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The Effects of Different Acid Treatment and Stratification Duration on Germination of *Cercis siliquastrum* L. Seeds

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Abstract

In this study, the effects of acid (H_2SO_4) treatment and moist stratification duration on the properties of seed coat, germination rate and duration of the *Cercis siliquastrum* L. were investigated. The rupture force and firmness of seed coat decrease during the acid treatment and moist stratification, stratification was for more than 30 minutes of these applications. Up to this treatment, rupture force and firmness were highly decreased. Contrary to this decrease, the germination rate was increased. The decrease in rupture force and firmness of seed coat were changed limitedly after 45 minutes and over acid treatments. From the results of this study, we conclude that eight weeks of moist stratification duration was optimal after 30 minutes of acid treatment to remove the physical and physiological dormancy of the seeds of the *C. siliquastrum* L.

Keywords: *C. siliquastrum* L., acid scarification, moist stratification, rupture force, firmness

Introduction

Cercis L. is a genus of eight species distinctly distributed in the temperate parts of China, North America and Mediterranean Area. In the Mediterranean from Pakistan and Afghanistan in the east to France, Greece and Turkey in the west, only one species, *C. orientalis* L., is used in landscapes (Dianxiang, 1999).

C. siliquastrum L. has potential as a landscape tree due to ornamental features including blackish bark, zigzag twigs, heart-shaped leaves and bright purplish-rose flowers (Gebre and Karam, 2004). Plants are valued particularly for their showy buds and flowers that appear before the leaves (Lamb *et al*., 1975; Dirr and Heuser, 1987; Clark and Bachtell, 1992; Zencirkiran, 2008). The plant is also used for borders, erosion control, windbreaks and wildlife plantings. Furthermore, the plants are well adapted to semi-arid conditions and are tolerant of air pollution and nutrient deficient soils (Gebre and Karam, 2004; Zahreddine *et al*., 2007). On the other hand, *C. siliquastrum* L. is one of the indigenous plant species comprising the Mediterranean elements of the flora of Turkey (Davis, 1970) and it is endangered due to different activities.

The seeds of *Cercis* have a physical (the hardness and impermeability of the seed coat) and physiological (embryo dormancy) dormancy. *Cercis* seeds generally require pregermination treatment to overcome dormancy, which is attributable both to a hard, impermeable seed coat and to some demonstrated embryo dormancy (Heit, 1967a,b; Hamilton and Carpenter, 1975; Zins, 1978; Riggio-Bevilacqua *et al*., 1985; Geneve, 1991; Tipton, 1992; Jones and Geneve, 1995; Rascio *et al*., 1998). Three pre-treatment, have proven satisfactory for overcoming *Cercis* L. seed coat impermeability: mechanical scarification; immersion in sulphuric acid; or in hot water. Acid treatment has generally produced more consistent or slightly better results (Afanasiev, 1944; Liu *et al*., 1981).

Acid treatments involves immersing *Cercis* seeds in concentrated sulphuric acid for 15 to 90 minutes at room temperature followed by through washing in water (Frett and Dirr, 1979; Liu *et al*., 1981; Dirr and Heuser, 1987; Zencirkiran, 2003, 2008; Gebre and Karam, 2004).

After scarification, cold stratification is generally required to overcome some degree of embryo dormancy and maximize seed germination. Germination differences between unstratified and cold-stratified seeds range from none, to fractional differences in the response of excised embryos, up to major differences for intact seeds (Afanasiev, 1944; Hamilton and Carpenter, 1975; Frett and Dirr, 1979; Geneve, 1991). Stratification of *Cercis* seed for 30 to 90 days at 1° to 5°C has proven satisfactory (Dirr and Heuser, 1987; Hartmann *et al*., 2002; Anonymous, 2003). On the other hand, the level of dormancy varies by species, seed source, seed lot, age of seed and perhaps other factors.

The aim of this study was determined that effects of H_2SO_4 and moist stratification treatments on rupture forces and firmness of *Cercis siliquastrum* L. seed coat and the most favourable treatments for germination rates.

Materials and methods

Plant material and treatments

Mature pods of *C. siliquastrum* L. were collected from a tree growing in its natural habitat in Bursa-Turkey. The seeds were extracted, cleaned and air dried in shade for 24 h.

The seeds were grouped and treated with concentrated (98%) H_2SO_4 for 0, 15, 30, 45, 60 and 120 minutes. After acid treatment, seeds were washed with water for 30 minutes. Seeds in the control group were soaked in water.

After these treatments, the seeds were divided into four main groups and a first batch of seeds was sowed into viols which were filled with peat and placed under the plastic greenhouse at 25°C day/15°C night temperature for the germination test. For the stratification treatment; a second batch of seeds for 30 days and a third batch of seeds for 60 days kept in moistened perlite at 4°C in darkness.

The seeds which completed the stratification duration, as made in the previous sowings, were sowed into viols filled with peat and placed under the plastic greenhouse at 25°C/15°C day/night temperature for the germination test. Experiments were made with three replications containing 30 seeds in each replications.

Viability test

In order to determine the viability of *C. siliquastrum* L. seeds, the topographical tetrazolium test of viability was used. The solution was triphenyl tetrazolium chloride 1% (TCC). For each species, 100 seeds were used in 4 replicates of 25 seeds (Moore, 1985; ISTA, 1993). The seeds were soaked for 20 h in tap water. Pre-moistening is necessary because staining is more intense and imbibed seeds are generally less fragile than dry seeds. The seed coat of pre-moistened seeds was carefully removed and the embryos were immersed in tetrazolium where they remained for 24 h in a dark chamber at room temperature. The embryos with red coloured radicle and cotyledons were considered to be viable.

Germination test and the means of germination durations

Seed germination was defined as the appearance of a radicle, at least 2 mm long, according to the guidelines of the International Seed Testing Association (1993). Germinated seeds were counted every 4 days for 5 weeks. Each germinated seed was removed in order to avoid counting confusion. At the same time, the means of germination durations were calculated at the end of experimental periods.

Rupture force and firmness

A used dynamometer (Sundoo, 500 SH, accuracy 0.1 N, China) with 500 N capacity to measure rupture force to evaluate treatment effect on seed coat rupture. The loading velocity of the dynamometer was constant at 35 mm/min during measurements. For each test, a single seed was placed on its minor axes on a flat steel plate and then compressed with a 12 mm-diameter probe. Maximum rupture forces were carried out with 30 repetitions.

Firmness values (Q) at rupture point were determined by using the following equation (Ünal *et al.*, 2009).

$$Q = \frac{F_r}{D_r}$$

where; F_r is the rupture forces (N); D_r is the deformation values at rupture point (mm). The average deformation values are used according to Ünal *et al.*, (2009).

Statistical analysis

The data obtained from acid treatment and stratification duration were statistically analyzed by using MINITAB 14.0, 1996. Significance of difference values were determined using Tukey's procedure for multiple range test at the 0.05 significance level.

Results and discussion

In the viability study, the seeds were rated viable or non-viable according to embryo staining. The embryos which were stained entirely red, or with small unstained areas were considered to be viable. The viability of *C. siliquastrum* L. seeds was 90%.

From the results of studies, we observed that acid treatment time and stratification duration and their interactions with each other have great effect on the germination ratio (Tab. 1, 2 and 3). On the other hand, acid treatment time and stratification duration are found statistically important on the germination time (Tab. 1 and 2), rupture

Tab. 1. Germination and mean germination duration changes occurred in different stratification duration of *C. siliquastrum* L. seeds

Stratification duration (weeks)	Germination (%)	Mean germination duration (days)
0 (Control)	27.91 a*	39.16 a*
4	53.15 b	25.16 b
8	57.21 c	18.33 c

* Values in the same group are not different according to Tukey's multiple range test at the 0.05 significance level.

Tab. 2. Germination and mean germination duration changes occurred in different acid treatment time of *C. siliquastrum* L. seeds

Scarification with H_2SO_4 (min)	Germination (%)	Mean germination duration (days)
0 (Control)	1.12 f*	43.00 a*
15	41.21 e	31.00 b
30	52.42 d	25.60 c
45	64.53 a	23.66 d
60	62.41 b	21.70 de
120	54.86 c	20.33 e

* Values in the same group are not different according to Tukey's multiple range test at the 0.05 significance level

Tab. 3. Germination of *C. siliquastrum* L. seeds as influenced by acid treatment time and moist stratification

Stratification duration (weeks)	Scarification with H_2SO_4 (min)	Germination (%)
0 (Control)	0 (Control)	0.016 j*
	15	0.35 j
	30	0.60 j
	45	58.60 fg
	60	56.60 gh
	120	51.30 ı
4	0 (Control)	0.016 j
	15	63.30 d
	30	71.66 b
	45	66.66 c
	60	62.30 de
	120	55.00 h
8	0 (Control)	0.016 j
	15	60.00 ef
	30	85.00 a
	45	68.33 c
	60	68.33 c
	120	58.30 fg

* Values in the same group are not different according to Tukey's multiple range test at the 0.05 significance level

Tab. 4. Rupture force and firmness values of *C. siliquastrum* L. seeds as influenced by moist stratification durations

Stratification duration (weeks)	Rupture force (N)	Firmness (N/mm)
0 (Control)	318.59 a*	1225.34 a*
4	139.79 b	274.09 b
8	88.41 c	160.74 c

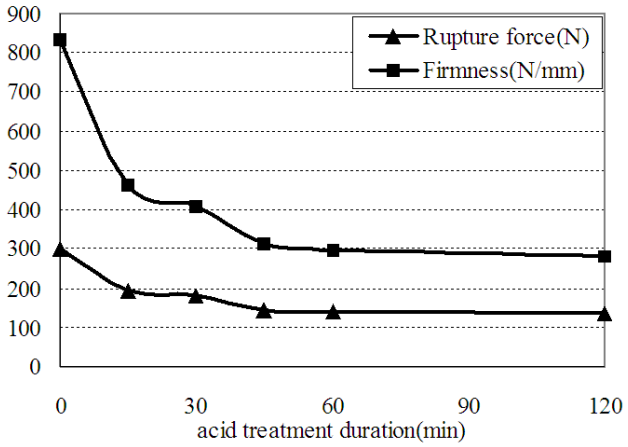
* Values in the same group are not different according to Tukey's multiple range test at the 0.05 significance level

force and firmness (Tab. 4 and Fig. 1), whereas interactions of their are not found important statistically.

Mean germination time decreases according to stratification duration and acid treatment time. Low value of germination time was obtained in two situations: 1) It was about 18.3 days in the seeds exposed to 8 weeks of stratification duration; 2) It was about 20.3 days in the seeds treated with acid for 120 minutes. These values were shown in Tab. 1 and 2.

In the control seeds, germination rate was determined about 27.9% meanwhile this value was determined about 57.2% for the seeds which had 8 weeks stratification duration (Tab. 1). On the other hand, acid applications have positive effect on germination rates and the highest value of germination rate was obtained about 64.5% for the seeds exposed to 45 minutes of acid treatment (Tab. 2).

Consideration of interactions with each other, we conclude that maximum value of germination was 85% in the seeds which exposed to 8 weeks of stratification duration after 30 minutes of acid treatment time (Tab. 3).



Acid treatment duration(min)	Rupture force(N)	Firmness(N/mm)
0	300.64 a*	835.11 a*
15	193.48 b	460.66 b
30	180.39 bc	409.97 bc
45	144.81 c	314.80 c
60	139.75 c	297.34 c
120	134.51 c	280.22 c

Fig. 1. Rupture force and firmness values of *C. siliquastrum* L. seeds as influenced by acid treatment durations. (* Values in the same group are not different according to Tukey's multiple range test at the 0.05 significance level)

Rupture force and firmness values were decreased according to increase in stratification duration and acid treatment time. For control seeds, rupture force was obtained about 318.59 N and after 8 weeks of stratification duration this value was decrease to 88.41 N. Firmness was determined about 160.74 N/mm at the end of the same stratification duration (Tab. 4 and Fig. 1).

Germination (%), rupture force and firmness distributions in different stratification durations are shown in Fig. 2-4. As shown in these figures, we observed that rupture force and firmness values are dramatically decreased in the first four weeks of the stratification duration. But germination values ranged from 30% to 60% and we can say that in contrast to rupture force and firmness, germination rates are highly increased in this stratification duration. Between four and eight weeks of stratification duration, these values are slowly changed with time and considered as constant values.

Tab. 5. Equations obtained regression analyses

Variable	Function	R^2
t (time)	$F_G = (-0.6619)t^2 + (8.9575)t + 27.91$	1
t (time)	$F_R = (3.9819)t^2 - (60.628)t + 318.59$	1
t (time)	$F_F = (26.184)t^2 - (342.55)t + 1225.30$	1

Stratification time: t (weeks), Germination: FG (%), Rupture force: FR (N), Firmness: FF (N/mm)

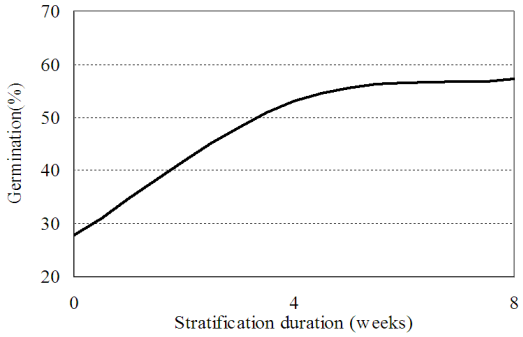


Fig. 2. Germination (%) distribution with stratification time (during stratification period)

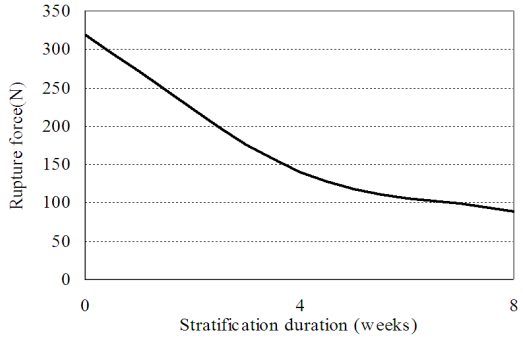


Fig. 3. Rupture force (N) distribution with stratification time (during stratification period)

According to results of regression analyses, we obtained three polynomial equations and were given in Tab. 5.

From the findings of this study, we determine the effects of stratification duration and acid treatment time on the mean germination duration, germination rates, rupture force and firmness of the seeds of the *Cercis siliquastrum* L.

In the acid treatments, we observed that rupture force and firmness values were decreased depend on diminish in the thickness of the seed coat. The decrease in the rupture force and firmness values was about 40% at 30 minutes of acid treatment and after 30 minutes of acid treatment, these values were obtained about 25%. Mean germination duration declined and germination rates increased due to decrease in the rupture force and firmness values (Tab. 2). The negative effects of hard seed coat on the germination

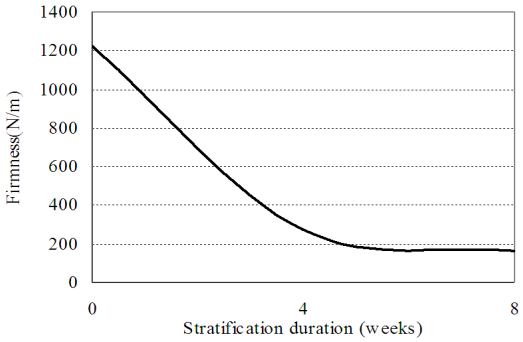


Fig. 4. Firmness (N/mm) distribution with stratification time (during stratification period)

can compensate with acid treatment (Riggio-Bevilacqua *et al.*, 1985; Rascio *et al.*, 1998; Smiris *et al.*, 2006; Afanasiev, 1944; Frett and Dirr, 1979; Liu *et al.*, 1981; Dirr and Heuser, 1987, Zencirkiran, 2003, 2008; Gebre and Karam, 2004) and this treatment positively affect the germination rate.

When the moist cold stratification duration taken into consideration; it is noticed that in 8 weeks time the germination is obtained at the highest rate and in the shortest time. Though the result gained from the germination rate is parallel to Rascio *et al.* (1998), it shows no similarity to the results of Gebre and Karam (2004), who reported that the moist stratification duration, has to be longer (16 weeks). Nevertheless, it is stated in the previous studies that, different stratification duration has different effects on the germination rate. Therefore, it is clear that, no matter the plants have the same origin; there exists a close relationship between the ecological condition of the place where the plant grows and the seed dormancy (Meyer *et al.*, 1990; Thampson, 1981).

On the other hand, rupture force and firmness values were decreased in parallel with the increase in moist stratification duration and these decreases in rupture force and firmness values showed that moist stratifications should be made before seed sowing.

As expressed in the previous researches, (Afanasiev, 1944; Heit, 1967a,b; Hamilton and Carpenter, 1975; Zins, 1978; Profumo *et al.*, 1979; Riggio-Bevilacqua *et al.*, 1985; Dirr and Heuser, 1987; Geneve, 1991; Tipton, 1992; Mcdonald, 1993; Jones and Geneve, 1995; Rascio *et al.*, 1998; Hartman *et al.*, 2002; Anonymous, 2003), the existence of double dormancy mechanism as physical and physiological of *C. siliquastrum* L. seed was confirmed once again with the results of stratification duration following the acid treatment. A maximum value of germination was obtained about 85% by 8 weeks of stratification following the 30 minutes of acid application (Tab. 3). These results are in agreement with Jones and Geneve (1995) and Liu *et al.* (1981). These researchers also reported that the moist stratification treatments following the scarification increase the germination.

Conclusions

We conclude that, the dormancy of *Cercis siliquastrum* L. seeds can remove when the acid and stratification applications were performed together.

Consideration of germination rate, mean germination duration, rupture force and firmness; the best germination rate was obtained about 85% by 8 weeks of moist stratification duration following the 30 minutes of acid treatment. These treatments (8 weeks of moist stratification duration following the 30 minutes of acid treatment) were found to be the best treatment for a nursery to use. However, findings may change with seed source, seed lot, age of seed and

the other ecological factors therefore researchers have to consider these factors in their later studies.

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