

SCIENTIFIC ARTICLE

Effects of dormancy breaking methods on germination of *Cercis siliquastrum* and *Spartium junceum* and seedling growth

Mina Taghizadeh 10°*, Fahimeh Sadat Sajadi 10

¹Arak University, Faculty of Agriculture and Environmental Science, Department of Horticultural Science, Arak, Iran.

Abstract

Seed dormancy is a mechanism of long survival that is ecologically important for seed propagation and dispersal and the expansion of plant populations. The impermeability of the seed coat in the Fabaceae family is due to a layer of sclerotic cells. Two experiments were conducted to investigate the effect of different seed treatment on germination parameters and seedling growth in *Cercis siliquastrum* and *Spartium junceum*. Experimental treatments comprised of chemical and thermal scarification treatment consisting of boiling water (2, 5, 10 min), H_2SO_4 (30, 60 min) and GA (0, 500 and 1,000 mg L⁻¹) in *C. siliquastrum* and boiling water (2, 5, 10 min), H_2SO_4 (2, 5 min) in *S. junceum*. The results presented here indicate that chemical scarification by soaking in sulfuric acid for 30 min and 2 min in *C. siliquastrum* and *S. junceum*, respectively were the most efficient methods to breaking the seed dormancy. The application of these methods promoted the highest values of indices seedlings. In the light of the found results, it revealed that *C. siliquastrum* and *S. junceum* seeds are affected by a coat dormancy, which can be removed by a chemical-thermal scarification with sulfuric acid and boiling water. The data obtained contribute to a better comprehension of propagation and establishment of these shrubs ornamental by seedling.

Keywords: boiling water, germination, gibberellic acid, scarification, sulfuric acid.

Resumo

Efeito de métodos de quebra de dormência na germinação e no crescimento de mudas de *Cercis siliquastrum* e *Spartium junceum*

A dormência das sementes é um mecanismo de longa sobrevivência que é ecologicamente importante para a propagação e dispersão das sementes e expansão das populações de plantas. A impermeabilidade do tegumento da semente da família Fabaceae se deve à camada de células escleróticas. Dois experimentos foram conduzidos para investigar o efeito de diferentes tratamentos de sementes nos parâmetros de germinação e crescimento de plântulas em *Cercis siliquastrum* e *Spartium junceum*. Os tratamentos experimentais consistiram de escarificação química e térmica com água fervente (2, 5, 10 min), H_2SO_4 (30, 60 min) e GA (0, 500 e 1.000 mg L⁻¹) em *C. siliquastrum* e água fervente (2, 5, 10 min), H_2SO_4 (2, 5 min) em *S. junceum*. Os resultados indicam que a escarificação química por imersão em ácido sulfúrico por 30 min e 2 min em *C. siliquastrum* e *S. junceum*, respectivamente, foram os métodos mais eficientes para quebrar a dormência das sementes. A aplicação desses métodos promoveu os maiores valores de índices de mudas. Diante dos resultados encontrados, revelou-se que sementes de *C. siliquastrum* e *S. junceum* apresentam dormência tegumentar, que pode ser removida por escarificação químico-térmica com ácido sulfúrico e água fervente. Os dados contribuem para melhor compreensão da propagação e estabelecimento por mudas desses arbustos ornamentais.

Palavras-chave: ácido giberélico, ácido sulfúrico, água fervente, escarificação, germinação.

Introduction

Seed dormancy is a mechanism of long survival that is ecologically important for seed propagation and dispersal and the expansion of plant populations. One of the most important reasons is the lack of germination stimulants and chemical inhibitors in the seed coat. Seed physical dormancy in the Fabaceae family is caused by impermeable seed shells to water. Break of scleroid cells or mechanical pressure can cause water absorbed by the seed and eventually lead to seed germination (Silva et al., 2021). Methods of breaking seed dormancy thermal scarification, mechanical scarification and acid scarification are effective in breaking the dormancy of hard-coated seeds. These treatments might crack the seed coat, increasing permeability and permitting absorption of water (Gao et al., 2020).

*corresponding author: m-taghizadeh@araku.ac.ir

https://doi.org/10.1590/2447-536X.v29i1.2528

Received June 20, 2022 | Accepted Feb 08, 2023 | Available online Mar 13, 2023 Licensed by CC BY 4.0 (<u>https://creativecommons.org/licenses/by/4.0/</u>) Editor: Maurecilne Lemes da Silva Carvalho Spanish broom (*S. junceum* L.) grows naturally on rocky ground or over steep slopes near the sea. Besides its xeromorphic adaptations, it has also adapted to higher salt concentrations in the soil. The vegetative organs of the spanish broom can adapt to dry areas and infertile soils and thus prevent soil erosion (Pulatkan et al., 2017). The seeds of this plant have an impermeable and very hard cover and prevent germination due to preventing water entry, gas exchange and root emergence (Yucedag and Gultekin, 2011). *Cercis siliquastrum*, commonly called Judas tree or Chinese redbud, is a deciduous, often multi-trunked, brush tree with a rounded crown (Sabeti, 1994). *C. siliquastrum* is propagated by seeds, but seed dormancy of this plant prevents its uniform and rapid germination (Koneshloo, 1994).

It has been reported that in some cases the seeds of this plant need 4 to 12 weeks of scarification after stratification, because in addition to the physical dormancy of the seed, it also has endogenous dormancy (embryo). Studies on *C. siliquastrum* seed dormancy have shown that sulfuric acid treatment with scarification had the highest germination percentage (Gebre and Karam, 2004). In another study, Zincirkiran et al. (2010) introduced the best seed germination treatment for *C. siliquastrum* seed soaking with 98% sulfuric acid for 30 minutes with scarification for eight weeks. In another study, to improve dormancy failure and germination of *C. siliquastrum* seeds, soaking the seeds in 90% sulfuric acid for 20 to 40 minutes with cold and wet scarification (in sand medium) for 90 to 120 days is mentioned (Pipinis et al., 2011).

Understanding of the germination of seeds is critical for by abundantly producing seeds of high viability. Therefore, the aim of this study was to determine the effects of soaking treatments with sulfuric acid, gibberellic acid and boiling water in removing the hard seed cover of *C. siliquastrum* and *Spartium junceum*, breaking the seed dormancy and determining the most optimal treatment for germination and seedling growth.

Materials and Methods

Plant material

The *C. siliquastrum* and *S. junceum* seed capsules material used in the study were collected from an adult shrub located in campus of Arak University, Iran, with a longitude of 496, latitude of 34 and an altitude of 1872 m above sea level during end-spring. Capsules were dried at room temperature (25 °C) for 1 week. Dried capsules were manually broken, and seeds were removed. Two independent experiments were conducted in the research laboratory of the Faculty of Agriculture and environmental science of Arak University, Iran.

Methods to break seed dormancy of C. siliquastrum

Before breaking the seed dormancy, two treatments were used to render the seed coat of *Cercis chinensis*. The

seeds were immersed in boiling water at 100 °C for 2, 5 and 10 min then cooled gradually to room temperature for 24 h in thermal scarification. Seeds immersed in sulfuric acid (H_2SO_4) (98%) in two times of 30 and 60 min and the solutions were periodically stirred during the treatment in the acid scarification. Then, the seeds were rinsed with running water for 24 h. Subsequent to these pretreatments, seeds were immersed in 0, 500 and 1,000 mg L⁻¹ gibberelic acid (GA) solution for 24 h at room temperature.

Methods to break seed dormancy of Spartium junceum

Thermal and mechanical scarification treatments were used to thinning the seed coat of *Cercis chinensis*. The seeds were immersed in boiling water at 100 °C for 2, 5 and 10 min then cooled gradually to room temperature for 24 h in thermal scarification. Seeds soaked in sulfuric acid (H_2SO_4) (98%) in two times of 2 and 5 min and the solutions were periodically stirred during the treatment in the mechanical scarification. Then, the seeds were rinsed with running water for 24 h. Immersion in water at room temperature (25 ° C) for 24 h was considered as a control in each experiment.

Conditions of seed germination

The seeds were disinfected after each treatment. They were immersed in 30% (v v⁻¹) Clorox TM solution (active chlorine 5%) for 10 minutes and then washed three times with sterile distilled water. The seeds were immediately placed to germinate in sterilized petridish containing whatman filter paper, and then maintained under growth chamber conditions (25 ± 1 °C in absolute darkness) for a period of 21 days.

Measurements of germination and seedling growth

Seed germination was recorded daily and expressed as a percentage of the total number of tested seeds. The seedling evaluation (root length, stem length, leaf length and width) was measured. In addition, at the end of the study (after three weeks), several germination parameters were calculated to characterize the seed dormancy. Germination Index (GRI), Coefficient Velocity Germination (CVG), Mean Germination Time (MGT), Mean Daily Germination (MGR), Final germination percentage (FGP), Seed Vigor Index (SV), Tolerance Index (TI) and Dormancy percentage (DP) in different treatments were calculated by equations (Table 1). The final germination percentage (FGP) represents the total number of seedlings at the end day of the test. The mean germination rate (MGR) is defined as the reciprocal of the mean germination time since the mean germination rate increases. MGT is a measure of the time spread of the germination while MGR is the reciprocal of MGT. GRI calculations merely show the percentage of germination per day, coefficient of velocity of germination (CVG) gives an indication of the rapidity of germination.

Germination indices	Equations	References
Germination Index (GI)	$GRI = \frac{G_1}{1} + \frac{G_i}{i} + \frac{G_2}{2} + \cdots$	Luo et al., 2018
Coefficient Velocity Germination (CVG)	$VG = \frac{\Sigma N_i}{(\Sigma N_i T_i)} \times 100$	Adetumbi et al., 2019
Mean Germination Time (MGT)	$G = \frac{\Sigma N_t}{\Sigma N_i}$	Seng and Cheong, 2020
Final Germination Percentage (FGP)	$FGP = 100 \times \frac{ng}{nt}$	Seng and Cheong, 2020
Seed Vigor Index (SVI)	$SVI = \frac{\text{GP} \times \text{SL}}{100}$	Adetumbi et al., 2019
Mean Germination Rate (MGR)	$MGR = \frac{\Sigma N_t}{\Sigma t}$	Seng and Cheong, 2020

Statistical analysis

A completely randomized experimental design (CRD) and four replications was used in this study.

The experimental data were analyzed using SAS version 19.1 software. The data were submitted to analysis of variance (ANOVA) used to compare the mean and to determine the significance of statistical differences in treatments at 1% level.

Results

Effect of different methods to break seed dormancy of *C. siliquastrum*

The effect of different methods to break seed dormancy on *C. siliquastrum* germination indices showed that the highest Coefficient Velocity Germination (CVG) (32.56) in 30 min of immersion in sulfuric acid + GA 1,000 mg L⁻¹ that this treatment was significantly different from other treatments. The lowest rate of Coefficient Velocity Germination (CVG) (12.44) in GA 500 mg L⁻¹ and 2 min of boiling water, was obtained (Figure 1A). There was no significant effect of GA+ boiling water (all of concentrations and times) on CVG trait, compared to each other while there was significant effect these treatments compared to GA+H₂SO₄. According to the results of this study, the highest mean germination time (8.39 days) in 1,000 mg L⁻¹ GA and 2 min immersion in boiling water while, this treatment was not significantly different from 500 mg L⁻¹ GA + 2 min boiling water, 1,000 mg L^{-1} GA + 2 and 5 min boiling water treatments. The lowest mean germination time (3.1 days) in 1,000 mg L⁻¹ GA and 30 minutes of immersion in 98% sulfuric acid was observed that this treatment was not significantly different from 500 and 1,000 mg L^{-1} GA + 60 min H₂SO₄ treatments (Figure 1B).

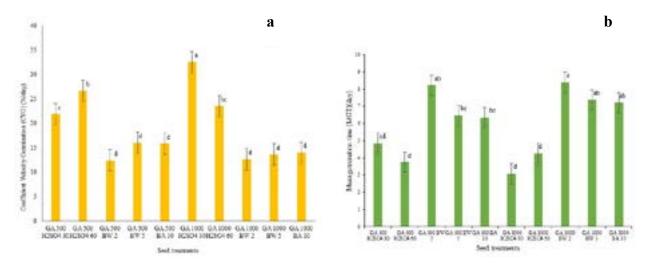


Figure 1. The effect of sulfuric acid, boiling water and gibberellic acid treatments on: a) Coefficient Velocity Germination (CVG) and b) Mean germination time (MG), seed of *Cercis siliquastrum*. The bar graph represents the mean and standard deviation values for three replications. Means with the different letters indicate significant differences based on Duncan's test at $\alpha = 0.01$.

According to a preliminary study, the final germination percentage (FGP) was 25% without any treatment in the seeds of Cercis siliquastrum. The results of this experiment showed that the highest final germination percentage (FGP) was obtained in 98% sulfuric acid (both 30 and 60 min). However, there were no significant differences in the treatments of GA and sulfuric acid on the final germination percentage. Boiling water had slight effect on seed germination. The lowest germination percentage (36%) was related to 2 min immersion in boiling water + 500 mg L⁻¹ GA, which was not significantly different from 5 and 10 min of immersion in boiling water (Figure 2A). According to the results, the highest Average Germination Rate (MGR) (4.86 seeds per day) in 30 min immersion in 98% sulfuric acid + 1,000 mg L^{-1} GA and the lowest Average Germination Rate (MGR) (0.63 seeds per day) in 2 min of immersion in boiling water + 500 mg L^{-1} GA were obtained (Figure 2B). There were no significant differences in the treatments of 500 mg L⁻¹ GA+ boiling water 2 min and GA 1,000 mg L⁻¹ + boiling water 2, 5 and 10 min on MGR. Also, the highest germination index (33.51) was obtained in 30 min of immersion in sulfuric acid + 1,000 mg L⁻¹ GA and the lowest rate of this trait (5.33) in 2 min of boiling water + 500 mg L^{-1} GA. Treatment of 30 min of immersion in sulfuric acid + 1,000 mg L⁻¹ GA was significantly different from other treatments (Figure 2C). The effect of 98% sulfuric acid and boiling water treatments on seed dormancy index is inversely related to seed germination rate. Hence, that the lowest percentage of seed dormancy was observed in 30 min of immersion with 98% sulfuric acid + 500 mg L^{-1} GA and the highest percentage of seed dormancy was detected in boiling water at 100 ° C for 2 min + 500 mg L^{-1} GA, nevertheless, this treatment was not significantly different from treatment of 1,000 mg L⁻¹ GA+ 2 min Boiling water (Figure 2D).

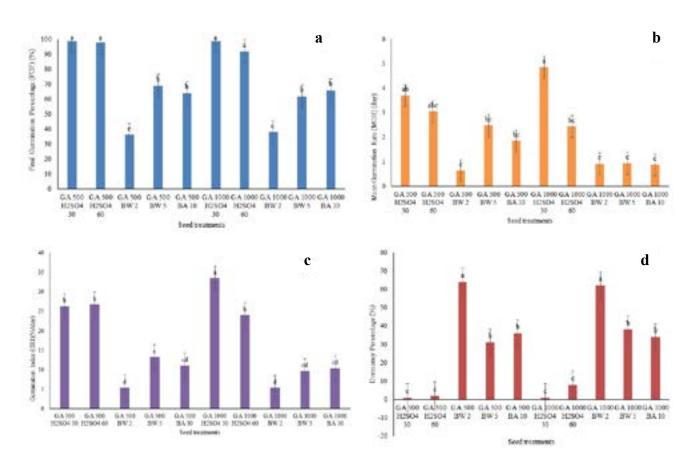


Figure 2. The effect of sulfuric acid, boiling water and gibberellic acid treatments: a) Final Germination Percentage (FGP), b) Mean Germination Rate (MGR), c) Germination Index (GRI) and d) Dormancy Percentage seed of *Cercis siliquastrum*. The bar graph represents the mean and standard deviation values for three replications. Means with the different letters indicate significant differences based on Duncan's test at $\alpha = 0.01$.

According to the results of comparing the mean effect of different treatments on seed dormancy of *C. siliquastrum* seeds, the highest and the lowest rate of root growth (4.42- 0.39 cm) and hypocotyl growth (8.24- 1.26 cm) was observed in the treatment of sulfuric acid for 30 min+ GA 500 mg L^{-1} and 10 min of boiling

water+ GA 1,000 mg L⁻¹, respectively. Also, the highest length (1.22 cm) and width (0.62 cm) of leaves in 60 min sulfuric acid treatment+ GA 500 mg L⁻¹ and the lowest length (0.3 cm) and width (0.15 cm) of leaves in 10 minutes of boiling water+ GA 1,000 mg L⁻¹ was obtained (Table 2).

Table 2. The effect of sulfuric acid, boiling water and gibberellic acid tr	reatments on growth of <i>C. siliquastrum</i> seedling.
---	---

Treatments	Root length(cm)	Shoot length(cm)	Leaf length(cm)	Leaf width(cm)
$GA_{500}H_{2}SO_{4}30$	$4.42 a \pm 0.69$	$8.24 a \pm 2.03$	$1.07 \text{ abc} \pm 0.05$	$0.55 \text{ ab} \pm 0.01$
$GA_{500}H_{2}SO_{4}60$	$3.01 \text{ ab} \pm 0.58$	7.45 ab ± 1.11	$1.12 \text{ abc} \pm 0.07$	$0.57 \text{ ab} \pm 0.02$
GA ₅₀₀ BW 2	$2.14 \text{ bc} \pm 0.38$	$6.7 \text{ abcd} \pm 1.01$	$1.22 a \pm 0.11$	$0.62 \ a \pm 0.07$
GA ₅₀₀ BW 5	$2.44 b \pm 0.40$	$6.9 \text{ abc} \pm 0.51$	$1.2 \text{ ab} \pm 0.04$	$0.6 \text{ ab} \pm 0.02$
GA ₅₀₀ BW 10	$2.42 b \pm 0.73$	$5.87 \text{ abcd} \pm 0.86$	$1.06 \text{ abc} \pm 0.41$	$0.62 a \pm 0.01$
$GA_{1,000} H_2 SO_4 30$	$1.82 \text{ bc} \pm 2.10$	$3.5 \text{ cde} \pm 4.05$	$0.51 \text{ cd} \pm 0.59$	$0.28 \text{ bc} \pm 0.32$
$GA_{1,000} H_2 SO_4 60$	$1.59 \text{ bc} \pm 1.97$	$3.09 \text{ de} \pm 3.57$	$0.53 \text{ bcd} \pm 0.61$	$0.28 \text{ bc} \pm 0.32$
GA _{1,000} BW 2	$2.24\ b\pm0.75$	$5.97 \text{ abcd} \pm 0.98$	$1.19 \text{ ac} \pm 0.07$	$0.58 \text{ ab} \pm 0.02$
GA _{1,000} BW 5	$1.56 \text{ bc} \pm 1.21$	$3.97 \text{ bcde} \pm 2.66$	$0.88 \text{ abcd} \pm 0.59$	$0.47 \text{ ab} \pm 0.31$
GA _{1,000} BW 10	$0.39 c \pm 0.78$	$1.26 e \pm 2.53$	$0.3 d \pm 0.60$	$0.15 c \pm 0.30$

Means followed by the same letters are not significantly different from each other (p < 0.01) as determined by Duncan's Multiple Range Test (DMRT).

Effect of different methods to break seed dormancy of *Spartium junceum*

The application of different methods to break dormancy did not affect most traits related to seedling growth of the shoot and roots. Figure 3 shows the effect of the different treatment with sulfuric acid and boiling water on the germination *S. junceum* of seeds. Treatments of sulfuric acid and boiling water on seed germination indices in *Spartium junceum*, showed that the highest Coefficient Velocity Germination (CVG) (16.77) was observed in sulfuric acid treatment for 2 min. Increasing the time of immersion in sulfuric acid (5 min) presented a decrease in Coefficient Velocity Germination however this treatment was not significantly different from sulfuric acid for 2 min. The lowest Coefficient Velocity Germination (8.21) was related to the control. There was no significant difference between control and boiling water treatments for this trait (Figure 3A). The sulfuric acid treatment reduced the Mean Germination (MG) in *S. junceum* seeds, while boiling water treatment enhanced the MG in seeds. The maximum (12.37 days) and lowest (6.59 days) Mean Germination were observed in the control (which was not statistically significant different from boiling water treatments) and two min of sulfuric acid, respectively (Figure 3B).

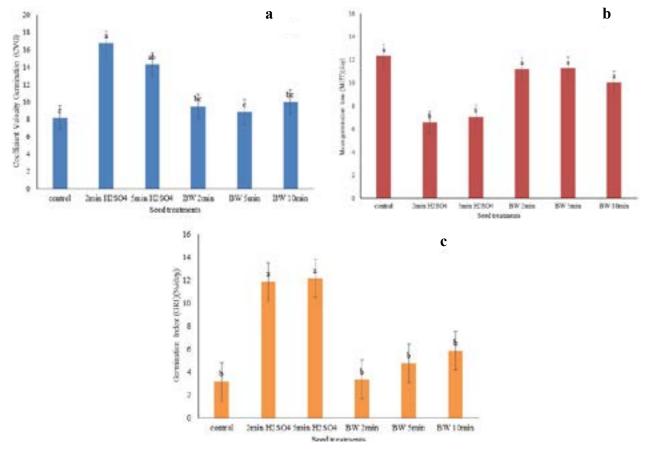


Figure 3. The effect of sulfuric acid and boiling water treatments: a) Coefficient Velocity Germination (CVG), b) Mean germination time (MG) and c) Germination Index (GRI) seed of *S. junceum* L. The bar graph represents the mean and standard deviation values for three replications. Means with the different letters indicate significant differences based on Duncan's test at $\alpha = 0.01$.

A comparison of the mean effect of sulfuric acid and boiling water treatments on *S. junceum* seed Germination Index (GRI) is shown in Figure 3C. Immersion of *S. junceum* seeds in 98% sulfuric acid increased the seed Germination Index (GRI). The minimum Germination Index (GRI) (3.15) in the control and the highest Germination Index (GRI) in 5 min of sulfuric acid (12.15) was obtained. The sulfuric acid for 2 and 5 had no significant difference on this trait. No significant difference was observed between control and boiling water treatment.

Discussion

In this study, it was found that sulfuric acid and gibberellic increased the germination rate of *C. siliquastrum* L. seeds. The highest Coefficient Velocity Germination (CVG) and Average Germination Rate (MGR) were obtained in 30 min of immersion in sulfuric acid with 1,000 mg L⁻¹ gibberelic. This conclusion agrees with Gao et al. (2020) pointed out that treatment with exogenous GA₃ following scarification significantly improved germination in *C. chinensis* seeds. Thermal scarification, mechanical scarification, and acid scarification are effective in breaking seed-coat dormancy because the treatments cause disruption of the seed coat, resulting in rapid imbibition. The hard seed coat of *C. chinensis* severely delayed water imbibition, which was one cause of seed dormancy. Structural studies of hard-coated seeds are significant for enhanced considerate of the reasons and release of seed shown a thin condensed layer of colloid material located between the inner surface of the test and the embryo, which is the remnant tissue of the endosperm (Gao et al., 2020).

The encouraging effect of GA_3 in the current study designates that seeds may also have 'physiological' dormancy also entitled 'combined' dormancy, which was broken by the application of GA_3 (Iralu and Upadhaya, 2018). Gibberellin encourage germination by prompting hydrolytic enzymes that weaken the obstacle tissues such as the endosperm or seed coat, inducing mobilization of nutrition reserves in seed and motivating development of the embryo. Major improvement in seed germination might be due to enhanced breakdown of reserve metabolites existing in seed. Furthermore, the gibberellic acid has encouraging effect on germination due to its phytohormone regulation capability and retarding effect against abscisic acid present in dormant seeds (Ullah et al., 2020).

Sulfuric acid is supposed to interrupt the seed coat and expose the lumens of the macrosclereids cell, allowing imbibition of water which activates the release of simple sugar that could be readily used for protein synthesis, thereby encouraging germination (Rusdy, 2017). The time required for seed germination plays an important role in plant survival (Bareke, 2018). It is very valuable to choose a technique that can improve seed germination traits and at the same time be inexpensive and unassuming to increase the chances of successful seedling production and establishment. The use of various substances such as sulfuric acid can have a significant effect on improving and increasing seed germination (Mehdadi et al., 2017; Venâncio and Martins, 2019). Beneficial effects of hot or boiling water washing on seed germination of Fabaceae plants as well as facilitating and stimulating the germination of sulfuric acid-treated seeds in plants of this family have been reported (Kheloufi, 2017; Kheloufi et al., 2017;). Sulfuric acid treatment has been introduced as a commercial method for scraping seeds (Nowruzian et al., 2022).

In this study, scarification using sulfuric acid and GA soaking was revealed as well encouraging the germination rate and percentage of *C. siliquastrum* seeds. The highest germination index was obtained in the treatment of 1,000 ppm GA + 30 min of immersion in sulfuric acid treatment. However, with increasing sulfuric acid soaking time, the germination index decreased significantly. The highest average germination time in *C. siliquastrum* seeds was obtained in the treatment of 2 min immersion in boiling water + 500 mg L⁻¹ GA. It can be stated that the reduction of the average germination time in *C. siliquastrum* seeds

treated with sulfuric acid was due to increased germination rate and proper water uptake, which led to rapid germination. According to the results of this study, the effect of sulfuric acid on these two indices of germination was the opposite. Thus, immersion in sulfuric acid at different times (30 and 60 min) and with concentrations of 500 and 100 mg L^{-1} GA increased the seed germination percentage and decreased the seed germination percentage. Boiling water treatment did not have a significant effect on the germination percentage of C. siliquastrum seeds, while the highest seed dormancy percentage was obtained in this treatment. Also, the results of this study showed that sulfuric acid increased the Germination Rate, Coefficient Velocity Germination and seed Germination Index of Spartium junceum. This is consistent with the results of previous studies on increasing the germination rate of seeds treated with sulfuric acid (Atiku et al., 2021).

Similar observations were noted by Mehdadi et al. (2017) whose demonstrated that the chemical scarification had permitted the coats softening of *Retama raetam* seeds which promote the germination. The same results were demonstrated in our study after pretreatment with sulfuric acid during 30 and 60 min. The use of chemical acids, especially sulfuric acid, improves seed germination in many legumes with hard shells and increases the permeability of the seed coat as well as the uplifting water and oxygen permeability of the testa (Mojeremane et al., 2018). The efficiency of this pretreatment on removing the tegumentary inhibition of Leguminoseae species of Sahel (Mehdadi et al., 2017).

According to the results of this study, the length of stem and root of C. siliquastrum seed increased in 30 min of immersion in sulfuric acid + 500 mg L^{-1} GA and the highest leaf length and width were obtained in 2 min of immersion in boiling water + 500 mg L⁻¹ GA treatment. These results are in agreement with Rusdy (2017) that the highest percentage of seedling emergence, plant height, number of leaves, biomass dry weight was recorded when seeds Leucaena leucocephala treated with sulfuric acid for 20 and 24 min. Sulfuric acid is thought to disturb the seed coat and expose the lumens of the macrosclereids cell, permitting imbibition of water which triggers the release of simple sugar that could be readily used for protein synthesis, thus encouraging germination (Rusdy, 2017). Gibberelic acid inducing germination by encourage hydrolytic enzymes that deteriorate the hurdle tissues such as the endosperm or seed coat, inducing mobilization of nutrition reserves in seed and stimulating development of the embryo (Luera et al., 2021).

However, the current results reach agreement with those of previous studies in which scarification combined with GA_3 application is described to have a synergistic effect in accelerating seed germination and to be effective in increasing seed germination in many species (Gao et al., 2020; Rusdy, 2017; Pipinis et al., 2011). Since, the physical and chemical treatments improved germination, it is proposed that there was an external dormancy which was perhaps due to the impermeable seed coat (Maleki et al., 2007). According to the results of this study, since

the seeds of the *C. siliquastrum* and *S. junceum* treated by sulfuric acid and hot water, germinated and the problem of seed dormancy was explained, it can be concluded that the dormancy of the seeds of this plant is most likely related to physical factors (Mechanical resistance of seed coat against germination). The present study revealed that of *C. siliquastrum* and *S. junceum* seeds exhibited a combination of physical and physiological dormancy.

Conclusions

The results presented here indicate that chemical scarification by soaking in sulfuric acid for 30 min and 2 min in *C. siliquastrum* and *Spartium junceum*, respectively were the most efficient methods to breaking the seed dormancy. The application of these methods promoted the highest values of indices seedlings. In the light of the found results, it revealed that *C. siliquastrum* and *S. junceum* seeds are affected by a coat dormancy, which can be removed by a chemical-thermal scarification with sulfuric acid and boiling water. The data obtained contribute to a better comprehension of propagation and establishment of these shrubs ornamental by seedling.

Author Contribution

MT: Participated in all of experiments, coordinated the dataanalysis and contributed to the writing of the manuscript. **FSS**: Coordinated the laboratory work.

References

ADETUMBI, J.A.; ORIMADEGUN, I.O.; AKINYOSOYE, S.T.; AKINTAYO, O.T.; AGBELEYE, O.A. Enhancing planting value of rice seed through priming with humic substance. Journal of Experimental Agriculture International, v.29, n.6, p.1-8, 2019. https:// doi.org/10.9734/JEAI/2019/46194

ATIKU, M.A.; ABDULRAHMAN, A.S.; AMBURSA, M.; MUHAMMED, I.; UMAR, F.; ALMUSTAPHA, A.G.; MOHAMMAD, A.; DOGO, A. Effect of presowing treatment on the seed emergence of *Diospyros mespiliformis*. African Journal of Sustainable Agricultural Development, v.2, n.2, p.11-19, 2021. https://doi.org/10.46654/2714.2257

BAREKE, T. Biology of seed development and germination physiology. Advances in Plants and Agriculture Research, v.8, n.4, p.336-346, 2018. https://doi.org/10.15406/apar.2018.08.00335.

GAO, Y.; ZHU., M.; MA, Q.; LI, S. Dormancy breakage in *Cercis* chinensis seeds. **Canadian Journal of Plant Science**, v.100, n.6, p.666-673, 2020. https://doi. org/10.1139/cjps-2019-0229 GEBRE, G.H.; KARAM, S.N. Germination of *C. siliquastrum* seeds in response to gibberellic acid and stratification. **Seed Science and technology**, v.32, n.1, p.255-260, 2004.

IRALU, V.; UPADHAYA, K. Seed dormancy, germination and seedling characteristics of *Elaeocarpus prunifolius* Wall. ex Müll. Berol.: a threatened tree species of northeastern India. **New Zealand Journal of Forestry Science**, v.48, n.1, p.1-10, 2018. https://doi.org/10.1186/s40490-018-0121-y

KHELOUFI, A. Germination of seeds from two leguminous trees (*Acacia karroo* and *Gleditsia triacanthos*) following different pre-treatments. **Seed Science and Technology,** v.45, n.1, p.259-262, 2017. https://doi.org/10.15258/SST.2017.45.1.21

KHELOUFI, A.; MANSOURI, L.M.; BOUKHATEM, F.Z. Application and use of sulphuric acid pretreatment to improve seed germination of three *Acacia* species. **Reforesta**, v.3, p.1-10, 2017.

KONESHLOO, H. Afforestation in arid zone. The Institute of Forest and Rangeland Research, v.1, p.516, 1994.

LUERA, P.; WAHL-VILLARREAL, K.; CHRISTOFFERSEN, B.O.; TREVIÑO, A.; SOTI, P.; GABLER, C.A. Effects of scarification, phytohormones, soil type, and warming on the germination and/or seedling performance of three Tamaulipan thornscrub forest species. **Plants,** v.10 n.8, p.489, 2021. https://doi.org/10.3390/ plants10081489

LUO, Y.; LIANG, J.; ZENG, G.; CHEN, M.; MO, D.; LI, G.; ZHANG, D. Seed germination test for toxicity evaluation of compost: Its roles, problems and prospects. **Waste Management,** v.71, p,109-114, 2018. https://doi. org/10.1016/j.wasman.2017.09.023

MALEKI, A.; ERFAN, M.B.; MOHAMMADI, A.S.; EBRAHIMI, R. Application of commercial powdered activated carbon for adsorption of carbolic acid in aqueous solution. **Pakistan Journal of Biological Sciences**, v.10, n.14, 2348-52, 2007. https://doi.org/10.3923/ pjbs.2007.2348.2352

MEHDADI, Z.; BENDIMERED, F.Z.; DADACH, M.; AISSET, A. Effects of temperature and salinity on the seeds germination of *Retama raetam* (Forssk.) Webb. scarified with sulfuric acid. **Journal of Fundamental and Applied Sciences**, v.9, n.3, p.1284-1299, 2017. https://doi. org/10.4314/jfas.v9i3.3 MOJEREMANE, W.; MATHOWA, T.; TEKETAY, D. Effects of different pre-Sowing treatments on germination of *Peltophorum africanum* Sond seeds from two provenances in Botswana. Journal of Biodiversity and Environmental Sciences, v.12, n.2, p.230-236, 2018.

NOWRUZIAN, A.; AALAMI, A.; FATEMI, F. Effect of different treatments on dormancy breaking for seed germination enhancement and metabolite analysis of *Capparis spinosa* L. Journal of Medicinal plants and Byproduct, Article online. 2022. https://doi.org/10.22092/ JMPB.2022.357295.1443

PIPINIS, E.; MILIOS, E.; SMIRIS, P.; GIOUMOUSIDIS, C. Effect of acid scarification and cold moist stratification on the germination of *C. siliquastrum* L. seeds, **Turkish Journal Agriculture and Forestry**, v.35, p.259-264, 2011. https://doi.org/10.3906/tar-1003-848

PULATKAN, M.; YILDIRIM, N.; TURNA, İ. Effects of different medium on seed germination of *S. junceum* L. with medicinal and aromatic importance. **International Journal of Secondary Metabolite**, v.4, n.3, 376-383, 2017 https://doi.org/10.21448/ijsm.374627

RUSDY, M. Enhancement of seedling emergence and early growth of *Leucaena leucocephala* by hot water, mechanical and acid scarification pre-treatments. **International Journal of Applied Environmental Sciences**, v.12, n.5, p.857-863, 2017.

SABETI, H. Forests, trees, and shrubs of Iran. Yazd: Yazd University Publication, 1994. 876p.

SENG, M.; CHEONG, E.J. Comparative study of various pretreatment on seed germination of *Dalbergia cochinchinensis*. Forest Science and Technology, v.16, n.2, p.68-74, 2020. https://doi.org/10.1080/21580103.202 0.1758801

SILVA, R.C.; CASTRILLON, S.K.I.; FERNANDEZ, J.R.C.; DE MORAIS, F.F.; MORINI, A.A.E.T.; DA SILVA NUNES, J.R. Overcoming dormancy in seeds of *Pterodon pubescens* (Benth.) Benth. (Leguminosae, Papilionoideae). **Research, Society and Development,** v.10 n.1, p.1-8, 2021. https://doi.org/10.33448/rsd-v10i1.9144

ULLAH, A.; ALI, M.; SHAHZAD, K.; AHMAD, F.; IQBAL, S.; RAHMAN, M.H.U.; AHMAD, S.; IQBAL, M.M.; DANISH, S.; FAHAD, S.; ALKAHTANI, J. Impact of seed dressing and soil application of potassium humate on cotton plants productivity and fiber quality. **Plants**, v.9, n.11, p.1444, 2020. https://doi.org/ 10.3390/plants9111444

VENÂNCIO, R.S.D.S.; MARTINS, A.C.G. Overcoming dormancy of *Senna multijuga* seeds with an ultrasonic probe the comparison with ultrasound and sulfuric acid baths. **Ciência Rural**, v.49, n.9, p.1-7, 2019. https://doi. org/10.1590/0103-8478cr20180904.

YUCEDAG, C.; GULTEKIN, C. The effect of sowing time on germination of twenty-two Leguminosea species. **African Journal of Agricultural Research**, v.6, n.16, p.3809-3816, 2011. https://doi.org/10.5897/AJAR11.880.

ZINCIRKIRAN, M.; TÜMSAVAŞ, Z.; ÜNAL, H. The effects of different acid treatment stratification duration on germination of *C. siliquastrum* L. seeds, **Botany Horticulture Agronomy**, v.38, n.1, p.159-163, 2010.