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Influence of biogeography and nine chemical and physical pre-treatments on the germination of *Juniperus phoenicea L.* seeds in the northeastern region of Morocco

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Abstract

Juniperus species plays a crucial ecological role by inhabiting sand dunes along the coast and arid mountainous regions. It contributes significantly to the stabilization and protection of these areas against erosion. However, due to excessive exploitation, this species is currently facing a continuous decline in its population. The *Juniperus phoenicea* L. is a species that naturally occur in Morocco and belong to the Cupressaceae family. *Juniperus phoenicea* exhibits different flowering periods, releasing pollen during the winter or spring seasons in terms of the region and climat. To address this issue, a study was conducted to enhance the germination *of Juniperus phoenicea* L. seeds by evaluating the impact of biogeography and various pretreatment methods. Mature red cones were collected in February-March 2021 from three different locations in northeastern Morocco, representing coastal, semi-continental, and continental regions, based on the distribution patterns of these shrubs. The gathered seeds underwent nine distinct pretreatment methods, including physical approaches such as mechanical and thermal shock, as well as chemical treatments involving sulfuric acid and acetic acid, alongside a control. Subsequently, the treated seeds were incubated at a consistent temperature of 20°C. Before the germination step we proceeded to a viability test and we found that the seed viability was 87%, although it varied depending on the geographical origin of the seeds. The cold-hot stratification had the highest seed germination rate of the semi-continental, with a percentage of 66%, followed by the seeds of the continental site with 60%. The boiling water test recorded a Gr of 54% in semi-continental seeds, while 51% was recorded in continental seeds. Coastal seeds had the lowest viability and Grs regardless of stratification applied. Due to their better germination potential, the use of continental seeds to generate seedlings of *J. phoenicea* L. in eastern Morocco should be considered. Despite biogeographic changes, cold-warm stratification can increase germination potential at a significant rate up to 66%.

Keywords: *Juniperus phoenicea L.*, Cupressaceae, cold-warm stratification, seeds germination.
Abbreviations: J_*Juniperus*, Grs_Germination, Lp_latency time.

Introduction

With roughly 67 species, the genus *Juniperus* (Cupressaceae) is distributed in four continents: America, Africa, Europe, Asia and the Northern Hemisphere (Adams, 1998; Hazubska-Przybył, 2019; Neale et al., 2019). The genus species possess prickly needles and inflexible branches which are separated from those with scaly foliage and flexible branches (Adams, 2004). Over Eurasia and North America, the genus *Juniperus* thrives in temperate and subpolar climates and the highlands of the tropics. Broadly, *Juniperus* species can adapt to harsh environments and are resistant, they expand in regions with persistent drought and a dry climate with significant fluctuations in temperature (Pavon et al., 2020). When compared to other Mediterranean countries, Morocco likely has *J. phonieca's* most significant areas of its occurrence, because the species is present both inland and along the Atlantic and Mediterranean coasts, also colonising exposed slopes of the Moroccan mountain ranges i.e. the High, the Middle and the Anti-Atlas (Benabid, 2000). The *Juniperus phoenicea* L. populations in Morocco face numerous threats. These trees have historically experienced significant human-induced pressures, including the destruction of tourist facilities along the coast, illegal logging, and intensive local utilization in mountainous and inland regions. Moreover, *J. phoenicea* L. is susceptible to pest and fungal attacks due to its humid coastal habitat in northeastern Morocco, while *J. phoenicea* L. populations face climate change and consequent droughts, especially in arid and semi-arid areas (Arar et al., 2020).

The *Juniperus phoenicea L.,* also known as the red juniper, holds various meanings and significances depending on ecological, cultural, and social contexts. Here are some essential aspects of this species:

In ecological terms, *Juniperus phoenicea L.* plays a crucial role in Mediterranean ecosystems by contributing to local biodiversity, providing a unique habitat for various plant and animal species. As a native species, its conservation is essential for maintaining ecological balance. The cones of *Juniperus phoenicea L.* are consumed by various frugivores such as the blackbird (Turdus merula) and thrushes (Debussche & Isenmann, 1985; Mandin, 2010). This species, belonging to the Cupressaceae family, plays a significant role in forest and ecological stabilization of soils, as emphasized in numerous studies (Benabid & Fennane, 1994; Boudy, 1950; Lemoine-Sebastian, 1965). *Juniperus phoenicea L.* thrives in thermal conditions ranging from -12 °C to 45 °C and flourishes in arid areas with only 200 mm of annual rainfall. It shows notable indifference to soil types and offers remarkable wind resistance (Lemoine-Sebastian, 1965). The red juniper is a favorable choice for stabilizing coastal dunes and reforesting dry mountains, with the ability to be planted up to 2000 m in altitude (Boudy, 1950). It can also be used for ornamental purposes and as a windbreak, thanks to its dense crown and bushy branches arranged in a goblet shape from the trunk's base. In addition to these ecological roles, *Juniperus phoenicea L.* has medicinal properties. It is considered an important medicinal plant by (Allali et al., 2008; Bellakhdar, 1997). Other economic values of red juniper include its production of high-quality yellow cabinet wood with white sapwood (Boudy, 1950). This wood is remarkably durable and a high-quality fuel. It is used for the construction of roofs of mountain houses (Wattiez, 1930).

The population of *Juniperus phoenicea L.* in Morocco faces numerous threats, historically experiencing significant anthropogenic pressures, including destruction of tourism infrastructure along the coast, illegal logging, and intensive local use in mountainous regions and interiors. Additionally, *Juniperus phoenicea L*. is vulnerable to attacks by pests and fungi due to its humid coastal habitat in northeastern Morocco, while populations face the challenges of climate change and droughts, particularly in arid and semi-arid zones(Arar et al., 2020). Red juniper forests are at imminent risk of extinction due to lack of natural regeneration. Despite its exceptional forest character, it is often neglected in foresters' reforestation projects. They cite as justification its secondary nature, it's very slow growth, the difficulty of natural regeneration and the fact that its seeds have almost negligible germination in the nursery (El Abidine et al., 2003).

The level of protection afforded to the red juniper species varies depending on their geographical location. In certain Mediterranean countries like Spain and Tunisia (Dakhil et al., 2022), the species is considered rare and receives protection status. However, on *Juniperus thurifera* compared to other juniper species (Ferradous et al., 2013; Jouaiti et al., 1997).

The best way to conserve red juniper stands in Morocco is to produce plants for reforestation and to conduct germination experiments. Thus, it is vital to have access to improved seed germination methods, which guarantee appropriate germination percentages, and to acquire strong and healthy seedlings for the conservation of the species. Having effective protocols for seed germination is crucial to ensure acceptable rates and healthy plant growth, limited seed dispersal ability is another challenge for survival(El-Bana et al., 2010; Nuet Badia & Romo, 2021; Quevedo et al., 2007) and its low germinative power (Khatamovich et al., 2023), due to a physiological dormancy, not yet broken by effective treatments, in which the embryo is unable to develop a radicle due to a physiological inhibition mechanism(Baskin & Baskin, 1998; García-Fayos et al., 2002). Moreover, only a few of its harvested cones bear viable seeds (Wesche et al., 2005) which are characterized by a very persistent seed coat (Adams et al., 2003).

Seed nursery programs in Morocco are a basement for forestry productivity by providing managers with high-quality seeds, forestry nursery programs involve the production and management of tree seedlings for reforestation projects. As *J. phoenicea* in north-eastern Morocco can grow in a variety of conditions, our long-term target is to cultivate it in nurseries for local conservation forestry. However, because to scares seed germination, establishing a steady supply of the species seedlings from seed can be difficult for nursery managers. Applying different pre-treatments in thick semi-permeable seed coat such as heating in water, treating with sulfuric acid or citric acid, or cold-warm stratification aims to overcome the dormancy of the embryo and allow water absorption. Pre-treatment of tree seeds is an important step in ensuring successful germination and growth of seedlings. The choice of efficient pre-treatment is critical to achieving the desired outcomes. The most used pre-treatment methods include scarification, stratification, and soaking in water or chemicals. The choice of pre-treatment depends on the species of tree being cultivated, some species require scarification to break their hard seed coat, while others require stratification to simulate winter conditions. The current study aims to determine the most effective pre-treatment method for breaking *J. phoenicea L.* seeds dormancy from north-eastern Morocco and examines how seeds from coastal, semi-continental, and continental as three different geographic populations will react regarding different dormancy breaking pre-treatments.

The study is in addition to improving seed germination techniques, resulting in better forest production and improved seed management for conservation both in natural habitats and in nurseries. Moreover, it helps to understand the ecophysiology of germination in red juniper.

Results

Viability of the Juniperus phoenicea L. seeds

The viability test was conducted to assess the germination capacity of seeds in different geographical regions. The results indicate that, respectively, 7%, 8%, and 13% of seeds from semi-coastal, continental, and coastal varieties were eliminated. These percentages of loss vary significantly depending on the geographical location of the tested seeds (Table 4).

This variation underscores the potential impact of geographical location on seed viability, suggesting that factors such as climate, soil, or other environmental conditions specific to each region can significantly influence the germination capacity of seeds. These findings highlight the importance of considering geographical diversity when assessing seed viability, which could have significant implications for the selection and conservation of plant varieties adapted to specific conditions.

Germination rate of the Juniperus phoenicea L. seeds

T3 and T5 pretreatments resulted in higher germination rates for *J. phoenicea* L. seeds in all three groups. This suggests that these treatments were more effective in promoting seed germination, regardless of seed geography. Other pre-treatments were not as effective, for coastal seeds of *Juniperus phoenicea* L., 70% of which pre-

Table 1. Climatic characteristics of the sampling sites.

Site	Altitude (m)	year	Latitude	Longitude	M(°C)	m(°C)	P (mm)	Q2	Bioclimates
Coastal (Saidia)	4	2021	35°11'	2°28'	31.83	7.03	315.04	44.4	Semi-arid Hot winter
Semi-continental (Oued El Himer)	951	2021	34°41'	1°88'	33.2	0.14	359.16	37.9	Semi-arid Cool winter
Continental (Figuig)	1850	2021	32°22'	1°66'	41.31	2.02	12.6	12.6	Saharan Cool winter

Table 2. Pre-treatments performed on *Juniperus phoenicea L.* seeds.

Nature of the pretreatment	Abbreviation	Number of seeds/sites	Code	Description
No pre-treatment	**T1**	**90**	Coastal1 (Coastal) SC1 (Semi-continental) C1 (Continental)	Control
Stratification at 4°C for 30 days	**T2**	**90**	Coastal2 (Coastal) SC2 (Semi-continental) C2 (Continental)	Physical pre-treatment
Stratification at 4°C for 30 days followed by 60 days at 20°C	**T3**	**90**	Coastal3(Coastal) SC3(Semi-continental) C3 (Continental)	Physical pre-treatment
Stratification at 4°C for 60 days followed by 60 days at 20°C	**T4**	**90**	Coastal4(Coastal) SC4 (Semi-continental) C4 (Continental)	Physical pre-treatment
Immersion in boiling water (100°C) for 2 minutes	**T5**	**90**	Coastal5 (Coastal) SC5(Semi-continental) C5(Continental)	Chemical pre-treatment
Immersion in citric acid* for 5 days, followed by 30 days at 4°C	**T6**	**90**	Coastal6 (Coastal) SC6 (Semi-continental) C6 (Continental)	Chemical pre-treatment
Immersion in citric acid* for 10 days, followed by 30 days at 4°C	**T7**	**90**	Coastal7 (Coastal) SC7 (Semi-continental) C7 (Continental)	Chemical pre-treatment
Immersion in sulfuric acid** for 15 min.	**T8**	**90**	Coastal8 (Coastal) SC8 (Semi-continental) C8 (Continental)	Chemical pre-treatment
Mechanical scarification***.	**T9**	**90**	Coastal9 (Coastal) SC9 (Semi-continental) C9 (Continental)	Physical pre-treatment
Mechanical scarification*** followed by cold stratification at 4°C for 30 days	**T10**	**90**	Coasta10 (Coastal) SC10 (Semi-continental) C10 (Continental)	Combined physical and cold pre-treatment

*Citric acid 5,000ppm ** Sulfuric acid 70%*** Rubbing with abrasive paper

treatments did not promote seedlings. On the other hand, seeds that were not exposed to any T1 pretreatment had a much lower germination rate, with less than 21% of seeds germinating at all three sites. i) Coastal Seeds of *Juniperus phoenicea L.*, T5 cold stratification had the highest germination rate of 3% ± 1, it was the most effective in breaking seed dormancy compared to no pretreatments (T1), which showed with the cold-hot stratification T3 the highest germination rate with values of 2% ± 0.57. All other applied pretreatments (T2, T4, T6, T7, T8, T9 and T10) were effective. ii) Semi-continental seeds of *Juniperus phoenicea L.* reached 66% germination rate; cold-hot stratification pretreatment was the most effective in breaking seed dormancy. The T3 pretreatment has the highest germination rate with a value of 66% ± 1.15.

Pre-treatment T5 also has a relatively high mean germination rate of 54% ± 5.19. Finally, pre-treatments of cold stratification T2 and mechanical scarification T9 have much low germination rates of 16% ± 1.52 compared to the control (T1) 21% ± 2. The other pre-treatments did not exceed 10% of the rate of germination, while T7 destructed completely the seeds. iii) Continental seeds of *Juniperus phoenicea L.* reached 60% of germination rate, the pre-treatments cold-warm stratification T3 and immersion in

Table 3. Measured parameters in seeds of *Juniperus phoenicea L. in* the coastal, semi-continental and continental geographic locations under the applied treatments

	Site 1 Coastal			Site 2 Semi-continental			Site 3Continental		
	Gr	Lp (day)	T_{50} (day)	Gr	Lp (day)	T_{50} (day)	Gr	Lp (day)	T_{50} (day)
T1	2 ±0.57 ^{b}	181±7.54^{a}	198.33±2.88^{a}	21±2^{a}	99.66±13.42^{c}	153±6.55^{b}	18±1^{a}	139.33±6.65^{b}	156.66±7.02^{b}
T2	0±0 ^{b}	-	-	16±±1.52^{a}	154±13.52^{a}	186±4^{a}	24±2^{a}	137±11.78^{a}	192±7.54^{a}
T3	2 ±0.57 ^{b}	153.33±13.50^{a}	192.66±6.42^{a}	66±1.15^{a}	146.66±11.06^{a}	189±7.54^{a}	60±0.57^{a}	44.33±6.65^{b}	132±13.07^{b}
T4	0 ±0 ^{c}	-	-	3±0^{a}	135±6.24^{a}	168±4.35^{a}	16±0.57^{a}	140±7.93^{a}	158.33±7.09^{a}
T5	3±1 ^{b}	155.33±10.11^{a}	166.33±5.5^{a}	54±5.19^{a}	155.33±8.02^{a}	184±13.52^{a}	51±1.15^{a}	133±11.26^{a}	164.33±14.01^{a}
T6	0±0 ^{a}	-	-	3±1^{a}	152±8^{a}	188±13.52^{a}	6±0.57^{a}	101.66±8.32^{b}	125.66±3.21^{b}
T7	0±0 ^{b}	-	-	0±0^{b}	-	-	16±0.57^{a}	197±11.13^{b}	223.33±10.4^{a}
T8	0±0 ^{a}	-	-	6±0.57^{a}	274±12.49^{b}	287.66±2.08^{b}	3±1^{a}	312.33±11.23^{a}	320±17.32^{a}
T9	0±0 ^{b}	-	-	16±1.52^{a}	187.33±9.45^{a}	203±7.54^{a}	16±1.15^{a}	163.66±7.57^{b}	192.66±6.42^{a}
T10	0±0 ^{b}	-	-	6±0.57^{ab}	264.66±6.02^{b}	290.66±8.32^{b}	9±1.15^{a}	283.33±9.45^{a}	322±9.84a

Value followed by different letters are significantly different (p<0.05). Value with the same letters indicates no significant difference (p>0.05). (column comparison). **Gr**: number of germinated seeds. **Lp**: the time required for the first germination. **T50**: average time for 50% of the seeds to have germinated. **-** : no germination detected.

boiling water T5 have the highest mean germination rate with a value of 60% ± 0.57 and 51% ± 1.15 respectively. Compared to control T1 which showed a rate of 18%± 0.57, the cold stratification T2 showed good results in dormancy breaking as well with 24% ± 2 rate of germination. Whereas; the chemical stratification T8 destructed the seeds (Supplemental Figure 1).

Germination kinetics

The latency period varied significantly ($p < 0.05$) among the three different geographic locations (Table 3). Coastal seeds exhibited the highest level of dormancy, while seeds from semi-continental locations were the least dormant. In the control T1, the semi-continental location had the shortest latency period of 99.66±13.42 days, which was the lowest among the three locations the applied tests did not effectively lower this period. Test T3 specifically, reduced the latency period to 44.33±6.65 days for the continental location (Table 3). The difference in t_{50} among the three geographic locations continental, coastal, and semi-continental was significant ($p < 0.05$). For test T3, t_{50} was shortest in the continental 132 ±13.07 days, followed by coastal 192.66±6.42 days and semi-continental 189±7.54 days. For test T5, t_{50} was shortest in the coastal location 166.33±5.5 days, followed by the continental 164.33±14.01 days and semi-continental 184±13.52 days location (Table 3). Among the seeds of *Juniperus phoenicea L.*, the coastal seeds had the slowest germination speed, with cold stratification T5 being the most effective in reducing the latency time and having the highest kinetics. Cold-warm stratification T3 improved the germination kinetics of seeds, but the other pre-treatments (T2, T4, T6, T7, T8, T9, and T10) did not have a significant impact on breaking dormancy in coastal seeds (**Fig 3A)**. The semi-continental seeds of *Juniperus phoenicea L.* had a moderate germination speed, with the highest kinetics observed with pre-treatment T3, and T5 is the most effective in reducing the latency period of seeds (**Fig 3B)**. The continental seeds of *Juniperus phoenicea* L. had the fastest germination speed, with T3 showing the earliest start of germination compared to coastal and semi-coastal seeds, as well as other sites. Pre-treatments T3, T5, and T2 were observed to improve seed kinetics compared to the control T1 (**Fig 3C).**

Effect of geographic location and pre-treatments

Geographic location, pre-treatment and their interaction had a significant effect ($p < 0.001$) on germination rate, Latency period, and t_{50}. The geographic location of sites significantly influenced: the germination rate with the pre-treatments had a significant impact on the Latency period and t_{50}. *i)* Germination rate: both "Site" and "pre-treatment" have a significant effect on the Gr ($p < 0.001$) and their interaction is also significant ($p < 0.001$), the origin of the seeds and the pre-treatment received both have an impact on the Gr, and the effect of pre-treatment may depend on the sites. *ii)* Seeds viability: Only "pre-treatment" has a significant effect on seeds viability ($p \leq 0.001$), and there was no significant effect of "Site" or their interaction, the pre-treatment applied to the seeds influences their viability. *iii)* Latency period (F=68.839, $P \leq 0.000$) depended significantly on the site, indicating that the seed's geography influenced significantly the dormancy of seeds, the effect of pre-treatment also has a significant effect on Lp (F=261.669, P=0.000) also the interaction (site x pre-treatment) is also significant (F=28.146, P=0.000), implying that the pre-treatment effect on dormancy varies depending on the seed's origin, iv) t_{50}: the factor Site has a significant effect on t_{50} (F=26.328, P=0.000), implying that the geography of the seeds influences the germination time, pre-treatment also has a significant effect on t_{50} (F=244.141; P=0.000), indicating that the pre-treatment applied to the seeds affected significantly the time of germination, also their interaction was significant (F=20.106, P=0.000), implying that the pre-treatment effect on germination time varies depending on the seed's geography (Table 4).

Effect of geographic location

The compound analysis (CA) revealed four groups with a statistical significance of $p \leq 0.05$. The initial two factors accounted for 72.01% of the overall variability, with F1 contributing 43.18% and F2 contributing 28.83% (**Fig. 4)**. The F1 of PCA showed a positive correlation with latency period (Lp), t_{50}, P (mm) and m (°C) but a negative correlation with seeds viability, germination rate, and M (°C). Conversely, the F2 exhibited a positive correlation with Lp, t_{50}, M (°C) and viability, but a negative correlation with germination rate, precipitation P(mm) and m (°C). There is significant

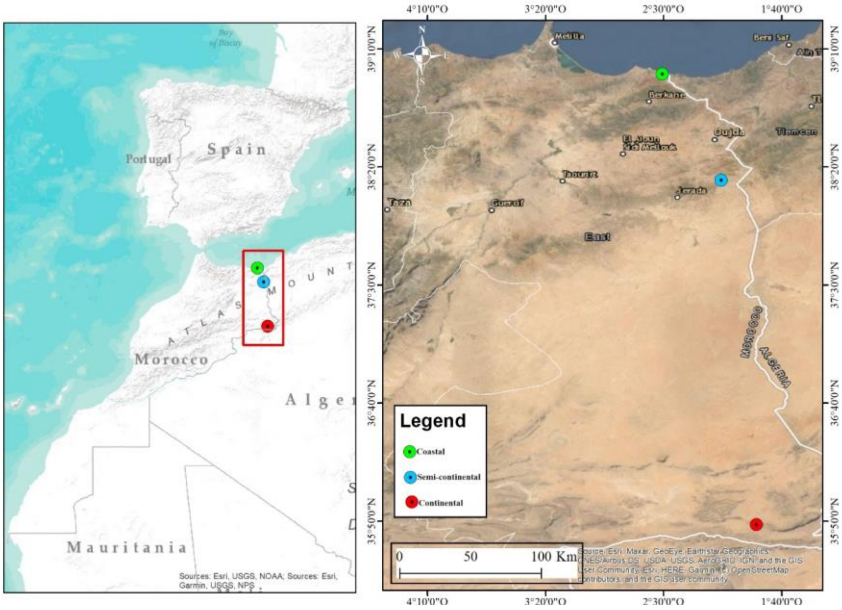

Figure 1. Map of the three sampling locations.

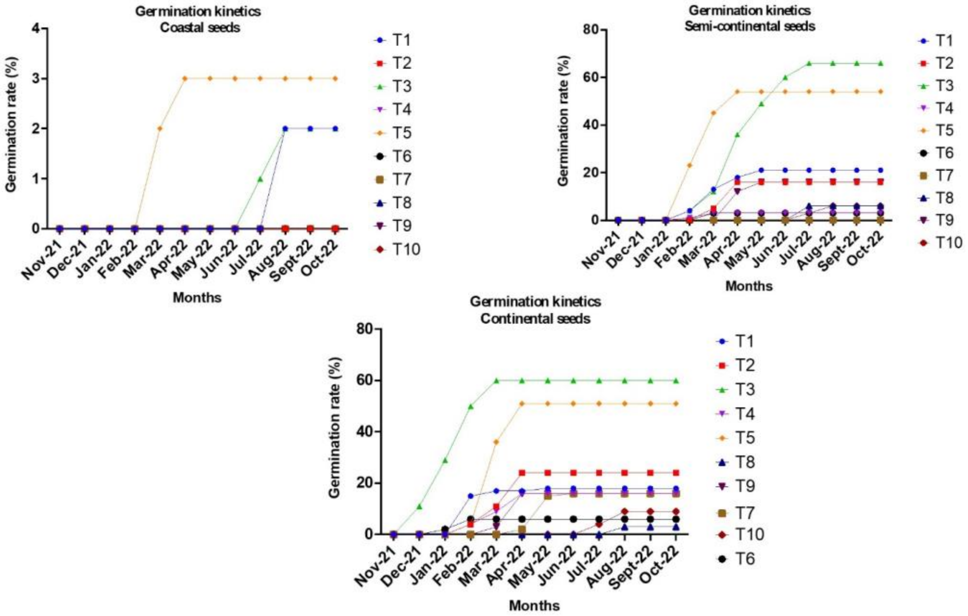

Figure 2. Germination kinetics and evolution of the latency time of *Juniperus phoenicea L.* seeds in the three sites A: seeds from Coastal; B: seeds from semi-continental; C: seeds from continental.

variability among the various samples (including the geographic location and pre-treatments) regarding their germination parameters. semi-coastal and continental samples were negatively aligned with the first axis, indicating high germination rate, high seeds viability and high M (°C), as well as low latency period, low t50, low m (°c) and low precipitations (P mm). Coastal and semi-continental seeds treated with specific pre-treatments had a positive correlation with the F1, meaning they had high Latency period, high t_{50}, high precipitations (P mm) and high m (°C) but low Germination rate, low viability, and low M(°C). Some semi-coastal and continental seeds had medium to low seeds viability, low latency period and low t50, while coastal samples had low germination. The first group contains thirteen samples: SC1, SC2, SC3, SC4, SC5, SC6, SC9, C1, C2, C4, C6, C7, and C8. These samples exhibit high to medium levels of germination rate and high to medium seeds viability; low latency period; low t_{50}; high M (°C); medium to low precipitation (P mm). The second group consists of two samples: C3 and C5, which have a low latency period and low t50, but the highest germination rate of seeds, the highest M(°C) and the low precipitation (P mm). The third group includes four samples: SC8, SC10, C9, and C10, which have low viability of seeds, germination rate that ranges from low to medium, and medium latency period and medium t_{50}. Lastly, the fourth group comprises three samples of Coastal 1, 3, and 5, which display high t_{50} and high latency period, but low germination rate and low seeds viability: high precipitation, high m (°C).

The analysis of correlation (Table 5) indicates that there is a strong negative correlation -0.97 (significant at the 0.01 threshold) between m(°C) and seed viability, this implies that in continental areas with decreasing temperature during winter are the most viable, which is confirmed by the results of T3 (Table 3). As a result, a strong positive correlation of 0.94 (significant at the threshold of 0.01) between the

Table 4. Tests between-subjects effects.

	Site		Pre-treatment		Site X Pre-treatment	
	F	P	F	P	F	P
Germination rate Gr	211.393	0.000	88.573	0.000	9.89	0.000
Latency period L_p	68.839	0.000	261.669	0.000	28.146	0.000
50% de germination t_{50}	26.328	0.000	244.141	0.000	20.106	0.000

Table 5. Correlation matrix between germination parameters and climatic conditions.

	Gr	**Lp**	**t50**	**m(°C)**	**M(°C)**	**P(mm)**
Viability	0.46**	-0.58**	0.11	-0.97**	0.52*	-0.38*
Gr		-0.47*	-0.35	-0.49**	-0.39*	-0.34
Lp			0.94**	0.088	-0.27	0.28
t50				-0.38	-0.17	0.18
m(°C)					-0.71**	0.59*
M(°C)						0.00

** Correlation is significant at the 0.01 level (two-tailed). m(minimum mean temperature); M (minimum mean temperature), Lp (latency time) and t50 (the time needed for 50% germination)*.

latency period and t50. This implies that seeds from the coastal location that have a longer latency period (Table 3) are more likely to have a longer t50.

Second, there is a negative correlation of -0.71 (significant at the 0.01 level) between m (°C) and M (°C), which is explained by a warm winter semi-arid bioclimate in coastal regions and a cold winter semi-arid bioclimate in continental regions (Table 1).

The correlation of -0.49 (significant at the 0.01 level) between germination rate and m(°C) tells us that cold weather favours seed germination, which is confirmed by T3 (Table 3) and by germination rate in seeds from continental regions (Table 3).

The correlation of 0.46 (significant at the 0.01 level) between viability and germination rate suggests that seeds with higher viability from the semi-continental are more likely to germinate and have a shorter latency period. This is supported by the negative correlation of -0.58 (significant at the 0.01 level) between viability and lag period.

In addition, there is a negative correlation between germination rate and lag period, indicating that pretreatments that shorten the lag period result in the best germination rate (Table 3).

Discussion

The study evaluated the efficiency of physical and chemical pre-treatments on the germination of *Juniperus phoenicea L.* as well as the effect of geographic location and climatic conditions on the germination rate and latency period of *Juniperus phoenicea* L. seeds. The viability of seeds depended on their site of sampling (Table 4), consequently, it influenced the rest of the measured parameters after the application of pre-treatments such as germination rate, latency period and t_{50}.

The viability test eliminated 7%, 8%, and 13% of the seeds from the Semi-coastal, continental, and coastal seeds respectively that varied significantly according geographic location, which varied significantly according to the applied pre-treatments and geographic location (Table 4) the limited viability of *Juniperus phoenicea L.* seeds is a significant barrier to their recruitment, as it reduces the number of viable seeds dispersed that can potentially germinate.

(Gruwez et al., 2013) identified it as a critical factor in the seed development of *J. communis*. The findings of this study demonstrated that the geographic location had a significant impact on the viability of *Juniperus phoenicea L.* seeds. The disparity between the viability of *Juniperus phoenicea L.* seeds and the germination rate of seeds from North-Eastern Morocco, imply that seeds have different levels of dormancy(Baskin & Baskin, 1998). Some applied pre-treatments in the current study could improve but selectively the germination rate depending on the location and levels of dormancy.

Among the different types of pre-treatments used, such as cold-warm stratification (T3 and immersion in boiling water (T5) as physical pretreatments, both were found to be the most effective in promoting the germination of seeds of *Juniperus phoenicea L.*, regardless of the geographic location, while other pre-treatments were found effective depending on the origin of the population.

Cold-hot stratification was found to be the most effective method for inducing germination in *Juniperus phoenicea L.* seeds that exhibited morpho-physiological dormancy required only 30 days of stratification at 4°C followed by 60 days at 20°C, which resulted in a germination rate that reached 66%. This process involved breaking physiological dormancy by cold stratification and promoting embryonic growth by prolonged warm stratification.

Cold stratification breaks integumentary dormancy and embryonic dormancy (Willan, 1992). which is confirmed by the following studies, According to(El Abidine et al., 2003), subjecting red juniper seeds to a 30-day cold stratification period at a temperature of 5°C greatly enhanced their germination rate, resulting in an increase of up to 97%. (Tilki, 2007) conducted a study where a cold pretreatment period of 3 months significantly improved the germination rate of Juniperus oxycedrus L. In the study, this pretreatment resulted in a germination rate of 63%.

Our results on the effect of cold-warm stratification on improving germination rate align with the natural environmental conditions i.e. cold of winter is followed by warm of spring. In situ *Juniperus phoenicea L.* cones are mature during the autumn season; the female buds start to develop just prior to flowering, which occurs when the temperature begins to decrease (middle of autumn to start

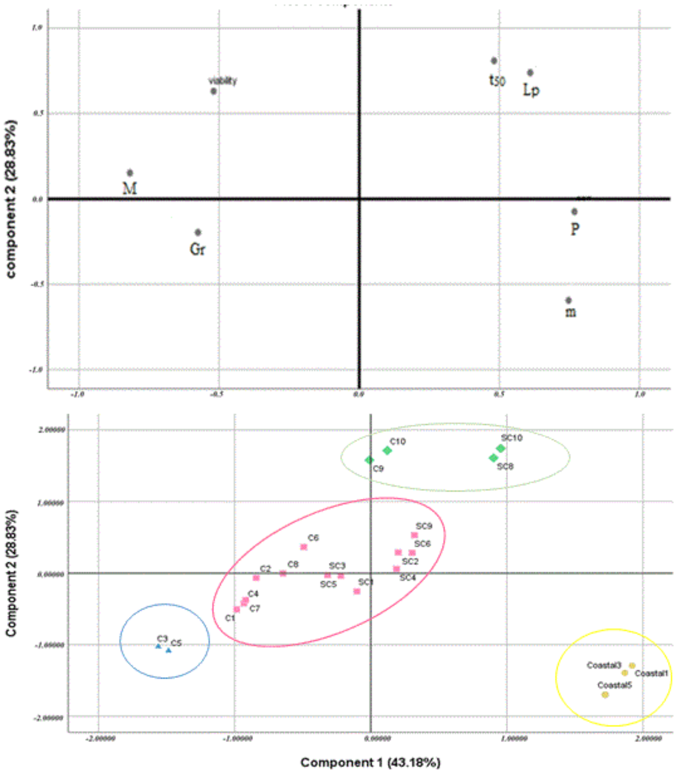

Figure 3. Principal component analysis A: Distribution of variables, B: Distribution of samples.

of winter). The morpho-physiological dormancy of the cones is broken by the low winter temperature low m(°C). As a result, the seeds can germinate in either the spring or early summer.

Immersion in boiling water (100°C) might be efficient because the heat softens the seed coat of coastal seeds, allowing water and oxygen to penetrate and reach the embryo, thus initiating the germination process.

Chemical pre-treatments such as immersion in citric and sulfuric acid lowered germination rates than the control and had a destructive effect on the seeds,(Sy et al., 2001) The immersion of seeds in sulfuric acid for 15 minutes provides worse results than the control. The pretreatment T8 (immersion of seeds in sulfuric acid for 15min) allowed to increase in the germination rate in *Juniperus phoenicea L.* seeds according to (El Abidine et al., 2003), This effect is related to the origin of the seeds and the duration of immersion; coastal seeds (Mehdia and Essouira in the West of Morocco) did not tolerate the immersion in the sulfuric acid (destructive effect), on the other hand, the seeds resulting from the continental areas (Toufliht in the high Atlas & Berkine in the Middle Atlas) which was beneficial for the increase of germination. This difference in tolerance is probably related to the hardness of the teguments which characterizes the seeds in the arid zones, a mode of conservation during the dry periods (Audin, 1993).

The combination of immersing *Juniperus phoenicea L.* seeds in citric acid for 5 days/10 days followed by 30 days in the cold (4°C) gives less satisfactory results than the control. on the other hand, these two pretreatments tested on seeds of *Juniperus oxycedrus* L. from Turkey gave significant results which is explained by the bioclimatic difference between the origin of the seeds (Tilki, 2007).

Mechanical scarification was found to damage the embryo and raise the possibility of fungal development. However, in the case of *Juniperus oxycedrus* seeds, this procedure was beneficial and led to increasing germination (Tilki, 2007). In the current study, it was found that seeds with high kinetics exhibited a high protection against the risk of fungal development, lowering the latency period was associated with an increasing risk of seed rot and low germination rates (Butler & Kempton, 1987; Johnson et al., 1995) as the correlation between latency period and germination rate was very significant (Table 5).

Cold stratification (T2) cold-hot stratification (T3) and boiling water immersion (T5) the three pretreatments favored the germination of continental seeds*;* cold-hot stratification (T3) and immersion in boiling water (T5) favored the germination of semi-continental seeds; finally, only immersion in boiling water (T5) favored it in coastal*,* it illustrates the importance of choosing the appropriate test for each population (genotype) of seeds and displays the effectiveness of a test to follow to raise the seedling of red juniper. In today's changing environment, finding the appropriate dormancy-breaking test to match local genotypes adapted to their local climatic conditions (Table 1) is critical, especially for breeding programs and creating migration plans. Managers would then be able to move the best genotypes to regions where the environment is expected to change (Locarnini et al., 2018).

The *Juniperus phoenicea* L. is found in the northeastern region of Morocco, extending from the coast in sand dunes along the littoral to the arid mountains of the interior, but it is restricted to specific and isolated ecological niches. The distribution of vegetation in the region is mainly determined by local climatic conditions. It was observed that the geographical location had a significant impact on the germination parameters, *Juniperus phoenicea L.* seeds are characterized by low germination rate, but there is difference within seed lots, there could be two groups of seeds one of which has non-deep dormancy (that is i.e. semi-coastal seeds) and the other is dormant (i.e. continental) for which pre-treatments could solve the problem.

Only immersion in boiling water at 100°C for 2 minutes was effective in decreasing lag time and increasing germination rate, among the various pretreatments that were used. This finding is consistent with the results reported by El Abidine et al. (2003), where the effectiveness of this pretreatment depended on the duration of immersion and the origin of the seeds. In the case of *Juniperus phoenicea* L. from the Moroccan coast, germination was significantly improved by immersing the seeds in boiling water (100°C) for shorter periods (ranging from 1 to 5 minutes). However, for seeds from mountainous regions, this pretreatment was effective only when the immersion times were longer (10 to 15 minutes).(El Abidine et al., 2003).

Or they were not able to reproduce (infertile) in situ male buds develop before female ones. The male buds started forming in late spring and bloomed during the summer, while the female flowers appeared just prior to the male flowers' blooming (Supplemental Figure 2).

The coastal location has the smallest number of seeds compared to the other two areas, but they are also the longest and widest (Sahib et al., 2022). This suggests that a significant portion of the energy resources in this location may be allocated towards developing the morphology of the seeds rather than reproduction.

Semi-continental populations have the highest number of seeds and leaves among all three populations, and it displayed intermediate values for most of the morphological features (Sahib et al., 2022). This could be attributed to the fact that the semi-continental *Juniperus phoenicea L.* grows in a more exposed environment and receives more sunlight, leading to a greater number of seeds with good quality. *Juniperus* is generally considered a genus that thrives in areas with abundant sunlight. The semi-continental population's positive response to increased light was evident in the significant increase in the quality of seeds with non-deep dormant characteristics (Table 2), the latency period of the control lot was 100 days the lowest among the three lots of *Juniperus phoenicea L*. populations. the semi-continental population has a greater number of seeds, which could enhance its pollination success, the continental climate seems to provide better pollination conditions for red juniper north-eastern Moroccan populations The semi-continental population showed intermediate values of the dimension of cones red juniper of north-east Morocco had an adapted morphology (Sahib et al., 2022)to the unique environmental conditions found in semi-continental location areas.

Continental population seeds were triggered by pre-treatment T3 specifically, which reduced the latency period to 44.33±6.65 (table 2) days and trigged the germination immediately (fig.3) consequently seeds had the fastest germination. Three applied pre-treatments T5, T3 and T2 were observed to improve seed kinetics compared to the control T1. In a selection local program, continental seeds are the best lot of selection. The continental population is located at 1,850m asl, previous studies in *Juniperus* exhibited that seeds originating from high-altitude populations had a higher germination rate compared to those from low-altitude populations i.e. coastal. The species *J. drupacea* exhibited better germination results in seeds collected from an altitude of 1400 m(Yücedağ et al., 2021). This finding is consistent with a previous study by (Douaihy et al., 2017) on the same species, which reported similar results for seeds collected between 1500 and 1600 m. The continental population shows the smallest cones dimensions (Sahib et al., 2022).

The origin of the seed population significantly influenced the emergence of seedlings, the three populations come from different geographical locations. The variability in germination rates and dormancy levels within the seed lot could be related to differences in species, seed characteristics, size being the most important genetic and environmental influences. The ability of red juniper seeds from northeastern Morocco to adapt morphologically to their specific bioclimatic conditions was demonstrated in a previous study (Sahib et al., 2022). There are morphological variations of the characteristics among the cones of the three sampled populations which surely had an impact on their behaviour vis-à-vis the pre-treatments.

For the genus Juniper, temperature and precipitations as external climatic factors may be necessary to break embryo dormancy (Pinna et al., 2014). The negative correlation between viability and m (°C) indicates that lower viable seeds come from the coastal location where winters are hot (m=7.03°C) while continental (m=2.02 °C) and semi-coastal seeds that grow under cool winters and (m=0.14 °C) exhibited better viability and less dormancy. Probably the cold temperature of winter triggers physiological processes that facilitate germination(Mao et al., 2010), These processes involve a reduction in the level of ABA in the embryonic axis and a decrease in the expression of the protein phosphatase gene that is present in dormant seeds (Lorenzo et al., 2001), that impacts the potential for growth of the embryo (Alvarado and al. 2000). Furthermore, the good number of precipitations in semi-continental location (P=359.16mm) was a natural break of seeds dormancy, it was enough to provide the necessary moisture for the seeds to germinate. Decreased levels of drought stress are particularly crucial during the initial years of seedling growth in semi-arid areas.

Material and methods

Seeds sampling and processing

Cones of *J. phoenicea* L. were collected from three sites, representing a biogeographic gradient of typical Mediterranean conditions. The geographical locations and climatic conditions of the three sites are shown in Figure 1 and the table. 1. i) *J.phoenicea* L. in Saïdia in the province of Berkane represents the coastal range of the species; ii) *J. phoenicea* at Oued el Himer in the province of Jerada represents the semi-continental range of the species; and iii) *J. phoenicea* at Abou Lkhel in the province of Figuig represents the continental range of the species in northeastern Morocco. Cones were collected between February and March 2021 from all accessible branches of infection- and insect-free shrubs.

In the laboratory, the hard pericarp of the fruits was softened by soaking the cones of *J. phoenicea L.* in sterile distilled water for 24 hours. With a binocular magnifier, the cone's fleshy scales were manually removed to reveal the seeds. Following 48 hours of air drying, 2700 seeds were collected according to their biogeography, with 900 seeds per site.

Pre-treatment and viability test

The viability of seeds was tested using specific gravity (SG) separation in water, which effectively sorted nonviable seeds of *Picea abies* L. (Karst.)(Dumont et al., 2015). Seeds were placed in a bowl containing sterile distilled water and stirred to facilitate separation; the floating and sunken fractions were collected separately after 24h (20°C temperature). The floating test separated a fraction of floating seeds (empty seeds) that was eliminated, and a fraction of sunken seeds (viable and ungerminated seeds), seeds were air-dried at room temperature for 48 h to reduce the moisture content (Daneshvar et al., 2017). Before the application of the pre-treatment seeds, disinfection was performed using a 10-minute treatment with sodium hypochlorite (NaClO), followed by rinsing with sterile distilled water.

To determine the most effective method for breaking the dormancy of *J.phoenicea L.* seeds, we carried out a series of 9 pretreatments plus a control test; T1 control test, T2 Stratification at 4°C for 30 days, T3 Stratification at 4°C for 30 days followed by 60 days at 20°C, T4 Stratification at 4°C for 60 days followed by 60 days at 20°C, T5 Immersion in boiling water (100°C) for 2 minutes, T6 Immersion in citric acid for 5 days, followed by 30 days at 4°C, T7 Immersion in citric acid for 10 days, followed of 30 days at 4°C, T8 Immersion in sulfuric acid for 15 min, T9 Mechanical scarification and T10 Mechanical scarification followed by cold stratification at 4°C for 30 days. as explained in (Table 2).

Germination

The germination of *Juniperus phoenicea* L. seeds collected from three sites was investigated, three replicates of 30 seeds each were randomly sampled from each seed population, and seeds were enclosed in 9.5 mm Petri dishes on cotton that were kept continuously moist with distilled water. Petri dishes containing seeds were placed on a germination table at a phytotron (**Fig. 2**) as a controlled chamber at 18°C with 16/8 Day/Night photoperiod in a completely randomized design. A copper oxychloride fungicide (5g/l) was applied to prevent any fungal attacks during the experiment. The germination process was monitored every week, and germinated seeds were counted when the radicle reached 10mm and had a normal appearance. The experiment lasted 12 months starting from the day of sowing until no further germination was observed, indicating the end of the experiment. The following parameters were determined: *Germination rate* (Gr %) expressed in percentage was computed as: $R = \frac{GS}{IS} * 100$; (GS) number of germinated seeds at the end of experiment. (IS) a few sown viable seeds. *Viability of seeds*: considered as the sum of the number of germinated seeds added to the number of viable ungerminated seeds; viability of the ungerminated seeds was assessed by a cut test (Osticioli et al., 2013) considered based on the total number of firm seeds that had developed embryos. The latency period (L_p days): germination delay was considered as the time between when the seeds are sown and when their germination started; *50% germination* (t_{50} days): mean germination time it takes for 50% of germination; *Germination Kinetics*: accumulation of the germination rate over time from the sowing of seeds until the end of the experiment.

Experimental design

After eliminating non-viable seeds, a total of 2700 seeds were subjected to nine different pre-treatments, in addition to the control group. Each pre-treatment was applied to 30 seeds, and this procedure was repeated three times, totalling 90 seeds tested per treatment. In total, 900 seeds were tested for each origin site. The boxes containing the seeds were placed in incubation in a growth chamber, maintaining a constant temperature of 20°C, with a photoperiod of 16 hours.

Statistical analysis

All tests were performed by "SPSS for Windows version 23" software. The mean germination rate was calculated for each population and each pre-treatment, the differences were considered significant at $p \leq 0.05$, and differentiation between populations in terms of germination rate was verified using Tukey's T-test, and the differences were considered significant at $p \leq 0.05$. ANOVA two-way univariate was performed to reveal the relation between site and pre-treatment and their interaction with Gr, seeds viability, Lp and $t_{50.}$ Principal component analysis (PCA) is performed to assess the impact of geography. The results were presented as mean ± SD. A correlation matrix was performed to assess to the relation between germination parameters and climatic conditions.

Conclusion

Juniperus phoenicea L. play a crucial role in safeguarding mountains and coastlines. However, the natural habitats of red juniper stands are experiencing severe degradation due to human activities, exacerbating the challenges associated with the species' natural regeneration and climate change. Nevertheless, this study demonstrates that overcoming germination difficulties in Juniperus can be achieved through appropriate pretreatment methods. The germination rate of the seeds was significantly influenced by both the origin of the seeds and the pretreatment techniques employed. These findings hold significant importance as they contribute to the restoration efforts of degraded areas populated by *Juniperus phoenicea* L. along coastal regions and in semi-continental and continental regions. The outcomes of this study can serve as an asset in reforestation programs conducted by water and forest agencies, aiding in the combat against desertification.

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