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Effects of Sulphuric Acid and Cold Stratification Pretreatments on Germination of Pomegranate (*Punica granatum* L.) Seeds

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Abstract: This study was carried out to determine the effects of some pretreatments including submersion in concentrate H_2SO_4 for 15 and 30 min, cold stratification (20, 40 and 60 days) and submersion in concentrate H_2SO_4 for 15 min with 60 day cold stratification on seed germination and to investigate how to overcome dormancy of *Punica granatum* (L.) seeds. The seeds were sown in polyethylene pots in greenhouse and on seedbeds under open field conditions. The statistical approach was a randomized complete block design with three replications. Germinated seeds were observed periodically during 90 days to determine germination percent and rates. Both the highest germination percent (84.8%) and the best germination rates (30 and 36 days) were obtained in seeds which were soaked in H_2SO_4 for 15 min with cold stratification for 60 days. While the lowest germination percent (6.9%) was determined from seeds that were soaked in H_2SO_4 for 30 min the slowest germination rate (71 days) were obtained in control seeds sown under open field conditions.

Key words: *Punica granatum*, germination, pretreatments, seed dormancy

INTRODUCTION

Vegetation cover is one of the most important factors in preventing and controlling soil erosion. It promotes long-term soil surface protection by providing leaf cover that reduces rain-drop effects. In addition, it helps soils to develop a better structure through establishing root system, thereby increasing infiltration and soil stability (Pritchett and Fisher, 1987; Balcı, 1996). Woody vegetation provides better soil protection and lasts longer than annual plants because their roots deepen and improve the soil and the shade they provide facilitates ecosystem metabolism. These functions are essential for ensuring the soil stability and continuity of agricultural activities (FAO, 1989).

Seeds of many woody plant species can not germinate even if they are sown under the correct moisture, oxygen and soil conditions on that year (Ürgenç and Çepel, 2001). This inability to germinate is called seed dormancy and there are different types of seed dormancies that occur due to various reasons. Baskin and Baskin (2004) have classified the types of seed dormancy as physiological, morphological, morphophysiological, physical and combination dormancies. Some of the biological reasons for dormancy, listed by ISTA (1966 and 1993), are hard and impermeable seed coat, immature or dormant embryo, absence of endosperm and fleshy part of fruit. The degree of seed dormancy varies both among and within species. There are different methods and techniques to overcome seed dormancy depending on these factors. For example, in general, such pretreatments like floating on hot water, mechanical and chemical scarification and hot aeration are used for seed coat dormancy while the pretreatments of cold and warm stratifications are applied to dormancy caused by restrictions at the embryo level (Landis *et al*., 1996).

The most important step in the bio-preventive measures for checking erosion is the selection of suitable stabilizing plants. This procedure must also take the climatic and slope conditions into consideration. For example, plant species that develop taproot hold excess water and prevent landslides. In order to achieve effective protection in erosion control areas, the problems should de determined correctly and the required plant species should be chosen accordingly (Üçler *et al*., 2002).

The *Punicaceae* comprise several species of trees and shrubs within the genus *Punica*. *P. granatum* L., pomegranate, provides an edible fruit and the roots, fruit rind and seeds have medicinal uses (Ellis *et al*., 1985). Occurring in rocky and steep landscapes *P. granatum* is drought-tolerant plant that is important in preventing soil erosion. It is cultivated in temperate climates primarily for ornamental purposes (Güngör *et al*., 2002). According to some researchers, there are germination obstacles in the *P. granatum* (Riley, 1981; Piotto *et al*., 2003;

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Olmez *et al*., 2007) and thus, there are propagation difficulties of their seedlings. Cold stratification between 30 and 60 days at 1-5°C is well-known method to increase germination percent of *P. granatum* seeds (Riley, 1981; Olmez *et al*., 2007).

The aim of the present study was to examine the influence of some pretreatments on seed germination percent and rate of *P. granatum* seeds.

MATERIALS AND METHODS

Ripe fruits of the species were collected from wild in the Artvin region, located in the northeastern part of Turkey, between the altitudes of 200 and 1200 m, in August and September 2004. The seeds were separated from the fruit material, rinsed in tap water, dried in the shade and stored at stored at 4±1°C in plastic bags after ratios of full seed were determined.

The pretreatments for *P. granatum* seeds are below:

- Cold stratification (CS) for 20, 40 and 60 days
- Submersion in concentrate (98%) H_2SO_4 for 15 and 30 min
- Submersion in concentrate H_2SO_4 for 15 min+60-day CS
- Control (no treatment)

These were applied to determine the effects of the pretreatments on seed dormancy, Germination Percent (GP) and Germination Rate (GR).

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 CS was applied on the seeds was about 5±1°C. The moisture of the sand and the seeds were checked regularly so that the seeds would not get mouldy.

Seeds were sown in polyethylene pots in the greenhouse and on seedbed under open field conditions in the spring (March) of 2005. Polyethylene pots were filled with growing medium composed of forest soil, creek sand and manure (1:1:1). The experimental design was a randomized complete block with three replications (30 seeds for each replication) for every treatment. Numbers of germinated seeds (evaluation done according to ISTA Rules (1993) were recorded for 7th, 10th, 14th and 21st days after the sowing and recording continued weekly after the 21st-day for about 70 days counting. Calculation of germinating seeds was then made for 7th, 10th, 14th, 21st and so on days. Germination Percent (GP) and Germination Rate (GR) were determined for each pretreatment and filled seed ratios were used to determine GP. The formula used in determining GR values is as follows (Pieper, 1952);

$$GR = \frac{(n_1 \times t_1) + (n_2 \times t_2) + (n_3 \times t_3) + (n_i \times t_i)}{T}$$

Where:

GR : Germination rate
n : Number of days for each counting of germinated seeds
t : Number of germinated seeds in each counting day
T : Total number of germinated seeds

The whole experiment lasted for about 90 days when it was observed that the seeds stopped germinating. Data from the treatments was analyzed by the SAS and SPSS statistical softwares. The ANOVA and Duncan tests were used to compare treatment groups whether they showed any statistically significant differences with significance level (α) set at 0.05. Approximate account of Satterthwaite was used to compute the differentials denominator degree of freedom to test greenhouse and open field conditions (Satterthwaite, 1946; Milliken and Johnson, 1984).

RESULTS AND DISCUSSION

P. granatum seeds germinated both in the greenhouse and open field conditions. The highest GP was 84.8% in seeds that were soaked in H_2SO_4 for 15 min with cold stratification for 60 days and sown in the greenhouse (Table 1). On the other hand, while 75.6% and 69.9% of GPs were obtained from submersion in sulfuric acid for 15 and 30 min pretreatments in the greenhouse, respectively, 6.9% of GP was determined from open field conditions for both two durations of sulfuric acid treatments. CS pretreatments in the greenhouse also gave better GP results than CS pretreatments under open field conditions. The highest GP (64.2%) was obtained from seeds that were stratified for 60 days and sown in the greenhouse among the CS pretreatments (Table 1 and Fig. 1).

Riley (1981) reported that CS pretreatments at 1-5°C for 30 to 60 days gave good germination (%) within 14-30 days for *P. granatum* seeds. Olmez *et al.* (2007)

Table 1: Results of statistical analyses showing the relationship of the germination percent and rate with different pretreatments (Means in column with the same letter are not significantly different at $\alpha = 0.05$, G: Greenhouse, OF: Open field)

Pretreatments	F-ratio	GP (%)	F-ratio	GR (day)
Submersion in H_2SO_4 for 30 min (OF)		6.87a		53c-e
Submersion in H_2SO_4 for 15 min (OF)		6.87a		51c-e
Control (OF)		8.02a	14.29*	71f
40-day CS (OF)	22.28***	10.31a	7.37**	49cd
60-day CS (OF)		12.60a	8.28***	46bc
20-day CS (OF)		13.75a		54c-e
Submersion in H_2SO_4 for 15 min + 60-day CS (OF)		32.07b		30a
40-day CS (G)		52.69c		54c-e
Control (G)		59.56cd		61d-f
20-day CS (G)		60.71cd		62ef
60-day CS (G)		64.15cd		50cd
Submersion in H_2SO_4 for 30 min (G)		68.87cde		49c
Submersion in H_2SO_4 for 15 min (G)		75.60de		57c-e
Submersion in H_2SO_4 for 15 min+60-day CS (G)		84.77e		36ab

*VS: Greenhouse (Treatment), significantly different at $\alpha = 0.05$, **VS: Open Field (Treatment), significantly different at $\alpha = 0.05$, ***VS: Greenhouse *Open Field (Treatment), significantly different at $\alpha = 0.05$

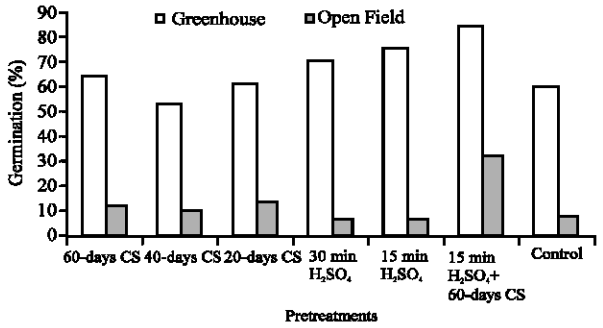

Fig. 1: Germination % of the seeds with different pretreatments

reported that *P. granatum* seeds germinated only in the greenhouse and the highest GP was 11.2% in seeds that were cold stratified for 60 days. They implied that germinations in the cold stratification medium can be reason of low GP for the seeds.

The best GR under open field conditions (30 days) and in the greenhouse (36 days) were obtained from seeds submersed in sulphuric acid for 15 min with 60 day-CS pretreatment. On the other hand, the slowest GR was 71 days for control seeds sown under open field conditions (Table 1).

Consequently, among all the pretreatments applied to the *P. granatum* seeds, soaking in H_2SO_4 for 15 min with CS for 60 days resulted in the best germination rate (30 days) and the highest germination percent (84.8%), followed by submersion in H_2SO_4 for 15 and 30 min (75.6 and 69.9%). Therefore, the results indicate that the pretreatment of submersion in H_2SO_4 for 15 min with 60-day cold stratification pretreatment and greenhouse conditions can be preferably used to overcome germination dormancy of *P. granatum* seeds.

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