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Study of the effect of pretreatments on the germination and growth of *Pistacia Atlantica subsp. atlantica*: investigations for the conservation of an endangered species in Morocco

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Abstract. The study delves into the critical issue of declining Moroccan endemic plant species of agro-sylva-pastoral and medicinal significance, impacted by various environmental degradation factors. With the aim of conserving and rehabilitating these species, the research focused on improving the germination and growth parameters of *Pistacia atlantica* subsp. *atantica* whose seeds were carried from different area of the Middle Moulouya; Oled Ali Youssef; El Orjane and Fritissa. In this study, nine different treatments were employed to overcome seed dormancy. These treatments included chemical scarification using gibberellic acid and sulfuric acid (98%), thermal scarification at 40°C for 24, 48, and 72 hours, mechanical scarification using manual sandpaper, and stratification at +4°C for 45, 60, and 75 days. The results showed that mechanical scarification yielded notably higher germination rates compared to the control group. Specifically, for seeds from Oled Ali Youssef, the germination rate was 81.11%, while for El Orjane and Fritissa, it was 38.88% and 71.11%, respectively, in contrast to 67.78%, 25.56%, and 44.44% in the control group. Moreover, the study revealed that treatments such as mechanical scarification, gibberellin application, and 30-day cold stratification significantly influenced the growth of seedlings, enhancing propagation practices for the species from its seeds. These findings underscore the importance of seed source, viability, and pre-germination treatments in achieving successful germination and growth.

Keywords: *Pistacia atlantica* subsp. *atlantica*; Scarification; stratification, Germination; Growth; Conservation; Middle Moulouya.

1. Introduction

For millennia, the conservation of forests and forest vegetation in the Mediterranean basin has posed a complex challenge due to the numerous uses and pressures exerted by the various cultural entities of the Mediterranean [1].The multiple pressures stemming from human activity, anthropogenic factors, and ecological influences have led to a noticeable degradation of our environment, necessitating concrete action in favor of its preservation. The Pistacia genus is primarily subtropical and comprises eleven species, some of which hold significant cultural and economic importance.

Taxonomic relationships among species like *Pistacia atlantica* Desf. are unclear due to genetic barriers. It is a dioecious species with unisexual flowers[2]. Pistacia atlantica plays a significant role as a rootstock in pistachio cultivation.

The *Pistacia Atlantica* subsp. *atlantica*, belonging to the botanical family Anacardiaceae, is commonly referred to as "Lbtom" in Darija, "Ijj," "Tijout," and "Tijft" in Berber of the Middle Atlas of morocco. It is a robust, woody tree endemic to North Africa, with an imposing silhouette when mature[3].*Pistacia Atlantica* Desf has been utilized for various purposes for a long time, including culinary, medicinal, fodder, and wood-related applications [4].

Its resin is utilized for medical, cosmetic, and artisanal purposes, including as chewing gum, antiseptic, and digestive tonic. Additionally, it is employed in repairing artworks and in the production of cosmetics and perfumes. [4-6].

Crude extracts and isolated compounds from *Pistacia Atlantica* Desf. exhibit a wide range of pharmacological properties, including antibacterial [6], antifungal [7], anti-inflammatory [8], and antidiabetic effects [9].

This species is known for its richness in valuable compounds, including volatile compounds, flavonoids, phenolic compounds such as oleic acid, linoleic acid, and palmitic acid [10], as well as fatty acids, tocopherols, and phytosterols [11] .*P. atlantica* also contains minerals and trace elements like iron, lead, copper, potassium, sodium, and calcium [12] , along with fat-soluble vitamins such as α-, β-, γ-, δ-tocopherol, and phytosterols [13] .

P. atlantica Desf inhabits a vast region spanning North Africa, the Middle East, Iran, and Afghanistan [2].

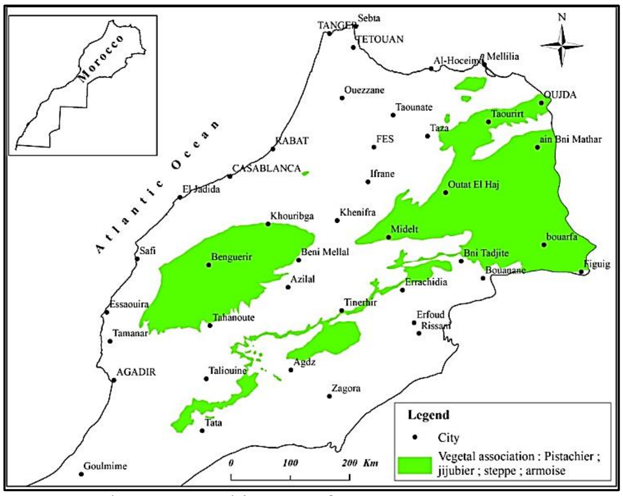

Figure.1.phytogeographic map of Morocco **(Northern region of Morocco)**

According to the International Union for Conservation of Nature (IUCN), the presence of *Pistacia atlantica Desf.* varies from rare and occasional to abundant in only a few locations.

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However, the number of mature individuals and population density have significantly declined over the past decades due to numerous threats, including ruthless domestic use, collection for fodder or firewood and trade, harvesting practices, overgrazing, deforestation, desertification, sand encroachment, erosion, and insect attacks. Given the extent of species exploitation, it is projected that the global population size will decrease by 25% over the next 100 years [14].

The distribution map of *Pistacia Atlantica* subsp. *atlantica* in Morocco indicates that this species occupies extensive areas in the southern and eastern regions but in a scattered and isolated manner or clustered as isolated patches in maraboutic sites or protected areas, across various bioclimates ranging from semi-arid to Saharan on different soil types [15].

As a result, the reintroduction of *Pistacia Atlantica* Desf. has become a significant concern for semi-arid and arid regions in Morocco. However, this can only be achieved through the mastery of its propagation processes. Natural regeneration of this species remains challenging and highly unpredictable, mainly due to the hardness of the seed coats that inhibit germination[16]. Threats to unprotected populations primarily come from overgrazing, anthropogenic pressure, and drought. The lack of interest in this species will inevitably lead to its extinction. Hence, the importance of this study is to optimize seed germination and seedling growth of *Pistacia Atlantica* Desf., aiming to overcome the obstacle of tegumentary inhibition that affects the natural regeneration of this species.

2. Material and Methods

2.1.Study area

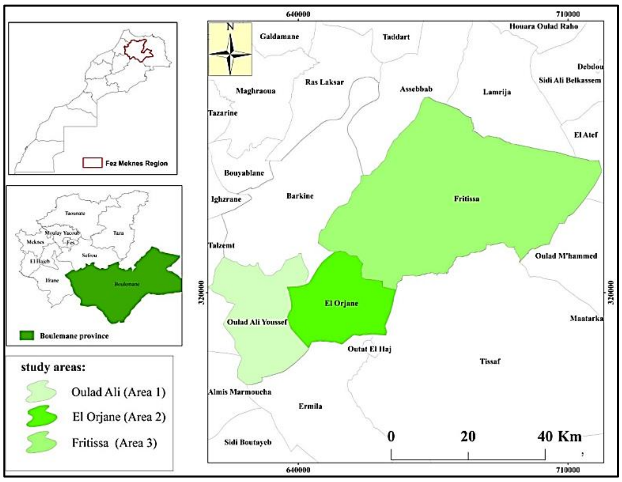

Figure.2.Location of the study area.

The study area is situated within of the Middle Moulouya, recognized as one of the geomorphological areas within the Moulouya watershed, geographically located in Area 1 of Morocco (northern Morocco, Merchich). Administratively, this sub-basin falls under the Fez Meknes region.

2.2.Site Selection

Within our study area, we identified three primary areas:
Area 1: Ouled Ali Youssef (33°27'46.8"N 3°58'10.5"W)
Area 2: El Orjane (33°34'05.8"N 3°47'04.1"W)
Area 3: Fritissa (33°37'25.0"N 3°36'37.4"W)

2.3.Plant Material

The seeds used in this study belong to the species Pistacia atlantica Desf. They are harvested when mature, and the selected trees for harvesting are in good vegetative condition. After harvesting, the seeds are air-dried, and a sorting process is carried out to remove any infected seeds. The seeds are then placed in bags and stored at room temperature, protected from light, until they are used.

2.4.Viability Testing

The viability of *Pistacia Atlantica* Desf. seeds is assessed using three methods:

2.4.1. Mechanical Test

Seeds are manually cracked open, and the number of full and empty seeds is recorded as a percentage [17] .

2.4.2. Floatation Test

This test provides a preliminary estimation of seed viability. Floating seeds are separated from sinking ones, and the percentage of seeds separated and those remaining at the bottom is calculated[18].

2.4.3. Standard Germination Test

Petri dishes containing seeds treated with fungicide are placed between two layers of filter paper soaked in water. The dishes are then placed in a germination chamber at room temperature. Results are expressed as a percentage of germination [19].

2.5.Determination of Moisture Content

The seeds are weighed, and then they are placed in an oven (80 ± 2°C) for 72 hours. Afterward, they are weighed again using a precision balance. The moisture content of the seeds is determined using the following expression [20].

$$* \quad TE(\%) = \left(\frac{Pi - Ps}{Pi}\right) \times 100 \qquad (1)$$

TE (%): Moisture Content; Pi: Initial Weight (fresh); Ps: Final Weight (dry).

2.6.Seed Treatments

Various batches of seeds underwent pre-germination treatments with the aim of breaking seed dormancy, thereby facilitating the exchange between the embryo and the external environment, especially water absorption 23] [22-23].

To investigate the effect of seed origin, soaking in water, the duration of stratification at 4°C, chemical and mechanical scarification, and gibberellin treatment on germination rates, 900 seeds from each site were treated with a fungicide (50% procymidone) and then divided into 10 seed lots. Each lot of 90 seeds underwent the following treatments:
T0: (Control);
T1: stratification at +4°C for 45 days;
T2: stratification at +4°C for 60 days;
T3: stratification at +4°C for 75 days;
T4: Chemical scarification with concentrated sulfuric acid (H_2SO_4, 98%) for 15 minutes;
T5: Thermal scarification (40°C) for 24 hours;
T6: Soaking in warm water (40°C) for 48 hours;
T7: Soaking in warm water (40°C) for 72 hours;
T8: Mechanical scarification (manual) using sandpaper;
T9: Chemical scarification with gibberellic acid (GA3) at 1000 ppm for 24 hours.

2.7.Cultivation Process

The pre-treated seeds are sown at a depth of 1cm in cells containing black peat (organic matter content > 90%). They are then covered with vermiculite to maintain substrate moisture. Daily watering is carried out using an automatic sprayer, and this operation is repeated as needed. Before placing them in a germination chamber set at 28°C, the cells are labeled with the sowing date, the type of pre-treatment, and the seed origin. The young seedlings that emerge are transferred to polyethylene bags containing a peat substrate. Subsequently, the seedlings undergo growth measurements under semi-controlled conditions.

3. Expression of Results

3.1.Measurement of the Germination Process

To express seed germination, we used the following parameters:

3.1.1.Germination Rate (GR) [22]

Represents the number of germinated seeds relative to the total number of seeds subjected to germination. This parameter is considered the best way to determine seed germination capability. It is expressed as a percentage and calculated as follows:

$$GR\% = (n/N) \times 100 \quad (1)$$

n: Number of germinated seeds; N: Total number of seeds subjected to germination.

3.1.2.Germination Kinetics (GK)

To specify the physiological significance of the germination behavior of *Pistacia Atlantica* Desf. seeds from the three studied origins, the number of germinated seeds was counted every 2 days until the end of the experiment [23].

3.1.3.Germination Velocity

Reflects the germination energy responsible for depleting the seed's reserves. It can be expressed by the median germination time (T50) (the time when 50% of seeds have germinated [24] . The coefficient of germination velocity (VC) [27-29] corresponds to the average reciprocal germination time, allowing for the determination of germination uniformity [26].

$$T50 = T1 + \left(0.5 - \frac{G1}{G2-G1}\right) \times (T2 - T1) \quad (2)$$

G1: Cumulative percentage of germinated seeds with a value closest to but less than 50%.
G2: Cumulative percentage of germinated seeds with a value closest to but greater than 50%.
T1: The time corresponding to the germination of seeds with a value closest to but less than 50%.
T2: The time corresponding to the germination of seeds with a value closest to but greater than 50%.

$$CV = \frac{\sum N_i}{\sum N_i Ti} \times 100 \quad (3)$$

Ni: The number of seeds that have newly germinated at time Ti and Ni+1: The number of seeds that have germinated between time Ti and Ti+1.

3.1.4.Germination Index (GI):

It reflects the percentage of germination on each day of the germination period [27]

$$GI = \sum \frac{Number\ of\ germinated\ seeds}{Number\ of\ days} \quad (4)$$

3.2.Measurement of Growth Process

For the study of growth, the following parameters were measured [28]:

3.2.1.Stem Height (HT)

Measurements of plant heights were taken from the base to the terminal bud. Measurements were made every 21 days over a period of 3 months.

3.2.2. Root Length (LR)

Measurements in centimeters were taken simultaneously with height growth measurements, using a ruler.

3.2.3. Number of Leaflets per Stem (NF)

The number of leaflets was calculated based on the developmental stage of the first stem of each seedling and concurrently with other growth measurements.

3.3.Statistical Analysis

The obtained results were subjected to analysis of variance using the software "GraphPad Prism 8.0.1," and significantly different means were separated using the "Tukey's test" at a 5% significance level.

4. Results

4.1.Seed Quality

4.1.1.Seed Viability Test

From the graph, we observe that the highest viability percentage is provided by the floatation test, followed by the mechanical test, and then the standard germination test. The highest viability rate was obtained in Area 1 for all types of treatments (75.77%), followed by Area 3 (37.44%), and finally, Area 2 (29.44%).

4.1.2.Determination of Moisture Content

Seeds from Area 3 exhibited the highest moisture content percentage (4.87%), followed by Area 1 (3.80%), and lastly, Area 2 (2.57%).

The results were subjected to analysis of variance, and significantly different means at a 5% threshold were indicated in the figure using different letters. The presence of the same letter indicates no significant difference. The findings indicate that *Pistacia Atlantica* Desf. seeds belong to the category of orthodox seeds, which can tolerate natural or artificial desiccation.

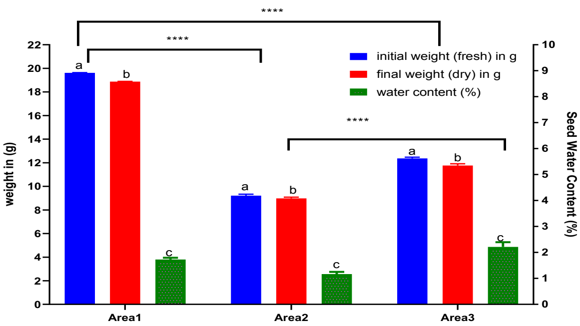

Figure.3. water content of the seeds (Values marked with the same letter are not significantly different; ****: significantly different)

4.2.Germination

4.2.1.Effect of Origin on Germination

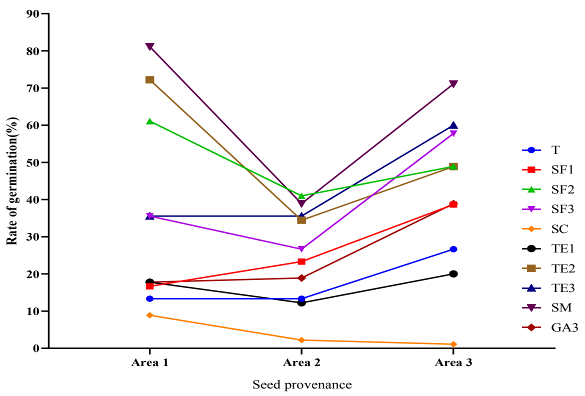

Figure.4.Germination rate of the three areas

The first parameter of germination considered is the final germination percentage.
We conducted a comparative "control" test of the germination potential of seeds from the three studied areas. Regular monitoring of germination allowed us to establish cumulative germination curves for these three seed lots. the results of these observations are expressed in the figure 5.

The examination of this figure shows that, regardless of the type of treatment, seeds from area 1 often exhibit the highest germination rates, while those from areas 3 and 2 have the lowest rates.

This figure also demonstrates that, except for seeds treated with chemical scarification using sulfuric acid, which maintain a low germination rate, all other seeds treated with various pre-treatments show an increase in germination rate.

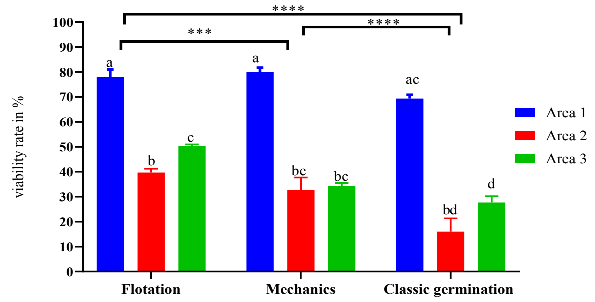

Figure.5. Seed *Pistacia Atlantica* Desf viability (Values marked with the same letter are not significantly different).

The results of the analysis of variance reveal that the seeds from the three selected areas are statistically significant at 5%, as shown in the following table:

Table.1.Comparing the number of germinated seeds of *Pistacia Atlantica* Desf. according to seed origin.

Tukey's test	Mean Diff.	Signification	Summary	P value
Area 2 vs. Area 1	0.07143	No	ns	0.8933
Area 3 vs. Area 1	1.738	Yes	***	0.0004
Area 3 vs. Area 2	1.667	Yes	***	0.0001

Germination tests of seeds from this tree exhibit variable responses depending on their origin and the type of treatment. Such a result suggests the potential to link the germination behavior of the *Pistacia Atlantica* Desf. to its genetic variability and ecology at the adult stage. The results of the statistical analysis reveal a significant difference at the 1% level between the seed origin and the various treatments applied.

4.2.2.Effect of Pre-treatments on Germination

The recorded results indicate that the pre-treatment (chemical or physical) of seeds has a notable effect on the number of germinated seeds for all three studied origins.

Area 1: Mechanical scarification, soaking seeds in lukewarm water for 48 hours, and cold scarification at 4°C for 60 days successively enhance the germination potential of *Pistacia Atlantica* Desf. seeds from area 1.

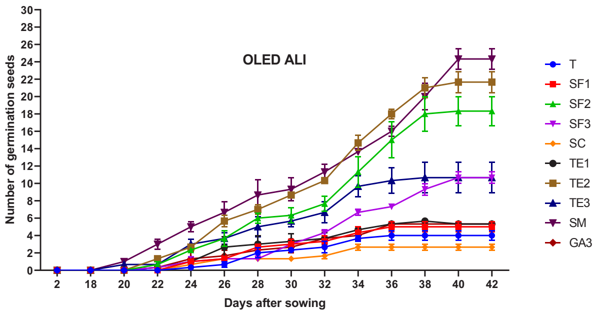

Figure.6.Germination kinetics seeds from area1

In fact, a comparison of the number of germinated seeds using the smallest significant difference method at a 5% significance level confirms the results shown in figure 6.

Area 2: The figure shows that seeds stratified scarified in the cold for 60 days had the highest number of germinated seeds compared with the other treatments.

The analysis of variance showed a highly significant effect ($p<0.0001$) between the treatment and the different treatments except for soaking the seeds in warm water for 24 hours.

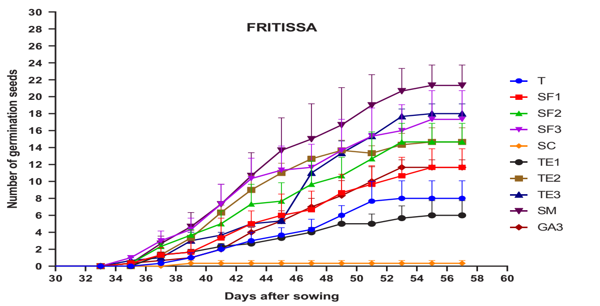

Figure.7. Germination kinetics seeds from area 2

Area 3: The highest number of germinated seeds was recorded in the batch of seeds mechanically treated with sandpaper, with an increase compared with the control of +44.43%.and the lowest values were observed in seeds chemically scarified with sulfuric acid, with a decrease compared with the control of almost -23.36%.

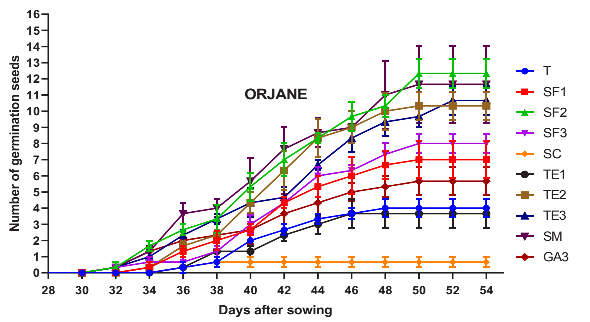

Figure.8.Germination kinetics of seeds from area 3

The differences observed are highly statistically significant ($p<0.0001$), except for the 24h warm water soaking. Scalding for (24h and 72h), cold scarification for 30 and 90 days gave a lower daily average and median germination time than seeds soaked in warm water for 48h and cold scarified for 60 days. chemical scarification with sulfuric acid has a negative effect on seed germination behavior and germination parameters.

The mechanical scarification of seed coats results in the highest germination rate (81%) for all seed lots from different sources; as the germinations continue to grow, they produce more vigorous plants (Figure.9).

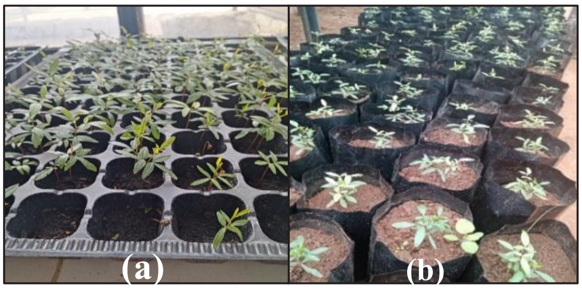

Figure.9. Young seedlings of *Pistacia atlantica* Desf. **a**: seedlings after 8 weeks on peat substrate **b**: seedlings transplanted into polyethylene bags.

Our results indicate that boiling and scarification are effective treatments. These treatments not only increase the final germination rate of seeds from all three sources but also reduce the average germination time. The results obtained with sulfuric acid can be attributed to its corrosive action on the seed coats as well as the embryos. Indeed, mechanical scarification with sandpaper is the most effective method as it improves all germination parameters compared to the control group for seeds from Ouled Ali (Area 1) and Fritissa (Area 3).

It's worth noting that cold scarification at 4°C for 2 months resulted in the highest germination rate compared to other pre-treatments for seeds from El Orjane. However, the average germination time was improved by mechanical scarification for seeds from the same source. previously, the characterization of germination kinetics was based on the work of [29].However, since 2005, a more comprehensive approach has emerged, involving an assessment of the germination process through a set of parameters. these parameters encompass germination capacity, the speed of this crucial process, the daily germination rates, and the overall germination index. This transition has led to a better understanding of the underlying mechanisms of the germination process.

Furthermore, pre-treatments such as mechanical and cold scarification, the use of warm water, or GA3, have a positive effect on the overall germination behavior, except for chemical scarification using sulfuric acid (H_2SO_4). This can be observed through variations in the different calculated parameters (GR: germination rate, T50: average reciprocal germination time, GK: germination Kenetics, VC: the velocity coefficient, GI: Germination Index); confirming their positive impact on the initiation of germination (Table 2).

Tableau.**2.** Averages of the main germination parameters of *Pistacia Atlantica* Desf. seeds from the three Area (A1, A2 and A3) according to the different pretreatments (SF1: cold stratification at 4°C for 45 days; SF2: cold stratification at 4°C for 60 days; SF3: cold stratification at 4°C for 75 days; SC: Chemical scarification by concentrated sulfuric acid (98%); TE1: Soaking in warm water (40°C) for 24 hours; TE2: Soaking in warm water (40°C) for 48 hours; TE3: Soaking in warm water (40°C) for 72 hours; TM: Mechanical scarification by sandpaper; GA3: Chemical scarification by gibberellic acid.

A1	**T**	**SF1**	**SF2**	**SF3**	**SC**	**TE1**	**TE2**	**TE3**	**SM**	**GA3**
GR	13.33±3.33	16.67±3.33	61.11±9.62	35.56±3.85	8.89±1.92	17.78±1.92	72.22±6.94	35.56±10.1	81.11±6.94	17.78±1.92
T50	27.45±1.50	26.00±1.01	27.40±2.95	29.00±0.00	23.68±1.52	24.45±1.39	26.15±0.64	23.20±6.28	20.67±10.9	24.67±2.08
GK	0.11±0.03	0.14±0.03	0.48±0.09	0.28±0.02	0.08±0.02	0.14±0.02	0.55±0.05	0.29±0.07	0.61±0.05	0.15±0.01
VC	2.81±0.06	2.84±0.02	2.77±0.03	2.73±0.05	2.86±0.06	2.86±0.10	2.79±0.00	2.87±0.04	2.83±0.03	2.83±0.12
GI	0.21±0.07	0.27±0.07	0.81±0.14	0.45±0.04	0.14±0.04	0.30±0.07	1.01±0.08	0.57±0.16	1.12±0.11	0.29±0.06
A2	**T**	**SF1**	**SF2**	**SF3**	**SC**	**TE1**	**TE2**	**TE3**	**SM**	**GA3**
GR	13.33±3.33	23.33±6.67	41.11±5.09	26.67±3.33	3.33±0.00	12.22±5.09	34.44±5.09	35.56±5.09	38.89±13.8	18.89±5.09
T50	38.22±1.35	38.00±0.67	37.67±20.3	38.40±0.96	42.94±8.76	37.01±2.64	37.89±0.84	38.67±2.67	34.50±2.60	35.11±5.39
GK	0.09±0.02	0.14±0.04	0.25±0.02	0.16±0.02	0.18±0.27	0.09±0.03	0.22±0.04	0.22±0.02	0.24±0.07	0.12±0.03
VC	2.11±0.04	2.12±0.02	2.13±0.03	2.10±0.03	2.08±0.20	2.12±0.07	2.11±0.02	2.12±0.02	2.14±0.02	2.16±0.08
GI	0.17±0.03	0.29±0.07	0.48±0.02	0.32±0.04	0.03±0.03	0.15±0.05	0.43±0.08	0.40±0.02	0.48±0.13	0.24±0.05
A3	**T**	**SF1**	**SF2**	**SF3**	**SC**	**TE1**	**TE2**	**TE3**	**SM**	**GA3**
GR	26.67±12.02	38.89±12.6	48.89±12.6	57.78±19.5	3.33±0.00	20.00±10.00	48.89±9.62	60.00±6.67	71.11±13.9	28.89±13.88
T50	35.79±7.23	39.81±2.77	39.38±3.07	36.94±0.59	50.67±9.24	37.99±5.20	38.71±0.50	43.09±2.01	36.08±4.70	40.94±2.58
GK	0.16±0.07	0.21±0.07	0.28±0.08	0.32±0.11	0.02±0.00	0.12±0.05	0.30±0.03	0.34±0.03	0.40±0.09	0.22±0.03
VC	1.97±0.04	1.98±0.04	2.00±0.01	2.02±0.01	1.88±0.17	2.03±0.06	2.02±0.03	1.97±0.02	2.00±0.03	1.96±0.03
GI	0.26±0.11	0.39±0.17	0.53±0.19	0.64±0.24	0.03±0.02	0.22±0.08	0.60±0.06	0.62±0.08	0.79±0.26	0.40±0.08

4.3.Seedling Growth

4.3.1.Average Stem Heigh

The data in Figure 10 illustrate the effect of pre-germination treatments on the average stem height evolution of seedlings from two sources of *Pistacia atlantica* Desf. Examination of this figure reveals that the growth in height has been influenced by the type of treatment used. Indeed*, Pistacia Atlantica* Desf. seedlings thrive better with cold scarification for 30 days and chemical scarification using GA3 for Area 1 and Area 2. scarification for 30 days and chemical scarification using GA3 for Area 1 and Area 2.

4.3.2.Average length of the main roots

The analysis of the results obtained shows that root growth is influenced by the treatments used. For the three studied sources, the most significant root elongation in Area 2 and Area 3 was observed in seedlings subjected to mechanical scarification and cold treatment for one month.

In contrast, Area 1 displayed the highest values when gibberellic acid was used as a treatment. However, chemical scarification with sulfuric acid appears to be less favorable for the root development of the pistachio tree. On the other hand, the effect of the source on the growth of the root system was not observed in the cultivated seedlings.

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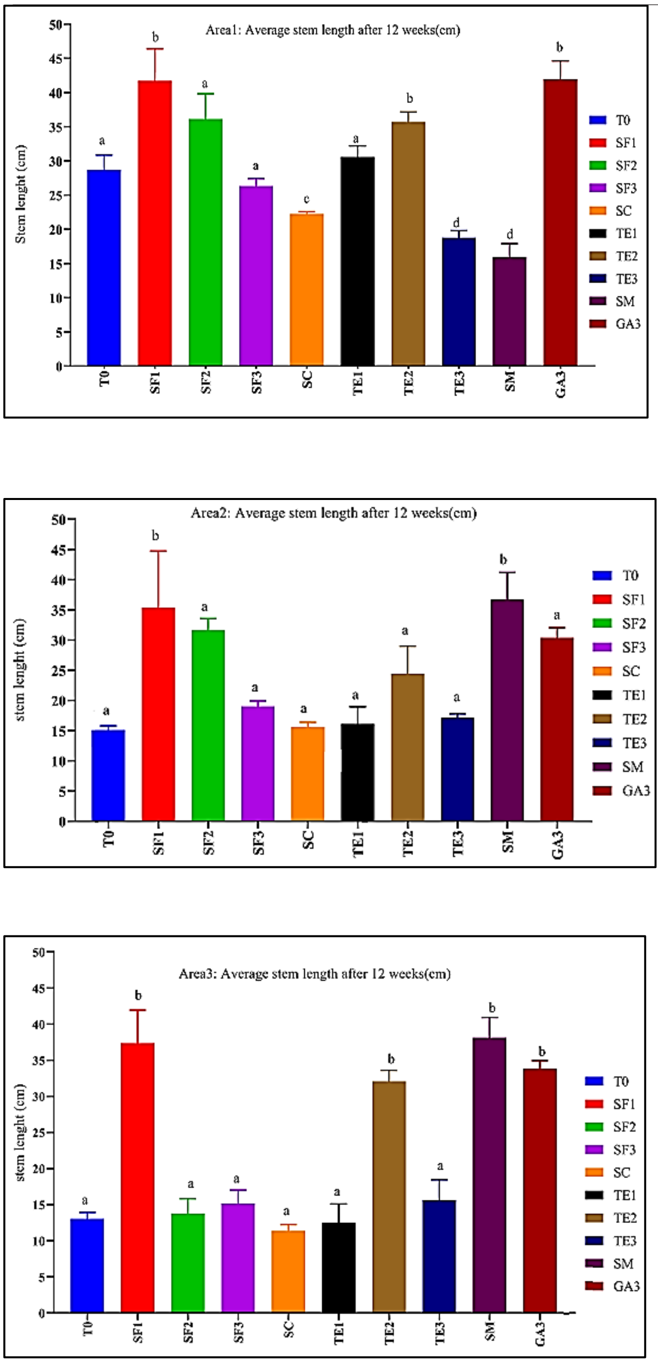

Figure.10. Evolution of aerial growth of Pistacia atlantica Desf in the three study areas according to the pre-germination treatment (Significant differences have been indicated in the figure by different letters a, b, c, d, The presence of the same letter indicates the absence of a significant difference).

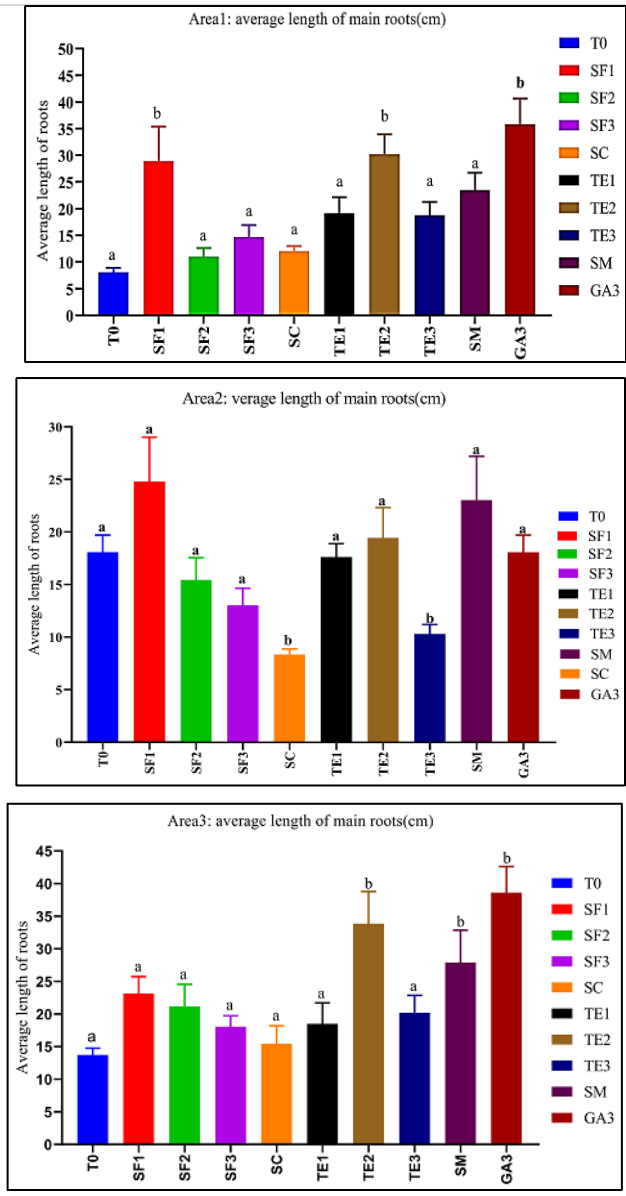

Figure.11. Variation of the average root length of young seedlings of the three provenances of Pistacia atlantica Desf. after 12 weeks of cultivation by different pregermination treatments (Values followed by the same letters within columns are not significantly different at 5%).

5. Discussion

5.1. Effect of Pre-germination Treatments on Germination

According to the results, it is evident that seed viability is influenced by their origin. Therefore, the assessment of seed viability using rapid and cost-effective tests is necessary during the storage and conservation of genetic resources or during their utilization in reforestation programs [30] .

The seeds of pistachio trees do not begin their germination process immediately after being released by the parent tree. They require a period of cold stratification to develop their germination capacity. In the case of temperate species, cold stratification is a common method to break seed dormancy [31][32].

Pistacia Atlantica Desf. seeds belong to the category of orthodox seeds, which can endure artificial or natural desiccation up to 4 to 6%. The moisture content of seeds from all three sources ranged between 2% and 5%. Quality criteria assessment indicates that they are of satisfactory quality.

The results of viability tests are pivotal as they mirror the true quality (either good or poor) of a batch [18]. Across all three sources, the classical germination test emerges as the most dependable.

The germination of Pistacia seeds is linked to the parent tree, their quality, successful pollination, and their recent harvest as "seeds of the year." Germination is generally straightforward for most Pistacia species, although it is influenced by the seed source [33].

The obtained results have indicated that pre-germination treatments (mechanical scarification, cold scarification at 4°C for two months, boiling, chemical scarification using gibberellic acid) have a positive effect on germination behavior, except for chemical scarification using sulfuric acid. This variation in results can be explained by the different calculated parameters.

The result of seed scarification is consistent with that of [34] who have shown that scarification significantly promotes the germination process, thereby involving the rapid inhibition of seed coat and the entry of water into reserves, allowing for the rapid emergence of the root and the initiation of metabolic reactions of the embryo and cotyledons.

The significant effect of stratification on the germination rate can be explained by its role in breaking seed dormancy, both embryo and possibly seed coat dormancy.

The cold and moist stratification process is known to soften the seed coats, facilitating the germination process. Stratification may also transform complex seed compounds into simpler, easily assimilable substances, leading to the resumption of seed physiological activity [35].

Studies conducted by [36] show that the germination rate of *Pistacia Atlantica* Desf. seeds increased from 40% to 90% with the duration of cold stratification. [33]. also report that the germination rate of various Pistacia species depends largely on the species itself: *Pistacia integerrima* 33.2%, *Pistacia palestina* 41.2%, *Pistacia vera* 53.1%, and *Pistacia terebinthus* germinated poorly, only 22.9%.

These authors suggest that the application of cold stratification in a humid environment improves results, especially since natural regeneration of the plant is absent in the area (due to uncontrolled grazing and dormancy).

Therefore, the use of treatments, particularly mechanical scarification, should be recommended to nursery workers and forest technicians to produce *Pistacia Atlantica* Desf. seedlings. These treatments offer the advantage of facilitating and promoting faster germination and the production of often more vigorous seedlings after transplantation.

Studies of [29] suggest that polyphenols and flavonoids produced in the fruit or seed may hinder germination. The inhibitory effect of phenolic compounds on seed germination is closely linked to the regulation of endogenous auxin, oxygen supply, and seed coat permeability. However, it has been observed that the concentration of phenolic compounds varies depending on seed treatments with gibberellic acid and scarification among the three species of Pistacia. The highest levels of phenolic compounds were found in P. *atlantica*. This finding could partially explain the low germination rate of untreated seeds observed in our experiment.[37]

The germination rate was also significantly influenced by the source of the seeds [38]. really the effect of geographic origin is highly significant among the three sites studied. This result can be explained by the fact that the seeds from Ouled Ali are of good quality.

Viability tests have indeed shown that the seeds from El Orjane have lower values compared to those from Ouled Ali and Fritissa. Differences in the edaphoclimatic conditions of the three sites or the state of fruiting could also explain this result. These differences among the studied sites are crucial for nursery workers and allow for better seed selection to achieve the best germination rates.

For these reasons, the study of other sites in the distribution areas of *Pistacia Atlantica* Desf. in Morocco could reveal genotypic differences among geographically distant populations.

5.2. Effect of Pre-germination Treatments and Source on Growth

The obtained rates may vary because initially, seedling growth measurements depend on the results of germination tests. Pre-germination treatments often affect the growth of the root system and stem height of *Pistacia Atlantica* Desf. seedlings.

However, we observed that there is no significant difference in growth due to the source. Similar results have shown a significant effect of substrate type and pre-germination treatments on stem height, stem diameter, and root system length [39].

6. Conclusion

Pistacia Atlantica Desf tree constitutes an important forest heritage in the Moyenne Moulouya region of Morocco. Following field surveys to collect plant material from the three studied sites (Ouled Ali Youssef, El Orjane, and Fritissa), it was found that the *Pistacia Atlantica* Desf. tree exhibits significant morphological variability in its natural habitat. Seed germination trials of this tree show varying responses depending on their origin and the type of pre-treatment.

Our results indicate the significant effect of mechanical scarification on germination and growth parameters of both underground and aerial parts. Indeed, cold stratification for 60 days in zones 1 and 2, and for 75 days in zone 3, appears to be more effective for inducing seed dormancy. However, further research is needed to confirm this finding, and additional studies are required to enhance the germination of Pistacia seeds.

At the juvenile stage, the growth of *Pistacia Atlantica* Desf. depends on the nature of the treatments applied during germination.

This species serves as an excellent barrier against desertification. Therefore, its rehabilitation and conservation are essential to contribute to the sustainable development of arid regions.

References

1. F. Médail, P. Quézel, *Conséquences écologiques possibles des changements climatiques sur la flore et la végétation du bassin méditerranéen*, B , **16**, n°1, pp. 397-422, (2003).
2. G. M. AL-Saghir et M. D. Porter, *Taxonomic revision of the genus Pistacia L. (Anacardiaceae)*, AJPS, **2012** (2011).
3. A. Monjauze, "*Connaissance du bétoum Pistacia atlantica Desf*", RFF, **32**, no 4, pp. 356-363 (1980).
4. J. Bellakhdar, *Contribution à l'étude de la pharmacopée traditionnelle au Maroc : la situation actuelle, les produits, les sources du savoir (enquête ethnopharmacologique de terrain réalisée de 1969 à 1992)*, TDUPVM, (1997).
5. N. Benhammou, F. A. Bekkara et T. K. Panovska, *Antioxidant and antimicrobial activities of the Pistacia lentiscus and Pistacia atlantica extracts*, AJPP, **2**, no 2, pp. 022-028 (2008)
6. L. Boulos, *Medicinal Plants of North Africa*, EM **9**, n°2 p. 168, (1983).
7. M. Azizian, I. Pakzad, R. Azizian, F.A. Jalilian, M. Taherikalani, N. Sadeghifard, M.M. Kartalaie, Raziehshirani, A. Delpisheh, N. Abassi, R. Havasian, J. Panahi, A. Hasanvand, *Antibacterial effect of hydro-extract of Pistacia atlantica on bacteria in vitro*, B P J, **6** , pp. 133–136 (2013).
8. M. Falahati, A. Sepahvand, H. Mahmoudvand, P. Baharvand, S. Jabbarnia, A. Ghojoghi, M. Yarahmadi, *Evaluation of the antifungal activities of various extracts from Pistacia atlantica Desf*, CMM, **1** (3), pp. 25–32 (2015).
9. G. Hajjaj, A. Bahlouli, M. Tajani, Y. Cherrah, A. Zellou, *In vivo analgesic and anti-inflammatory properties of the aqueous extract of Pistacia atlantica Desf. from Morocco*", JAIM. **3**, pp. 196–199 (2017).
10. I.I. Hamdan et F.U. Afifi, *Studies on the in vitro and in vivo hypoglycemic activities of some medicinal plants used in treatment of diabetes in Jordanian traditional medicine*, JEP, **93**, pp. 117–121 (2004).
11. M. Adams, I. Plitzko, M. Kaiser, R. Brun, M. Hamburger, *HPLC-profiling for antiplasmodial compounds: 3-Methoxycarpachromene from Pistacia atlantica*, PL, **2**, pp. 159–162 (2009).
12. M. Yousfi, B. Nedjmi, R. Bellal, D. Ben Bertal, G. Palla, *Fatty acids and sterols of Pistacia atlantica fruit oil*, JAOCS, **79** , pp. 1049–1050 (2002).
13. A. Asma, T. Boumediene, B. Mohammed, T. Nourelhouda, B. Mebrouka, *Antibacterial activity and physicochemical characteristics of Pistacia atlantica extracts*, DPC, **8** , pp. 162–168 (2016).
14. J.K. Lodge, M.G. Traber, A. Elsner, R. Brigelius-Flohé, *A rapid method for the extraction and determination of vitamin E metabolites in human urine*, JLR, **41**, pp. 148–154 (2000).
15. G.J. Rankou, H. M'SOU, S. Babahmad, R.A. Ouhammou, A. Alifriqui, M. Martin, *Red List Threat. Species. Pistacia atlantica*, IUCNNR (2018).
16. L. Emberger, *La végétation de la région méditerranéenne: Essai d'une classification des groupements végétaux*, RGB, **42**, pp. 641–662 (1930).
17. P. Boudy, *Guide du forestier en Afrique du Nord*, MRP, **1** (1952).
18. B.Suszka, C. Muller, M. Bonnet-Masimbert, *Graines des feuillus forestiers : de la récolte au semis*, INRA, pp. 55–56 (1994).
19. S.A. Boualem, D. Boukraa, D. Bouhadi, A. Elouissi, *Evaluation de l'effet de la période de greffage du pistachier vrai sur l'amélioration du taux de reprise et le développement*, AB, **12**, pp. 2961-2968 (2022).
20. C. Picasso, *Contrôle de la qualité des semences*, O , **38**, no 2 (1983)
21. R.L. Willan, *Guide de manipulation des semences forestières : dans le cas particulier des régions tropicales*, FAO, **20** (1992).
22. H. Dominique, *Ecologie de la germination des espèces indigènes de la Réunion*, TDUR (2012).
23. N. Nivot, *Essais de germination et de bouturage de six espèces indigènes sciaphytes du Canada*", TDUL (2005).
24. D. Come, *Les Obstacles à la Germination*, BMSLL, **40**, p. 108 (1970).
25. M.D.& M.B. H. Hajlaoui, *Étude de la variabilité intraspécifique de tolérance au stress salin du pois chiche (Cicer arietinum L.) au stade germination*, TC, **25** (2007).
26. H. Salehzade, M.I. Shishvan, M. Ghiyasi, "*Effect of Seed Priming on Germination and Seedling Growth of Wheat (Triticum aestivum L.)*, RJBS, **4** (5), pp. 629–631 (2009).
27. Y.A. El-kassaby, I. Moss, D. Kolotelo, M. Stoehr, *Seed Germination: Mathematical Representation and Parameters Extraction*, FS , **54** (2), pp. 220-227 (2008).
28. M. Gaudreault, *Amorçage et séparation des graines d'épinette noire (Picea mariana (Mill.) BSP): amélioration de la germination des lots de semences forestières*, UQC (2005).
29. M.A. Ranal, D.G.D.E. Santana, *How and why to measure the germination process*, RBB. **29**, pp. 1–11 (2006).
30. M.P. Carvalho, D.G. Santana, M.A. Ranal, *avaliada por meio de amostras pequenas*, RBB ,**2**, pp. 627–633 (2005).
31. J.D. Maguire, *Speed of germination - aid in selection and evaluation for seedling emergence and vigor*, CS, **2** , pp. 176–177 (1962).
32. B. Satrani, N. El Ouadihi, A. Guedira, P. Frey-Klett, M. Arahou, J. Garbaye, *Effet de la bactérisation des graines sur la croissance des plants de Cedrus atlantica Manetti*, BASE, **13**, pp. 367–372 (2009).

33. C.C. Baskin, J.M.Baskin, *Seeds: Ecology, biogeography, and evolution of dormancy and germination*, E, **86**, pp. 705–707 (1998).
34. F. Bonner,W.Yao & Y .Shen, *Effects of gibberellic acid and magnetically treated water on physiological characteristics of Tilia miqueliana seeds,* CJFR, **48** (5), pp. 554-558 (2018).
36. D. Chen, Z. Yuan, X. Luo, L. Yang, X. Chen, Z. Zhang, J. Wang, X. Hu, *Cold stratification requirements for seed dormancy-break differ in soil moisture content but not duration for alpine and desert species*, PS, **471**, pp. 393–407 (2022).
37. E. Germain, N. Aletà, A. Ninot, D. Rouskas, G. Zakinthinos, D. Avanzato, A.M. Gaspar, A. Nacional, I. Nacional, *La multiplication du pistachier*,EROMAEFCN,**16**,pp.121–132(1997).
38. N. Chebouti-Meziou, A. Merabet, Y. Chebouti, F.Z. Bissaad, N. Behidj-Benyounes, S. Doumandji, *Effect of cold and scarification on seed germination of Pistacia atlantica L. for rapid multiplication*, PJB,**46**, pp. 441–446 (2014).
39. A. Zine, E. Abidine, Y. Ammari, *La germination des graines du genévrier rouge (Juniperus phoenicea L.)*, ARFM, **29**, pp. 5–11 (1996).
40. A. Yaaqobi, L. El Hafid, B. Haloui, *Étude biologique de Pistacia atlantica Desf. de la région orientale du Maroc*, BE,**3**, pp. 39–49 (2009).
41. H. Abu-Qaoud, *Effect of Scarification, Gibberellic Acid, and Stratification on Seed Germination of Three Pistacia Species*, ANUJR, **21**, pp. 1–11 (2007).
42. C. Hessou, R.G. Kakai, A. Assogbadjo, T. Odjo, B. Sinsin, *Test de germination des graines de Caesalpinia bonduc (L.)*, IJBCS, **3**, pp. 310–317 (2009).
43. B. Benmahioul, F. Daguin, & M. Kaïd-Harche, *Cryopreservation of Pistacia vera embryonic axes*, JFS, **61** (4), pp. 182–187 (2015).