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Full Length Research Paper

Effects of cold stratification and H_2SO_4 on seed germination of sea buckthorn (*Hippophae rhamnoides* L.)

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This study was carried out to determine the effects of some pre-treatments including soaking in concentrated (98%) H_2SO_4 for 1, 2 and 3 min and cold stratification for 30, 45, 60 and 90 days on seed germination and to investigate how to overcome dormancy of *Hippophae rhamnoides* L. seeds. The seeds were sown at 22 ± 1°C under darkness in laboratory conditions. The statistical approach was a randomized complete block design with three replications. Germinated seeds were observed periodically during 30 days to determine germination percentages and germination rates. The highest germination percentage (100%) was obtained from the seeds soaked in H_2SO_4 for 1 min and the lowest one (96.7%) was obtained from the seeds soaked in H_2SO_4 for 2 min, although, there was no significant difference for germination percentages between pretreatments. In addition, the seeds which were stratified at 4 ± 1°C for 45 to 90 days germinated in the stratification medium. On the other hand, better germination rates (4 days) were observed from 30-day cold stratification and in H_2SO_4 scarification pretreatments than control (8 days) seeds.

Key words: *Hippophae rhamnoides*, germination, pretreatments, 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 for better soil structure development through establishing a root system, thereby increasing infiltration and soil stability (Balci, 1996; Pritchett and Fisher, 1987). *Hippophae rhamnoides* is native to northwestern Europe through central Asia to Altai Mountains, western and northern China, and the northern Himalayas (Krüssmann, 1984; Small et al., 2002; Li and Beveridge, 2003; Busing and Slabaugh, 2008). In addition, *H. rhamnoides* is also a native plant species distributed in different regions in Turkey (Davis, 1982; Öner and Abay, 2001).

fH. rhamnoides is a drought-tolerant plant occurring in sandy and salty landscapes and is known as an important species in preventing soil erosion (Ürgenç, 1998; Rodwell, 2000; Small et al., 2002; Li and Beveridge, 2003). The species is also considered as fine vegetation in improving soil fertility and restoring degraded sites in high hills (Li and Beveridge, 2003; Airi et al., 2009; Sourisseau et al., 2009). Sea buckthorn can withstand temperatures from -43 to +40°C. It is considered to be drought resistant, however, most natural populations of sea buckthorn grow in areas receiving 400 to 600 mm of annual precipitation. The plant tolerates high soil pH up to 8.0 and salt from sea water around the costal regions (Li and Beveridge, 2003). A very hardy deciduous shrub or a small tree, *H. rhamnoides* is used primarily for ornamental purposes. In Europe and Asia, it is used to form hedges and because of its nitrogen-fixing symbionts, it serves to enrich and protect soils (Bogdon and Untaru, 1967; Stewart and Pearson, 1967). The plant also has a variety of medicinal uses. It has been used medicinally in China for at least 12 centuries, and sea buckthorn oil is used clinically in hospitals in Russia and China (Mathews, 1994; Chauhan, 1999; Small et al., 2002). The berries of the species are sources of vitamins, and used in preparations of various products including beverages and marmalades (Valicek, 1978; Li and Schroeder, 1996; Chauhan, 1999; Gaur, 1999; Li and Beveridge, 2003; Yaldız and Tunç, 2010).

Seeds of many woody plant species cannot germinate even if they are sown under optimal moisture, oxygen and soil conditions (Landis et al., 1996; Yahyaoğlu and Ölmez, 2005). 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. There is a great deal of variation in germination ability of seeds even within the same species (Dirr and Heuser, 1987; ISTA, 1966, 1993; Baskin and Baskin, 2001; Özdemir, 2003; Olmez et al., 2007). Poulsen (1996) and Landis et al. (1996) reported that dormancy among and within seeds of the same species varies with provenance, crop year and individual trees.

There are various germination obstacles in *Hippophae* sp. seeds resulting in propagation difficulties (Li and Schroeder, 2003; Özdemir, 2003; Busing and Slabaugh, 2008; Airi et al., 2009; Frochot et al. 2009). 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 for 15 to 90 days at 2 to 5°C, submersion in concentrated H_2SO_4 and soaking in different concentration of KNO_3, GA_3, thiourea pretreatments are well-known and used to increase germination percentage of *Hippophae* seeds (Li and Schroeder, 2003; Busing and Slabaugh, 2008; Airi et al., 2009). In addition, stratify-cation for 15 days is sufficient when seeds are sown in the fall (Grover et al., 1962). The optimum temperature for the germination of *H. rhamnoides* seed is the variable temperature of 20 to 30°C and germination tests may be run in 40 days on stratified seeds in sand flats (Tayier et al., 2006; Busing and Slabaugh, 2008).

The aim of this study was to examine the influence of cold stratification (30, 45, 60 and 90 days) and soaking in concentrated sulphuric acid (1, 2 and 3 min) pretreat-ments on dormancy of *H. rhamnoides* seeds.

MATERIALS AND METHODS

Ripe fruits were collected from *H. rhamnoides* individuals in September 2009, in Uzundere-Erzurum located in the eastern part of Turkey (latitude: 40°33' N, longitude: 41°35' E, altitude: 1030 m). 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-treatment applications were used to determine their effects on germination percentage (GP) and germination rate (GR) of *H. rhamnoides* seeds:

1. Cold stratification (CS) for 30, 45, 60 and 90 days.
2. Submersion in concentrated (98%) sulphuric acid for 1, 2 and 3 min.
3. Control (no treatment).

The seeds were stratified at 4 ± 1°C 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 moisture of the sand and the seeds was checked regularly so that the seeds would not get mouldy.

The seeds were sown in the Petri dishes on sand at 22 ± 1°C under dark conditions. The experimental design was a randomized complete block with three replications (30 seeds in each replication) for each treatment. Numbers of germinated seeds (evaluation done according to ISTA Rules (1993)) were recorded on the 4th, 7th, 10th, 14th and 21st days after the sowing. Calculation of percent germinated seeds and germination rate was then made for the 4th, 7th, 10th, 14th and 21st 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 is the germination rate; n is the number of days for each counting of germinated seeds; t is the number of germinated seeds at each counting day and T is the total number of germinated seeds

The whole experiment lasted for about 21 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 assumptions. The 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 highest germination percentage (100%) was obtained from the seeds soaked in H_2SO_4 for 1 min and the lowest one (96.7%) was obtained from the seeds soaked in H_2SO_4 for 2 min; although, there were no significant differences between germination percentages for pre-treatments (Table 1). According to Li and Schroeder (2003), Busing and Slabaugh (2008) and Airi et al. (2009), cold stratification for 15 to 90 days at 2 to 5°C, submersion in concentrate H_2SO_4 and soaking in different concentration of KNO_3, GA_3 and thiourea pre-treatments are used to increase germination percentage of *Hippophae* seeds. On the other hand, according to Airi et al. (2009)'s results, the treatments before sowing had significantly increased germination percentages as compared to control (24 to 30%). They obtained 63 to 71% germination percentages from cold stratification for 30 days. The germination percentage was 98.8% in cold stratification for 30 days in this study (Table 1). In addition to this, it was observed that *H. rhamnoides* seeds were germinating, while the stratification treatments for 45 to 90 days were still underway (Figure 1). Thus, it can be said that the dura-tion of cold stratification for 30 days is sufficient to improve dormancy of sea buckthorn seeds.

Table 1. Results of statistical analyses showing the relationship of the germination percentage (GP) and germination rate (GR) with different pre-treatments.

Pre-treatment	F-ratio	GP (%)	F-ratio	GR (day)
Soaking in H_2SO_4 for 2 min		96.7		4^a
Soaking in H_2SO_4 for 3 min	0.924^{NS}	98.3	121.0*	4^a
Cold stratification for 30 days		98.8		4^a
Control		98.8		8^b
Soaking in H_2SO_4 for 1 minute		100.0		4^a

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

Figure 1. Germinated *H. rhamnoides* seeds in the stratification media.

According to Frochot et al. (2009), *H. rhamnoides* seeds have a weak dormancy. Busing and Slabaugh (2008) reported that germination of untreated sea buckthorn seeds ranged from 6 to 60% after 60 days. Özdemir (2003) also determined the germination percentages between 11 and 80% from untreated seeds in the laboratory conditions for different populations at the end of the 28th day. In this study, higher germination result (98.8%) was obtained from the control seeds after 10 days than Özdemir (2003)'s results (Table 1 and Figure 2). Reasons for the high germination percentage of control seeds (98.8%) can be explained by their high full seed ratio or provenance of this seeds. In addition, Grover et al. (1962) stated that untreated seeds should be sown in the fall but stratification treatment for 15 days was required before sowing the seeds. Gültekin (2007) and Genç (2007) also reported that cold stratification for 12 to 14 weeks was necessary before sowing the seeds in the spring.

All these results are also consistent with the results stated by Poulsen (1996), Landis et al. (1996), Baskin and Baskin (2001) and Olmez et al. (2007). They

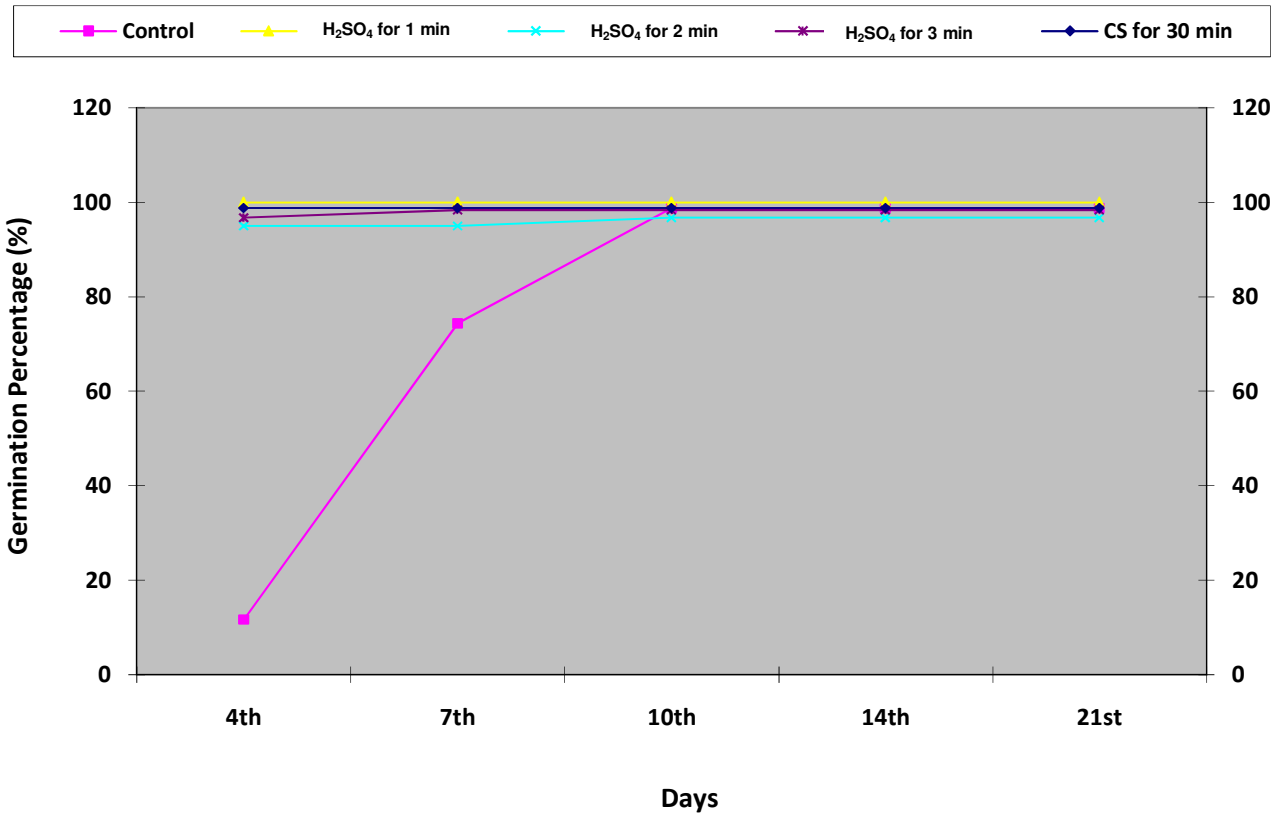

Figure 2. Germination rate of the seeds that were pre-treated with cold stratification for various days and sulphuric acid methods (CS: cold stratification)

reported that dormancy among and within seed of the same species varied with provenance, crop year and individual trees.

When the germination rate was considered, sulphuric acid and cold stratification treatments gave the best germination rate at 4 days, while it took 8 days for the control seeds to reach maximum germination rate (Table 1 and Figure 2).

Consequently, among all the pre-treatments applied to the *H. rhamnoides* seeds, soaking in sulphuric acid for 1 min resulted in the highest germination percentage (100.0%) and the shortest time before maximum germination rate (4 days). It is followed by pre-treatment of cold stratification (at 4 ± 1°C) for 30 days with 98.8% of germination percentage and 4 days before maximum of germination rate. Therefore, these results indicate that the pre-treatment by submersion in sulphuric acid for 1 min should be used to overcome dormancy of the *H. rhamnoides* seeds and 30 day cold stratification is sufficient to improve dormancy of sea buckthorn seeds.

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