin	Savsat	Ardanuc	
Control	1.5	2.5	1.0	1.7c
1 min	5.0	7.0	4.0	5.3b
5 min	7.5	6.0	10.0	7.9ab
10 min	9.5	8.5	12.5	10.2a
30 min	8.5	4.5	7.0	6.5b
50 min	6.0	6.5	3.0	5.2b
80 min	5.5	6.5	8.0	6.7ab
Population mean	6.2	5.9	6.4	

Means with the same letter(s) are not significantly different at $P < 0.05$.

MATERIALS AND METHODS

Seed collection

Mature fruits of *C. coggygria* from Artvin (lat. 41° 12' N, long. 41° 48' E, 600 m a.s.l.), Savsat (lat. 41° 30' N, long. 42° 20', 550 m a.s.l.), and Ardanuc (lat. 41° 08' N, long. 42°10' E, 650 m a.s.l.) were collected from its natural habitat of three different provenances in late August 2007. Collected fruits were packed in plastic bags and transported to the laboratory where the study was undertaken. The seeds were extracted manually, washed and allowed to dry on filter paper at room temperature (20°C) for one day and stored at +4°C until use.

Treatments

Tetrazolium stating was used for rapid estimates of seed viability. Seeds were soaked in water for 24 h before breaking open the seed coat and staining 24 h at 30°C in a 1% solution (Enescv, 1991). To evaluate some methods to overcome seed dormancy, seeds were subjected to the following treatments:

i.) control (no treatment).
ii.) Scarification with concentrated sulfuric acid for 1, 5, 10, 30, 50 and 80 min at room temperature (20°C), washing the seeds with distilled water to remove any trace of acid before being tested for germination.
iii.) Cold moist stratification on moist sand at 4 ± 1°C for 30, 60, 90 and 120 days.
iv.) Chemical scarification with concentrated sulfuric acid (1, 5, 10, 20, 30 and 40 min) followed by cold moist stratification (30 and 60 days).

Seed germination tests

Seeds were germinated in Petri dishes, 12 cm in diameter, (4 replicates of 50 seeds each) containing two layers of filter paper moistened with distilled water. Germination tests were conducted in a germination chamber kept at 20ºC at a 12 h photoperiod. Seeds were monitored every day and moistened with distilled water if necessary. Germination counts were recorded every day for 30 days and seeds were considered germinated when their radicle protruded. Germination percentage (GP) was calculated each day and as the final value after 30 days. Germination rate was calculated and expressed as peak value (PV), an index of germination speed which is the highest number obtained when percentage germination is divided by the number of elapsed days (Czabator, 1962).

Statistical analysis

All treatments were conducted in a completely randomized design using fifty seeds each in four replicates for all treatments. Data on percent germination were arcsine transformed prior to analysis of variance to stabilize any heterogeneous variance. Whenever significant differences were identified, means were tested by the Duncan's New Multiple Range Test ($p < 0.05$) (Zar, 1984).

RESULTS

Results of the study showed that viability of seeds was high (96 - 98%), indicating that seeds would be capable of germination under favorable conditions and that failure of germination would be attributed mainly to seed dormancy.

Germination response for different scarification treatments revealed a significant difference (Table 1) but seed germination responses under scarification treatments do not differ significantly among provenances. In general scarification was not very successful in enhancing seed germination, which did not exceed 10.2%, with maximum responses of a soaking duration of 10 min, when all 3 provenances were averaged.

Provenances and stratification durations affected seed germination percentage. Seeds from different provenances did not vary in responses under stratification treatment. Cold stratification treatment significantly improved ($p < 0.05$) germination (Table 2) over control and also all stratification treatments improved germination by far better than scarification treatments. On average for all provenances, germination percentages were significantly affected by 30 day cold-moist stratification. Furthermore, an increase in duration of stratification increased germination percentage, and stratification of seeds for 120 days resulted in the highest germination percentage (72.3%). When averaged over the stratification treatments, Artvin provenance had the lowest germination

Table 2. Effect of cold stratification treatment on germination percentage of *Cotinus coggygria* seed.

Cold stratification	Provenance			Treatment mean
	Artvin	Savsat	Ardanuc	
Control	1.5	2.5	1.0	1.7e
30 days	17.0	12.0	21.5	16.8d
60 days	37.5	45.5	38.5	40.5c
90 days	54.5	62.0	64.0	60.2b
120 days	66.0	80.0	71.0	72.3a
Population mean	35.3B	40.4A	39.2A	

Means in the column with the same letter are not significantly different ($p < 0.05$). Means in the row with the same letter are not significantly different ($p < 0.05$).

Table 3. Effect of sulfuric acid treatment + cold stratification on germination percentage of *Cotinus coggygria* seed.

Sulfuric acid + cold stratification	Provenance			Treatment mean
	Artvin	Savsat	Ardanuc	
Control	1.5f	2.5e	1.0f	1.7g
1 min + 30 days	26.0e	20.5d	37.5e	28.0f
1 min + 60 days	46.0d	37.5c	60.0d	47.8e
5 min + 30 days	65.5c	60.5b	75.5c	67.1d
5 min + 60 days	77.0b	63.0b	86.5b	75.5c
10 min + 30 days	82.5b	93.0a	96.0a	90.5b
10 min + 60 days	97.0a	94.0a	96.5a	95.8a
20 min + 30 days	97.5a	93.5a	97.5a	97.8a
20 min + 60 days	99.0a	96.0a	99.5a	98.2a
30 min + 30 days	100.0a	98.0a	99.0a	99.0a
30 min + 60 days	98.0a	96.5a	97.5a	97.3a
40 min + 30 days	98.0a	94.5a	98.5a	97.0a
40 min + 60 days	97.0a	94.0a	96.5a	96.0a
Population mean	75.0B	73.0B	80.2A	

Means followed by the same lowercase letter in each column are not significantly different ($p < 0.05$). Means followed by the same capital letter in the row are not significantly different ($p < 0.05$).

percentage.

Germination rate was also significantly affected by stratification treatment and provenances. Germination rate of *C. coggygria* increased significantly with increasing stratification duration (30 to 120 days) for all seed provenances, with optimum at 120 days, and stratification of seeds for 120 days resulted in the highest germination rate (PV = 8.2) when averaged over three provenances.

ANOVA also showed significant differences among scarification followed by stratification treatments, and seeds scarified with sulfuric acid followed by cold moist stratification showed significant improvement for germination (Tables 3 and 4). Seed germination responses under 20 - 40 min scarification + 30 or 60 days stratification do not differ significantly among provenances. 10 min scarification with 60 days cold stratification and 20 - 40 min scarification followed by 30 or 60 days cold stratification also appeared optimum for all provenances. This suggests that dormancy mechanisms are relatively uniform among provenances (with a few exceptions). When average all treatments, provenance Ardanuc had the highest germination percentage (80.2%). The provenance Savsat and Ardanuc required at least 10 days scarification with stratification for 30 days whereas the provenance Artvin did not show the highest germination under this treatment.

Scarification (10 - 40 min) followed by stratification (30 or 60 days) treatment improved the germination response significantly if compared to other pretreatments (Table 4). The best treatments also helped ($p < 0.05$) in increasing germination rate (Table 4). Germination rate of *C. coggygria* increased significantly with increasing scarification + stratification duration for all seed provenances. In spite of considerable germination variation among pro-

Table 4. Effect of sulfuric acid treatment + cold stratification on germination rate (PV) of *Cotinus coggygria* seed.

Sulfuric acid + cold stratification	Provenance			Treatment mean
	Artvin	Savsat	Ardanuc	
Control	0.1g	0.1g	0.1f	0.1g
1 min + 30 days	3.2f	2.9f	4.2e	3.4f
1 min + 60 days	5.7e	4.5e	6.8de	5.7e
5 min + 30 days	7.0de	6.4d	8.6cd	7.3d
5 min + 60 days	9.3cd	8.7c	10.3c	9.4c
10 min + 30 days	11.5bc	12.3b	12.1b	12.0b
10 min + 60 days	13.6b	13.1b	13.4b	13.3b
20 min + 30 days	17.3a	16.6a	17.2a	17.0a
20 min + 60 days	16.7a	16.8a	17.1a	16.8a
30 min + 30 days	16.6a	16.9a	17.1a	16.9a
30 min + 60 days	16.8a	16.2a	17.2a	16.7a
40 min + 30 days	17.0a	17.4a	18.0a	17.5a
40 min + 60 days	16.5a	16.2a	16.8a	16.5a
Population mean	11.7	11.4	12.2	

Means followed by the same letter(s) in each column are not significantly different ($p < 0.05$).

venances there was no significant difference in germination rate. Acid scarification (20 - 40 min) followed by cold stratification (30 or 60 days) produced the highest germination rate for all provenances (PV > 16).

DISCUSSION

Chemical treatments has been widely used to improve germination of several hard-seeded species (Bradbeer, 1988; Li et al., 1999; Bhatt et al., 2000; Tigabu and Oden, 2001; Tilki 2007), and the sulfuric acid effect is attributed to softening of seed coat, which accelerates water absorption and gaseous exchange (Everitt, 1983; Hartmann et al., 1990). But in case of *C. coggygria*, seeds showed poor response to sulfuric acid treatments. However, the beneficiary effects of concentrated sulfuric acid treatments have been seen as preliminary treatment to reduce length of cold stratification treatment in this study.

All stratification treatments improved germination by far better than scarification treatments. Stratification of seeds for 120 days resulted in the highest germination percenttage and germination rate in each provenance. This definite response to stratification is typical of dormant seed sources. However, the best responses under 120 days stratification were lower compared to the seeds subjected to scarification followed by cold stratification. Gokturk (2005) found that 60 days stratification increased germination percentages of common smoke-tree to around 44% under greenhouse condition. Kebesoglu (2008) stated that following 90 days stratification seed germination percentage of common smoke-tree reached to 57%. Pretreatments and seed germination can vary greatly among seed lots (Edwards and El-Kassaby, 1996; Baskin and Baskin, 1998; Tilki and Guner, 2007; Jull and Blazich, 2000). Seeds generally have an inherent high genetic variability, which results in great heterogeneity in their behavior and, in particular, in their germinability fol-lowing stratification procedures. In the present study there was considerable germination variation among pro-venances. For example, the highest mean germination (80%-Savsat) under 120 days cold stratification treatment was significantly higher than the responses of all other provenances. And Artvin provenance had the lowest germination percentage when averaged over the stratification treatments.

Seed coat-imposed and internal dormancy are the most important causes of the primary dormancy present in several species of the genus *Cotinus* (Rudolf, 1974; Dirr and Heuser, 1987; Dirr, 1990). Pretreatments with sulphuric acid scarification (20 - 80 min) followed by cold stratification (45 - 90 days) have a promotive effect on the germination of *C. coggygria* (Gonderman and O'Rourke, 1961; Heit, 1967; Rudolf, 1974; Dirr and Heuser, 1987; Dirr, 1990). Sulfuric acid scarification (20, 50 and 80 min) followed by stratification (60 days) increased germination percentage significantly and the treatment of sulfuric acid scarification for 20 min with cold stratification for 60 days gave the highest germination (87%) under greenhouse condition and in nursery bed (38%) in *C. coggygria* (Gokturk, 2005). Takos and Efthimiou (2003) found that soaking in concentrated sulfuric acid for 60 min followed by stratification for 60 days produced 73% germination in *C. coggygria* germinated at the alternating temperature of 25/20°C. Before spring sowing, mechanical scarification, or scarification with sulfuric acid (20 - 80 min) followed by cold stratification (60 - 80 days) was suggested in this species (Piotto et al., 2001). In the present study scari-

fication (10-40 min) followed by cold stratification (30 or 60 days) was successful in obtaining a higher germination percentage (>90%) under 20°C although cold stratification increased germination percentage significantly. Germination under these pretreatments was close to viability assessed for the different provenances under the study. This suggested that the pretreatment was quite effective to overcome dormancy. Sulfuric acid scarification for 20 min or more followed by cold stratification for 30 or 60 days gave the highest germination rate (PV > 12). This suggest that sulfuric acid treatment before cold stratification appears to be an essential requirement for improving germination performance in three provenances of *C. coggygria* as found by Kebesoglu (2008) for one provenance. It is presumed that *C. coggygria* seeds have internal dormancy and impermeable seed coats as stated by Rudolf (1974) and Takos and Efthimiou (2003).

In conclusion, the germinability of *C. coggygria* seeds can be enhanced significantly by cold stratification or scarification followed by cold stratification treatments. Concentrated sulfuric acid treatments reduced length of cold stratification treatment, and scarification (20 - 40 min) followed by cold stratification (30 or 60 days) gave the best results for all provenances. This suggests that the dormancy mechanisms are relatively uniform among the three provenances.

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