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Germination and dormancy breaking in the endemic juniper (*Juniperus thurifera* L.) of Algeria: an evaluation of acceleration techniques

Germinação e a quebra da dormência no zimbro endêmico (*Juniperus thurifera* L.) da Argélia: uma avaliação de técnicas de aceleração

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ABSTRACT

Juniper forests (*Juniperus thurifera* L.) are among the most valuable forest ecosystems in the mountains of Algeria. Numerous studies have focused on enhancing the germination capacity of juniper seeds. This particular study aimed at breaking the seed dormancy by employing pretreatment methods involving sulphuric acid and gibberellic acid (AG3). To distinguish viable seeds from non-viable ones, the seeds were submerged in sucrose solutions with increasing concentrations (0%. 20%. 40%. and 60%) and subjected to cold treatments at 4 °C for a period of 2 years. Additionally, two pre-treatments were conducted: the first involved immersing the seeds in concentrated sulphuric acid for 15 minutes, while the second pre-treatment utilized AG3 at various concentrations (100 ppm, 400 ppm, and 1000 ppm) for 24 and 48 hours. The results demonstrated the possibility of breaking seed dormancy and achieving germination, which varied significantly based on the pretreatments, seed fractions, cold treatment, and their interactions. The highest germination percentage (83.33%) was observed when applying the gibberellic acid pretreatment (400 ppm for 48hours) to the seeds of the heavy fraction previously stored in cold conditions. Overall, this research opens up new possibilities for effectively managing and protecting juniper forests, contributing to their long-term sustainability and conservation efforts.

Keywords: *Juniperus thurifera* L.; *Germination rate; Cold; Sulfuric acid; Gibberellic acid.*

RESUMO

As florestas de zimbro (*Juniperus thurifera* L.) estão entre os ecossistemas florestais mais valiosos das montanhas da Argélia. Numerosos estudos têm-se concentrado em melhorar a capacidade de germinação das sementes de zimbro. Para distinguir as sementes viáveis das não viáveis, as sementes foram submersas em soluções de sacarose com concentrações crescentes (0%, 20%, 40% e 60%) e sujeitas a tratamentos a frio a 4 °C durante um período de 2 anos. Adicionalmente, foram efectuados dois pré-tratamentos: o primeiro consistiu na imersão das sementes em ácido sulfúrico concentrado durante 15 minutos, enquanto o segundo pré-tratamento utilizou AG3 em várias concentrações (100 ppm, 400 ppm e 1000 ppm) durante 24 e 48 horas. Os resultados demonstraram a possibilidade de quebrar a dormência das sementes e conseguir a germinação, que variou significativamente com base nos pré-tratamentos, nas fracções de sementes, no tratamento a frio e nas suas interacções. A maior percentagem de germinação (83,33%) foi observada quando se aplicou o pré-tratamento com ácido giberélico (400 ppm durante 48 horas) às sementes da fração pesada armazenadas em condições de frio. De um modo geral, esta investigação abre novas possibilidades para gerir e proteger eficazmente as florestas de zimbro, contribuindo para a sua sustentabilidade a longo prazo e para os esforços de conservação.

Palavras-chave*: Juniperus thurifera* L.; *Taxa de germinação; Frio; Ácido sulfúrico; Ácido giberélico.*

1. INTRODUCTION

The thuriferous juniper (*Juniperus thurifera* L.) is a typical species found in plant formations within the semi-arid mountains of the western Mediterranean and is well known for its historical, ornamental, therapeutic, and economic worth as well as for the high quality of its

wood (Morsli et al., 2015). It belongs to the Cupressaceae family, which constitutes a significant part of the forest flora in the Atlas Mountains (Aouadj et al., 2020; Bigot et al., 1989). Three distinct varieties exist: *J. thurifera* var. gallica in France (the Alps and the Pyrenees); *J. thurifera* var. hispanica in Spain; and *J. thurifera* var. africana in North Africa. Additionally, *Juniperus thurifera* L. var. Aurasiaca is an endemic species found in the Aures region of Algeria (Chirio & Balanc, 1997).

The thuriferous juniper forests are found over vast areas but due to a combination of natural incidences (fires, for instance) and human activity (unrestricted exploitation of forests, etc.), the extent of many juniper-containing forests is currently decreasing (Khater & Benbouza, 2019). Furthermore, this species exhibits slow growth and faces challenges in natural regeneration. It is known for its ability to thrive on poor rocky soils (Adams et al., 2003). Additionally, the thuriferous juniper holds economic importance due to the quality of its rot-proof wood. In Algeria, *Juniperus thurifera* is primarily found in the Aures Mountains, with the best-preserved stands located in Djebel Chelia in Eastern Algeria (Aouadj et al., 2020; Hafsi et al., 2017).

The dysfunction in the spatiotemporal dynamics of the thuriferous juniper forest cover, like many other forest species in the Mediterranean environment, is mainly attributed to human activities (e.g.. grazing, logging), degradation of soil physicochemical and biological characteristics, and competition with surrounding vegetation (Hazubska-Przybył, 2019; Albaladejo et al., 1998).

In its natural range in Algeria, the thuriferous juniper exhibits almost no natural regeneration. Previous attempts at artificial regeneration have encountered difficulties in promoting seed germination under controlled conditions (Kostas et al., 2023; Salih et al., 2021; Hazubska-Przybył, 2019; Morsli et al., 2015). Seed dormancy is a common adaptive strategy employed by plants to ensure survival under unfavorable environmental conditions. It serves as a protective mechanism against factors such as competition and herbivory. However, while seed dormancy is beneficial for long-term seed viability and dispersal, it can also pose challenges to the germination process. The dormant state of seeds hinders their ability to germinate promptly, delaying the establishment of new plants and potentially impacting population dynamics. Therefore, understanding and effectively breaking seed dormancy is crucial for promoting successful germination and facilitating the regeneration of plant populations.

This research aims to address these challenges by determining favorable conditions for thuriferous juniper seed germination through various pretreatment methods to break seed dormancy, including the novel approach of sulfuric acid pretreatment. To achieve this, this study employed a combination of techniques. First, to separate viable from non-viable seeds, the seeds were immersed in sucrose solutions with increasing concentrations (0%, 20%, 40%, and 60%) and subjected to cold treatments at 4 °C for a period of 2 years. Additionally, two pre-treatments were carried out. The first involved immersing the seeds in concentrated sulfuric acid for 15 minutes, while the second pre-treatment utilized gibberellic acid (AG3) at varying concentrations (100 ppm, 400 ppm, and 1000 ppm) for 24 and 48 hours. The effects of these pretreatments on seed germination were evaluated and analyzed.

By employing these methods, the study aimed to provide valuable insights into breaking seed dormancy and enhancing thuriferous juniper seed germination. The findings will contribute to the development of effective artificial regeneration practices, aiding in the conservation and sustainability of these endangered and genetically unique juniper stands.

Little research has been done on the application of chemical products as a way to accelerate the seed germination of *Juniperus thurifera* L. in sustainable management systems to improve and restore degraded forests. Thus, the main objective of our study was to investigate how this method improves *Juniperus* germination response in regions where no previous studies on these species have been conducted in eastern Algeria.

2. MATERIAL AND METHODS

2.1. Plant material

The plant resources used are confined to seeds of the *Juniperus thurifera* L. species (Figure 1). Some of these seeds were harvested in November 2016 from the T'KOUT region with geographical coordinates

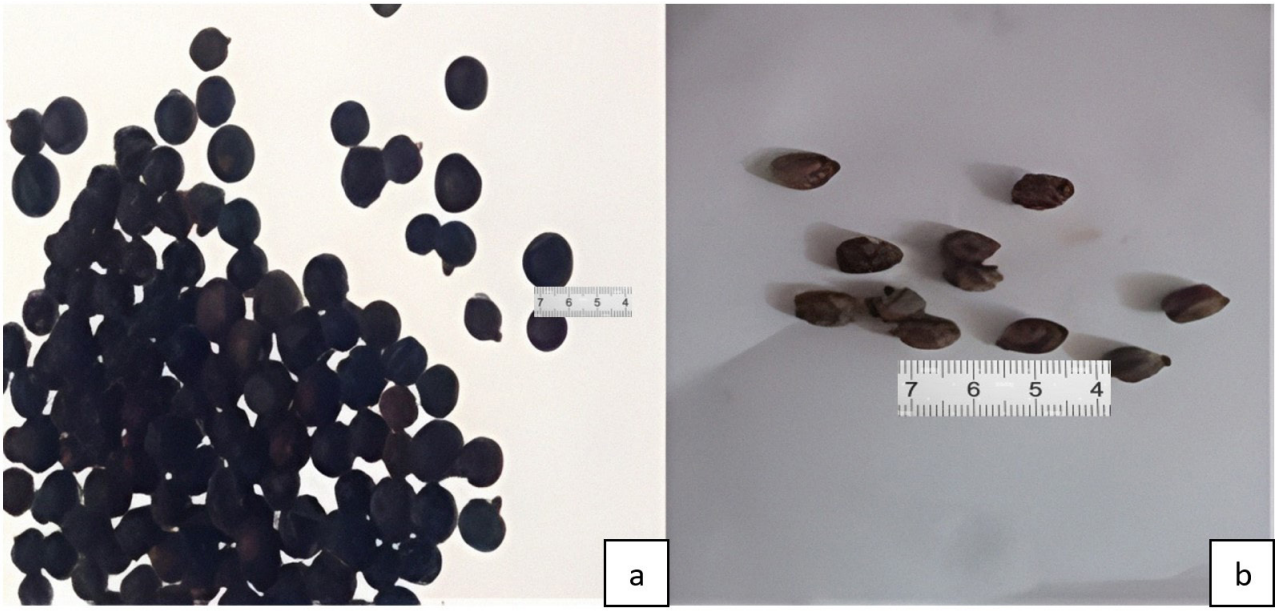

Figure 1. Galbula (a) and seeds (b) of *Juniperus thurifera* L.

35° 8' 19" North. 6° 19' 9" East, located in the Aures massif, 95 km southeast of Wilaya of Batna in eastern Algeria. Another batch of seeds was harvested two years ago in the same region and stored at 4 °C. The botanical identification was carried out based on the floras of Quezel & Santa (1962) which was validated by Dr. ZERAIB A., research professor at the Abbes Laghrour University of Khenchela. The seeds are extracted by removing the fleshy scales. A naked eye examination of a large proportion of shelled galbula showed no signs of pest attack. However, their observation with a binocular magnifying glass revealed that the majority of the seeds were devoid of viable embryos.

2.2. Seed separation method in a sucrose gradient

The method adopted by Bonner et al. (1994) makes it possible to separate viable seeds from non-viable ones by immersing them in a sucrose solution. The non-viable seeds that were floating on the surface of the solution were discarded and were called light fraction. The seeds that settled were called heavy fraction. This test went through at least four times by adding, each time, to the initial solution which was the 0% fraction. The proportions of sucrose varied between 20%, 40%, and 60% (Ferradous et al., 2013) as below:

- The first fraction of discarded light seeds is called: 0% light fraction;
- The heavy seeds that settled at the bottom of the container underwent a second immersion in the initial solution with 20% sucrose added; the seeds which floated were also discarded and were called 20% light fraction;
- The heavy seeds which settled at the bottom of the container following this second attempt underwent immersion in the same solution but by adding up to 40% of sucrose. The seeds that floated on the surface of the solution were removed and stored as the 40% light fraction;
- The seeds that remained heavy under the effect of 40% sucrose were again immersed in a 60% solution. The seeds which remained floating on the surface of said solution were called the 60% fraction. The fraction deposited at the bottom of the container was called the 60% heavy fraction.

2.3. The pretreatments used

Acid-based pretreatments were carried out on the seeds as defined below:

2.4. Pure sulfuric acid

Sulfuric acid is a chemical compound with the formula H_2SO_4. It is a mineral acid whose strength (pKa = -3.0) is exceeded only by few acids. It is miscible with water in all proportions, where it dissociates releasing hydronium cations (Afshar et al., 2014).

2.5. Gibberellic acid

Gibberellic acid or gibberellin (A3) is a plant hormone; its chemical formula is $C_{19}H_{22}O_6$. When pure, it is a white or pale yellow solid.

2.6. Germination tests

Germination tests were conducted to evaluate the germination capacity of *Juniperus thurifera* L. seeds. The seeds used in the tests were obtained from the November 2016 harvest and were two years old and stored under cold conditions, as previously described. To separate viable seeds from non-viable seeds, sucrose solutions were utilized at various proportions: 0%, 20%, 40%, and 60%. From each solution, new samples consisting of ten (10) seeds each were selected for further testing. The samples, consisting of ten seeds per Petri dish (in triplicates), were subjected to a series of three germination tests using sulfuric acid and in different concentrations gibberellic acid (AG3) treatments.

The sulfuric acid pretreatment involved immersing the seeds in concentrated sulfuric acid for the duration of fifteen minutes. For the gibberellic acid pretreatment, different concentrations were utilized: controls (no treatment). 100 ppm, 400 ppm, and 1000 ppm of gibberellic acid. The seeds were subjected to these treatments for 24 hours and 48 hours. The design of pretreatments was as follows:

A0: untreated seeds.

A1: seeds immersed in sulfuric acid for 15 minutes.

A2: seeds immersed in gibberellic acid at 100ppm for 24 hours.

A3: seeds immersed in gibberellic acid at 400ppm for 24 hours.

A4: seeds immersed in gibberellic acid at 1000ppm for 24 hours.

A5: seeds immersed in gibberellic acid at 100ppm for 48 hours.

A6: seeds immersed in gibberellic acid at 400ppm for 48 hours.

A7: seeds immersed in gibberellic acid at 1000ppm for 48 hours.

For a positive control, seeds were placed in Petri dishes on filter paper soaked in 10 ml of distilled water. After 15 days, observations were made for each treatment. Seeds were considered germinated when they showed visible signs of radicle emergence.

To calculate the germination rates or percentages, the number of germinated seeds was divided by the total number of seeds germinated and multiplied by 100, following the method described by Czabator (1962).

These germination tests were conducted with three replicates of 100 seeds each, in order to assess the effectiveness of the various treatments for breaking seed dormancy and promoting seed germination.

2.7. Statistical analysis

The analysis of variance was carried out separately for every factor (fraction; pretreatment. treatment) and was carried out for its interactions using a multifactor way, by the statistical software S.A.S v.9.1. The homogeneous groups were analyzed using the same statistical software.

3. RESULTS

3.1. ANOVA of the fraction; pretreatment; treatment effects and its interaction on the percentage of germination of *Juniperus thurifera* L. seeds:

ANOVA statistical analysis resulted in a very highly significant fraction, pretreatment and treatment effect ($p < 0.0001$. 1‰), and highly significant interaction (Fraction X Pretreatment) ($p = 0.0059$. 1%). The interaction effects for (Fraction X treatment) and (Treatment X Fraction X Pretreatment) were very highly significant but not significant for interaction (Treatment X Pretreatment) ($p < 0.0001$, 1‰). A highly significant fraction, pretreatment, and treatment effect indicated differences due to the level condition and chemical product concentration used to accelerate seed germination. Significant (Fraction X Pretreatment), (Fraction X Treatment) and (Treatment X Fraction X Pretreatment) interaction are indicatives of the differential responses of seed germination to the chemical methods used. However, there was no significant difference observed for the interaction between treatment and pretreatment ($p = 0.13$).

3.2. Effect of the fraction on the percentage of germination of *Juniperus thurifera* L. seeds

From the results obtained by the analysis of variance and value of the Least Significant Difference (LSD=3.36), it was found that the average percentage of germination varied according to the fractions. The highest value was recorded in the heavy fraction with 57.91±20.82%, followed by the light fraction at 60% with 12.91±11.66%; and finally the light fraction 40% with 6.66±10.98%. The 0% and 20% fractions showed no germination. This shows that the 20% fraction was not effective (Table 1).

3.3. Effect of the treatment on the percentage of germination of *Juniperus thurifera* L. seeds

Based on the results (Table 2), there was a statistically significant difference in the treatment effect on the percentage of germination of *Juniperus thurifera* L. seeds (LSD = 2.12). The germinated seeds can be divided into two homogeneous groups: the first group comprises untreated seeds with a germination percentage of 18.75 ± 27.67% and the second group consists of seeds exposed to cold storage for two years with a germination percentage of 12.25 ± 20.96%.

3.4. Effect of the pretreatment on the percentage of germination of *Juniperus thurifera* L. seeds

The pretreatments were divided into five groups according to the Least Significant Difference (LSD=4.25).The best includes the pretreatments A5, A6 and A7 of seeds immersed in gibberellic acid at 100,400 and 1000 ppm for 48 h respectively (Table 3).

Table 1. Means of germination rate for fractions effect.

Fractions	Means of germination rate (%)
Heavy fraction	57.91±20.82[a]
Fraction 60%	12.91±11.66[b]
Fraction 40%	6.66±10.98[c]
Fraction 20%	0.00[d]
Fraction 00%	0.00[d]

[a,b,c,d]homogeneous groups.

Table 2. Means of germination rate for fractions effect.

Treatments	Means of germination rate %
Treated seeds	12.25±20.96[b]
Untreated seeds	18.75±27.67[a]

[a,b]homogeneous groups.

Table 3. Means of germination rate for pretreatments of *Juniperus thurifera* L. seeds.

Pretreatments	Means of germination rate (%)
A0	8.33±18.21[c]
A1	15.66±25.55[ab]
A2	12.33±21.60[bc]
A3	15.66±25.55[ab]
A4	14±24.15[b]
A5	19.33±28.03[a]
A6	19.66±27.06.7[a]
A7	19±28.44[a]

[a,ab,b,bc,c]homogeneous groups.

3.5. Interaction effect on the percentage of germination of *Juniperus thurifera* L. seeds

3.5.1. Effect of the (Fraction X treatment) interaction on the percentage of germination of *Juniperus thurifera* L. seeds

According to the Least Significant Difference (LSD = 4.76) the effect of the (fraction X treatment) interaction, as shown in Figure 2, an increase in germination rate was observed in untreated seeds in Heavy fraction, fraction 60% and fraction 40% compared with the fraction 0% and 20% and treated seeds (stored under cold conditions). Germinated seeds could be classified into six homogeneous groups. The first two groups consisted of treated and untreated seeds from the heavy fraction, with the highest germination percentages observed: 68.33% for untreated seeds and 47.5% for treated seeds, respectively. The 60% fraction follows with 15.83% germination for treated seeds and 10% for untreated seeds. The 40% fraction shows 9.58% germination for treated seeds and 3.75% for untreated seeds. Lastly, the 20% and 0% fractions did not exhibit any germination.

3.5.2. Effect of the (Fraction X pretreatment) interaction on the percentage of germination of *Juniperus thurifera* L. seeds

According to the LSD (Least Significant Difference) value of 9.51 for the interaction effect of (fraction X pretreatment), as presented in Table 4, the germinated seeds can be categorized into six homogeneous groups. The first group included seeds from the heavy fraction that were pretreated with gibberellic acid at 100 and 1000 ppm for 48 hours, resulting in the highest germination percentage of 68.33%. The next group corresponded to the 60% fraction, with a germination percentage of 20% for seeds pretreated with 100 and 400 ppm gibberellic acid for

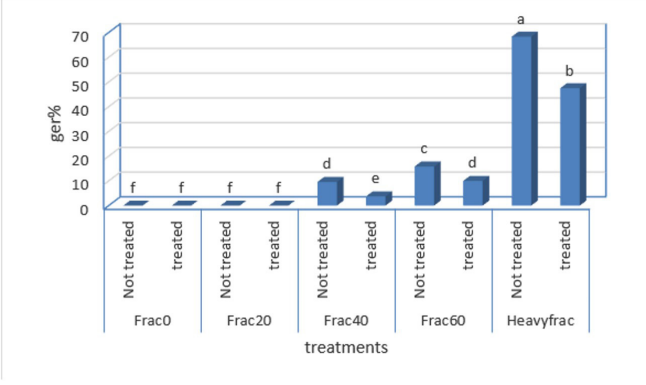

Figure 2. Means of the effect of the (Fraction X treatment) interaction on the percentage of *Juniperus thurifera* L. seed germination.

Table 4. Means of the percentage of *Juniperus thurifera* L. seeds germination of the fraction X pretreatment interaction effect

Preatretment	Fraction 0	Fraction 20	Fraction 40	Fraction 60	Heavy fraction
A0	0%[f]	0%[f]	0%[f]	3.33%[e]	38.33%[c]
A1	0%[f]	0%[f]	10%[e]	15%[d]	53.33%[b]
A2	0%[f]	0%[f]	3.33%[e]	10%[e]	50%[b]
A3	0%[f]	0%[f]	3.3%[e]	13.33%[d]	61.66%[a]
A4	0%[f]	0%[f]	6.66%[e]	6.66%[e]	56.66%[b]
A5	0%[f]	0%[f]	8.33%[e]	20%[d]	68.33%[a]
A6	0%[f]	0%[f]	11.66% [d]	20%[d]	66.66%[a]
A7	0%[f]	0%[f]	11.66% [d]	15%[d]	68.33%[a]

[a,b,c,d,e,f]homogeneous groups.

48 hours. In the subsequent group, the 40% fraction exhibited 11.66% germination for seeds pretreated with 400 and 1000 ppm gibberellic acid. Lastly, the 20% and 0% fractions did not exhibit any germination.

3.5.3. Effect of the (treatment X pretreatment) interaction on the percentage of germination of *Juniperus thurifera* L. seeds

The results of the effect of the (treatment X pretreatment) interaction are presented in Table 5, showing that there are no significant differences in the germination rate of pretreatments between the treated and untreated seeds; so this interaction does not have an effect on juniper seed germination percentage.

Table 5. Means of the percentage of *Juniperus thurifera* L. seeds germination of the (treatment X pretreatment) interaction effect

Treatment	Pretreatments	Means of germination rate (%)
Untreated	A0	4.75[a]
	A1	21.33 [a]
	A2	16 [a]
	A3	17.33 [a]
	A4	18.66 [a]
	A5	22.66 [a]
	A6	22.66 [a]
	A7	20 [a]
Treated	A0	18.88 [a]
	A1	8.66 [a]
	A2	8.66 [a]
	A3	14 [a]
	A4	9.33 [a]
	A5	16 [a]
	A6	16.66 [a]
	A7	18 [a]

[a]homogeneous groups.

3.5.4. Effect of the (Treatment X Pretreatment X Fraction) interaction on the percentage of germination of *Juniperus thurifera* L. seeds

According to the results of the Least Significant Difference (LSD= 13.45) of the effect of the interaction treatment X pretreatment X fraction presented in Table 6, the seeds of *Juniperus thurifera* L. are classified into six homogeneous groups of which the best percentage of germination (83.33%) is recorded for the seeds of the heavy fraction untreated with temperature (4 °C) and pretreated with 400ppm of gibberellic acid for 48 hours followed by the percentage of 76.67%, which was recorded in the seeds of the heavy fraction untreated with temperature and pretreated with pure sulfuric acid for 15 min.

The same group includes the interactions (heavy fraction X untreated X pretreatment with 400ppm of gibberellic acid for 24h) and (heavy fraction X untreated X pretreatment with 1000ppm of gibberellic acid for 24h) with a germination percentage equal to 73, 33% for both.

Concerning the seeds treated by 4 °C, the best percentage of germination was recorded in the seeds of the heavy fraction and pretreated with 1000 ppm of gibberellic acid for 48 hours with a percentage equal to 80% followed by a percentage of germination of 70% recorded by heavy fraction seeds and pretreated with 100 ppm gibberellic acid for 48 hours.

Despite their cold treatment and their pre-treatments with sulfuric acid and gibberellic acid at different concentrations, the grains of the 0 and 20 fractions did not germinate. This can be explained by zero germination which may be due to several possible factors. Further analysis of fraction components and germination conditions would be needed to understand more precisely the reasons for zero germination in these cases.

4. DISCUSSION

Given the results, it appears that there is a highly significant difference between the germination rates obtained after pretreatment. The results of the germination obtained after the application of different fractions and chemical treatments to the dormant seeds show

Table 6. Means of the percentage of *Juniperus thurifera* L. seeds germination of the (Treatment X Pretreatment X Fraction) interaction effect.

	Pretreatment	Fraction 0	Fraction 20	Fraction 40	Fraction60	Heavy fraction
Untreated	A0	0[f]	0[f]	0[f]	0[f]	50[c]
	A1	0 [f]	0 [f]	20 [d]	16.67 [e]	76.67 [a]
	A2	0[f]	0[f]	3.33 [e]	10 [e]	66.67 [b]
	A3	0[f]	0[f]	6.67 [e]	6.67 [e]	73.33 [a]
	A4	0[f]	0[f]	10 [e]	10 [e]	73.33 [a]
	A5	0[f]	0[f]	13.33 [e]	33.33 [d]	66.67 [b]
	A6	0[f]	0[f]	6.67 [e]	23.33 [d]	83.33 [a]
	A7	0[f]	0[f]	16.67 [d]	26.67 [d]	56.67 [b]
Treated	A0	0[f]	0[f]	0[f]	6.67 [e]	26.67 [d]
	A1	0[f]	0[f]	0[f]	13.33 [e]	30 [d]
	A2	0[f]	0[f]	0[f]	10 [e]	33.33 [d]
	A3	0[f]	0[f]	0[f]	20 [d]	50 [c]
	A4	0[f]	0[f]	3.33 [e]	3.33 [e]	40 [c]
	A5	0[f]	0[f]	3.33 [e]	6.67 [e]	70 [a]
	A6	0[f]	0[f]	16.67 [e]	16.67 [e]	50 [c]
	A7	0[f]	0[f]	6.67 [e]	3.33 [e]	80 [a]

[a,b,c,d,e,f]homogeneous groups.

a difference in the seed response. The fraction of so-called «heavy» seeds resulting from immersion in the 60% sucrose solution gave the best results. It was found that some Juniper species produced very few viable seeds or morphologically immature seeds. Moreover, one of the challenges to sexual reproduction in these trees is the small amount of fully developed seeds (Abdalrhaman et al., 2021). In this case we used this method to differentiate between vital and dead seeds. An increase in germination rate was observed in untreated seeds in the heavy fraction, fraction 60% and fraction 40% compared to fractions 0% and 20% and treated seeds (stored under cold conditions).

Our results are in agreement of those of Ferradous et al (2013) who found that the distribution of cone types in samples of *Juniperus thurifera* L. sorted by a sucrose gradient revealed that the heavy fraction with 60% sucrose contained over 75% of cones with viable embryos, while the light 20% sucrose fraction contained only 0.01% of cones with viable embryos. According to this research the results obtained with *J. thurifera* subsp. africana were higher than those generally cited in the literature. However, Badri et al. (2000) obtained very low rates (ranging from 0 to 2%) when compared to our study. According to García's (2001) results in *Juniperus communis*, the percentage of viable seeds did not exceed 3.5 to 5.5% mainly due to parasitic attacks. The heavy fraction of 60% that was pretreated with gibberellic acid at 100 and 1000 ppm for 48 hours, resulted in the highest germination percentage compared to the control of 0% and 20% fractions, which did not give any germination.

The seeds of the heavy fraction, untreated with cold (4ºC) and pretreated with 400 ppm of gibberellic acid for 48 hours presented the highest germination rate; followed by the seeds of the heavy fraction untreated with cold and pretreated with pure sulfuric acid for 15 minutes, and the seeds untreated and pretreated with 400 and 1000 ppm of gibberellic acid for 24 hours. Since no research has been done on the application of many factors to improve *Juniperus thurifera* L. regeneration, there are few references to compare our results to. According to Alberts & Mandel (2004), *J. monosperma* and *J. osteosperma* face difficulties in obtaining young regenerations as seed germination requires a period of hot stratification for 70 days followed by cold stratification for 120 days. *J. procera* seeds should be cold-moist stratified for six weeks in order to improve germination. It was discovered that the degree of dormancy in this juniper species was highly influenced by genetic and environmental factors (Tigabu et al., 2007). After being exposed to a limited range of red/far red light and a constant temperature of 20ºC, *J. procera* seeds showed a better germination response. On the other hand, *J. communis* seeds were warm-cold stratified at 15ºC/3ºC for 14+12 weeks to increase their germination potential and seedling emergence. Although *J. sabina* seeds responded similarly to this treatment, hot stratification was more effective at 20Cº (Yirdaw & Leinonen, 2002; Tylkowski, 2009, 2010).

However, our finding indicates that there is an increase of seed germination rate in the treatment with cold and treated by chemical products. According to the classification for seed dormancy of Baskin & Baskin (2014, 2021), in seeds with morphological dormancy, embryos do not need a pretreatment that breaks dormancy in order to germinate. Instead, they just need time to grow to maturity and eventually germinate. Morphological-physiological dormancy in seeds results in an embryo that is undeveloped and has a dormant physiological component. Therefore, such seeds need a pretreatment that breaks their dormancy in order to germinate. When compared to seeds with morphological dormancy, seeds with morphological-physiological dormancy (MPD) require a significantly longer time for embryo growth and radicle emergence. Our findings indicate that *J. thurifera* has a probable combined dormancy (physiological + morphological). In seeds with physiological dormancy, the biochemistry and biophysics of dormancy breakage and dormancy induction is regulated by metabolic pathways involving promoters, inhibitors, and membrane modifications (Hilhorst & Cohn, 2000).

The heavy fraction of 60% has the best percentage of germination from treated seeds with cold (4 °C) and pretreated with 1000 ppm of gibberellic acid for 48 hours, followed by the heavy fraction seeds and pretreated with 100 ppm gibberellic acid for 48 hours; when compared to the grains of the 0, 20 and 40% fractions, which didn't germinate. This can be explained by several possible factors, such as seed quality or nutrient availability, which is not conducive to germination in these specific fractions. In this case, it was discovered that very poor seed germination led to very low Juniperus plant production. The main cause of this in several of Juniperus species is the seeds' prolonged physiological dormancy (Pinna et al., 2014; Momeni et al., 2018; Mohammadi Zade et al., 2018; Bertsouklis et al., 2019). Further analysis of fraction components and germination conditions would be needed to understand more precisely the reasons for zero germination in these cases.

Acid treatments such as sulfuric acid (H_2SO_4) have been shown to enhance germination by breaking the seed coat allowing water absorption and reserve substances imbibitions (Ali et al., 2011). Sulfuric acid treatment has proven effective for various species in temperate and subtropical regions including *Gleditsia triacanthos* and *Ceratonia siliqua* (Kisou et al., 1983). Tropical tree species such as *Intsiapalembanica*, *Parkia javanica*, and *Dialiummaingayi* have also shown positive responses to sulfuric acid treatment (Sasaki, 1980). Other species like *Acacia albida*, *Acacia nilotica*. *Acacia Senegal*, *Acacia planifrons*, and *Prosopis tamarugo* have had successful storage after sulfuric acid treatment (Laurie, 1974; Habit et al., 1981; Willan, 1992). Negative results were found after *J. cedrus* seeds were exposed to concentrated nitric (HNO_3) or sulfuric acid (H_2SO_4), as well as after 30 to 60 days of cold stratification at 4 – 5ºC (Harry et al., 1995). Our results are in agreement with the cited data and show that breaking the dormancy of juniper seeds based on sulfuric acid treatment method is a good approach, especially considering that other approaches are rarely successful.

Gibberellic acid (GA3) has been reported to play a physiological role in promoting dormant seed germination by inducing hydrolytic enzymes in various plant species (Penfield, 2017; Baskin & Baskin, 2014; Zhang et al., 2006; Rogis et al., 2004). For example, *Eucalyptus delegatensis*, *E. fastigata*, and *E. regnans* seeds exhibited improved germination after treatment with gibberellic acid (Bachelard, 1967). *Nothofagus obliqua* seeds immersed in gibberellic acid for 24 hours resulted in rapid and complete germination in 14 days, whereas this species typically requires stratification for 28 to 42 days (Gordon, 1979). The strength of gibberellic acid also influences germination. Using 200 ppm resulted in 100 percent germination in 8 days, while 50 ppm achieved the same result in 12 days (Shafiq, 1980). Stratification treatments of 42 days at 3–5ºC resulted in 70% germination in 14 days and 88 percent in 28 days, compared to only 20 percent germination in control seeds soaked in distilled water without pre-refrigeration (Shafiq, 1980). Further research by Rowe & Gordon (1981) suggested that 4/7 gibberellic acid is preferred over 3 gibberellic acid as it shows better germination rates between 15 and 30 °C while the latter is more effective at temperatures above 21 °C.

The effectiveness of the stratification of seeds in Junipers in warmth at 20 °C for 16 weeks and cold at 1 °C for 12 weeks was recently proven by research on *J. polycarpos*. With this treatment of warm-cold, the capacity for germination increased from 8% to 72%. The treatment of seeds with gibberellic acid (GA3) and 6-benzyladenine (BA), either alone or in conjunction with cold stratification, did not produce significant results, according to Daneshvar et al. (2016). Moreover, according to the cited data by Abdalrhaman et al. (2021) and Darrudi et al. (2015),

a very low potential for regeneration from seeds was reported. Our results are satisfactory regarding the use of gibberellic acid, whether combined with cold treatment or not, especially the pretreatment of 48 hours, which presented the highest germination rate. Thereby, increasing attention was paid in our study to the possibilities offered by acid gibberellic and sulfuric acid technology, which could be an alternative method for conservation and natural regeneration.

The presence of inhibitors in the embryo, endosperm, or testa is one of the causes of seed dormancy to promote germination. Seeds lacking testa or isolated embryos which are incubated on media under carefully controlled in vitro culture conditions is also a strategy that some researchers have used to speed up the germination of some juniper species (Hazubska-Przybył, 2019; Khater & Benbouza, 2019).

Overall these findings highlight the challenges and potential solutions for germination in various *Juniperus* species, emphasizing the effectiveness of acid treatments and gibberellic acid in enhancing germination rates and offering insights for the long-term management and ecological restoration of these species (Bachelard, 1967; Gordon, 1979; Shafiq, 1980; Rowe & Gordon. 1981; García, 2001; Alberts & Mandel, 2004; Rogis et al., 2004; Zhang et al., 2006; Ali et al., 2011; Baskin & Baskin, 2014; Penfield, 2017; Khater & Benbouza, 2019). There should be a great deal more research done in this area, particularly investigations of other juniper species where seed germination is constrained by physiological dormancy.

Our findings contribute to the understanding of germination processes and provide valuable insights for future research aimed at improving germination and regeneration strategies for *Juniperus thurifera* L. and other challenging forest species facilitating seedling production and reforestation endeavors.

5. CONCLUSION

The results presented in this study have highlighted the effect of pretreatments with acid gibberellic and sulfuric acid on the germination of seeds of *Juniperus thurifera* L., which is distinguished by the almost total absence of natural regeneration. In this study an increase in germination rate was observed in untreated seeds in the heavy fractions; fraction 60% and fraction 40% compared to the fraction 0% and 20% and treated seeds (stored under cold conditions). The best effective pre-treatment for this species is with gibberellic acid which gave a very high germination rate (83.33%). This was recorded in the seeds of the heavy fraction untreated with cold (4 ºC) and pretreated with 400ppm of gibberellic acid for 48 hours. In treated seeds by 4 ºC the best percentage of germination is recorded in the heavy fraction and pretreated with 1000 ppm of gibberellic acid for 48 hours with a percentage equal to (80%). These results are very encouraging and can be used in the context of the ecological restoration of degraded ecosystems with highly endangered species in Algeria and around the world. The findings of this study serve as a starting point for additional research on *J. thurifera's* accelerated germination and regeneration for the long-term management of its particular environment in the Southern Mediterranean. In perspective it would be possible to test new techniques to increase the germination capacity of species with germination difficulties. This approach could also be applied to other forest species where germination is a major challenge for the production of seedlings and reforestation.

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AUTHOR CONTRIBUTIONS

SH, KK and ZG: design, performed the experiment, writing of the project; NH: laboratory experiments and writing; AZ, SB, SH and SL: help in writing and supervising the project; DA, KK and NM: statistical study. All authors have read and approved the manuscript.