

Article de recherche

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Effect of pretreatment on germination of seeds from different provenances of *Pistacia atlantica* Desf. in Algeria

K. Maamar kouadri*, K. Boubetra, A. Saifouni, A. Mihoubi, A. Kaddouri, S. Boudjada

National Institute of Forest Research, INRF, Baraki experimental station, Algiers, Algeria.

* Auteur correspondant : maamar_kouadri_kaddour@inrf.dz

ملخص

جمعت بذور الفستق الأطلسي *Pistacia atlantica* Desf. وعددها 3600 بذرة، بمعدل 450 بذرة لكل مصدر، من عدة مواقع تمثل التنوع البيومناخي في الجزائر. أجريت عملية انبات أولية في المشتل لتقدير معدل الانبات وتأثير مصدر البذور. بعد ذلك، اختبرت عدة معاملات لكسر السكون، شملت الخدش الكيميائي باستعمال حمض الكبريتيك بتركيزي 96% و50%، والمعاملة الهرمونية المسبقة بحمض الجبريليك بتركيزي 500 و1000 جزء في المليون، إضافة إلى التنضيد البارد عند درجة حرارة 5 درجات مئوية لمدة 30 و45 يوما، باستعمال بذور منزوعة القشرة أو سليمة. بالنسبة إلى مصدر بني سنوس، سجلت أفضل النتائج عند استعمال حمض الكبريتيك بتركيز 50%، حيث بلغ معدل الانبات 64.3%، وحمض الجبريليك بتركيز 1000 جزء في المليون بنسبة 52%، والتنضيد البارد للبذور منزوعة القشرة لمدة 45 يوما بنسبة 69.3%. أما في عين بن خليل، فقد بلغت معدلات الانبات 54.2% عند استعمال التنضيد البارد للبذور منزوعة القشرة لمدة 45 يوما، و48.6% عند استعمال حمض الجبريليك بتركيز 1000 جزء في المليون. وفي أولاد ميمون، سجل معدل انبات قدره 58.5% عند استعمال حمض الجبريليك بتركيز 1000 جزء في المليون. أعطت المعاملات الأخرى معدلات انبات تراوحت بين 12% و28.3%، مقارنة بنسبة قصوى بلغت 34% للبذور غير المعاملة، أي الشاهد، من مصدر بني سنوس. وتبرز هذه النتائج أهمية مصدر البذور واختيار المعاملات المسبقة الملائمة لتحسين عملية الانبات.

كلمات مفتاحية: فستق أطلس، إنبات، بذور، تنوع، سكون.

Abstract

Seeds of *Pistacia atlantica* Desf. (3,600 seeds, 450 per provenance) were collected from several representative localities of Algerian bioclimatic diversity. An initial nursery sowing was conducted to estimate germination rate and provenance effect. Various dormancy-breaking treatments were then tested: chemical scarification with sulfuric acid (96% and 50%), hormonal pretreatment with gibberellic acid (500 and 1,000 ppm), as well as cold stratification (5 °C, 30 and 45 days, dehulled or intact seeds). For the Beni Snous provenance, the best results were obtained with 50% sulfuric acid (64.3%), 1,000 ppm gibberellic acid (52%), and cold stratification of dehulled seeds for 45 days (69.3%). For Aïn Ben Khelil: 54.2% (cold stratification, dehulled seeds, 45 days) and 48.6% (gibberellic acid, 1,000 ppm). For Ouled Mimoun: 58.5% (gibberellic acid, 1,000 ppm). Other treatments yielded rates ranging from 12% to 28.3%, compared to a maximum of 34% for non-treated control seeds (Beni Snous). These findings highlight the importance of provenance and tailored pretreatments to optimize germination.

Keywords: Atlas pistachio, germination, seeds, diversity, dormancy.

Résumé

Des graines de *Pistacia atlantica* Desf. (3 600 graines, 450 par provenance) ont été collectées dans plusieurs localités représentatives de la diversité bioclimatique algérienne. Un premier semis en pépinière a permis d'estimer le taux de germination et l'effet de la provenance. Divers traitements de levée de dormance ont ensuite été testés : scarification chimique à l'acide sulfurique (96 % et 50 %), prétraitement hormonal à l'acide gibbérellique (500 et 1 000 ppm), ainsi que stratification à froid (5 °C, 30 et 45 jours, graines décortiquées ou non). Pour la provenance de Beni Snous, les meilleurs résultats ont été obtenus avec l'acide sulfurique à 50 % (64,3 %), l'acide gibbérellique à 1 000 ppm (52 %) et la stratification à froid des graines décortiquées pendant 45 jours (69,3 %). Pour Aïn Ben Khelil : 54,2 % (stratification à froid, graines décortiquées, 45 jours) et 48,6 % (acide gibbérellique, 1 000 ppm). Pour Ouled Mimoun : 58,5 % (acide gibbérellique, 1 000 ppm). Les autres traitements ont donné des taux de 12 % à 28,3 %, contre un maximum de 34 % pour les témoins non traités (Beni Snous). Ces résultats confirment l'importance de la provenance et des prétraitements adaptés pour optimiser la germination.

Mots clés : Pistachier de l'Atlas, germination, graines, diversité, dormance .

1. Introduction

The genus of *Pistacia* L. (Anacardiaceae) includes more than 600 species widespread in the Mediterranean and the Middle East, Western and Central Asia (Tutin et al., 1968; Pourreza et al., 2008; Aghili, 2009). Many species of *Pistacia* such as *P. vera* L., *P. atlantica* Desf., *P. terebinthus* L., *P. khinjuk* Stocks and *P. lentiscus* L. are known for their great economic and ecological importance (Mozaffarian, 2005).

P. atlantica (*Atlas pistachio*), is one of the most widely distributed wild species in the genus and is recognized as an excellent source of pharmacological and nutritional compounds. Traditionally, it has been used for various medicinal and pharmaceutical applications, including antibacterial, antifungal, anti-inflammatory, and antidiabetic effects (Boulos, 1983; Yousfi et al., 2002; Kordali et al., 2003; Azizi-an et al., 2013; Falahati et al., 2015; Hajjaj et al., 2017). In Addition, this tree is used as a rootstock for *Pistacia vera* in semi-arid and arid areas (Monastra et al., 1997; Kafkas and Kaska, 1998). The foliage is also used as forage (Niazi et al., 1999).

Four subspecies have been recognized: subsp. *atlantica*, *kurdica*, *mutica*, and *cabulica* (Ben Ahmed et al., 2021). *P. atlantica* subsp. *atlantica* is native to the Maghreb countries (Quézel and Santa, 1963). It is xerophilous, exhibit a high morphologi-

cal diversity and a strong capacity to adapt to extreme environments (El Zerey-Belaskri and Benhassaini, 2016; Ifticene-Habani et al., 2022).

Currently, the Atlas pistachio tree is threatened by several factors, including overexploitation, ecosystem degradation and low production of viable seeds. All these factors compromise the natural regeneration of the species. The seeds exhibit embryonic dormancy which is associated with integumentary inhibition (Boudy, 1952; Côme, 1970; Mozaffarian, 2005). Numerous studies have been showed that different pretreatments can significantly improve the seeds germination (Degdag et al., 2022; Bassouya et al., 2024).

In Algeria, Atlas pistachio is widespread in semi-arid and arid areas, particularly in steppe region (Ifticene-Habani and Abdoun, 2018). It occurs in isolated stands, often in association with other xerophilous species such as *Zizyphus lotus*, *Nerium oleander*, *Anabasis articulata*, *Zilla macroptera*, *Launaea arborescens*, *Gymnocarpos decandrus* (Benaradj et al., 2015). These species play an important role in protecting young pistachio plants from animals and strong winds. Atlas Pistachio can be used as a reforestation species in degraded lands and to fight against desertification (Chebouti-Meziou et al., 2014). The multiplication of this species in nurseries and of the conditions required of seed germination can assist in understanding how to successfully use rehabilitation and restoration program. In this context that the present study aims to improve the germination of seeds of Atlas pistachio through the use of various mechanical, chemical, and hormonal pretreatments.

2. Material and Methods

2.1. Plant Materials

The mature seeds were collected in September (2021) in Ouled Mimoun and Beni Snous (Tlemcen), Ain Ben Khelil (Naâma), Messad (Djelfa), Mergueb (M'sila), M'doukal (Batna), Ras El Miad and Oued El Besbes (Biskra) (Figure 1, Table 1).

The harvest was done on healthy, well-formed sub-

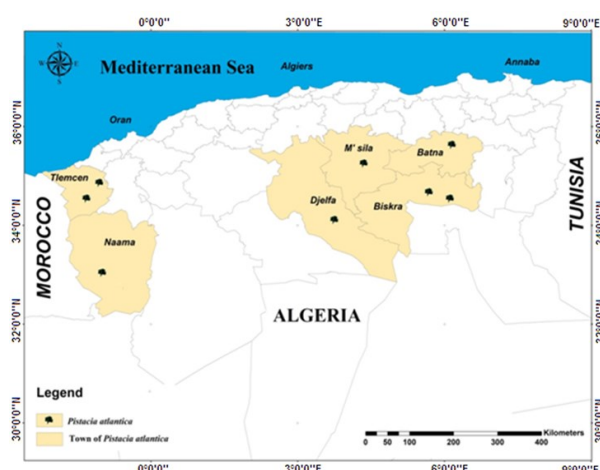


Figure 1. Geographic locations of the Atlas pistachio provenances used in the study.

Table 1. Origin and geographical information of sampled sites of Atlas pistachio in Algeria.

Sites	Number of trees	Altitude	Geographic coordinates	
Beni Snous	15	673	34°40'36.0"N	01°30'47.5"W
Ain Ben Khelil	30	1107	33°26'02.4"N	00°53'21.8"W
Ouled Mimoun	25	700	34°55'06.8"N	00°58'43.2"W
Messad	20	639	33°58'28"N	3°34'14"E
Mergueb	20	577	35°33'27"N	03°56'02"E
M'doukal	70	397	35°06'49.1"N	05°10'45.3"E
Ras El Miad	10	397	34°06'27"N	04°44'06"E
Oued El Besbes	20	324	34°07'59"N	04°57'48"E

jects. The seeds mature are carefully sorted and are air dried then placed in bags and conserved at room temperature (Figure 2).

2.2. Botanical description

Phenological monitoring and a botanical description of the Atlas pistachio tree were carried out throughout the study period. The main phenological stages of the species were observed and recorded regularly. Photographs were taken at each developmental stage and are presented in Figure 3.

2.3. Pretreatments of seeds

Chemical, hormonal and thermal pretreatments were applied to seed lots from different provenances of Atlas pistachio. A total of 3600 seeds were selected, with 450 seeds allocated to each provenance. Fifty seeds were used for each treatment, including the control. Seeds were first disinfected by bleach (13%) for 10 minutes and then rinsed twice with distilled water.

Each batch of seeds underwent the following pretreatments:

- Control T0;
- Chemical scarification with concentrated sulfuric acid (H_2SO_4 , 96%) (T1) and diluted to 50% (T2) for 15 minutes;
- Hormonal pretreatment with gibberellic acid applied on slightly cracked seeds at two concentrations 1,000 ppm (T3) and 500 ppm (T4) for 24 hours;
- Cold stratification at 5°C during 30 days for: hulled Seeds(T5), dehulled Seeds(T6);
- Cold stratification at 5°C during 45 days for:

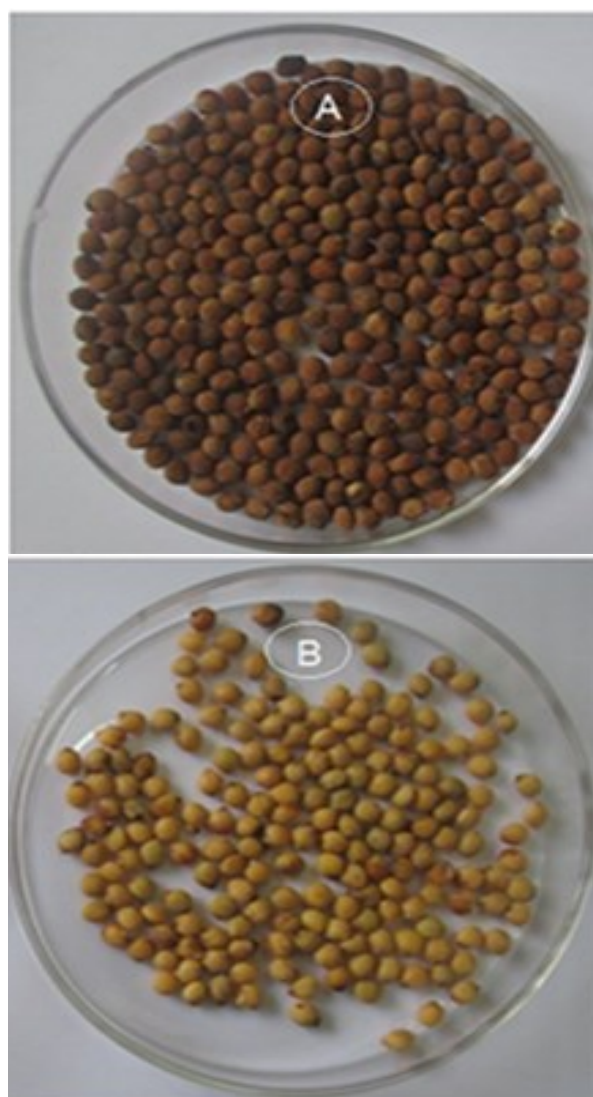


Figure 2. Seeds of Atlas pistachio **A:** Hulled seeds, **B:** dehulled seeds



Figure 3. Phenological development stages of Atlas pistachio (*Pistacia atlantica* Desf.)

Tree in its natural habitat (a), tree in an experimental plantation at the Baraki station (b), trunk (c), leaves (d, e), female flowers (f), male flowers (g), female flowers after pollination (h), immature fruits (i, j), mature fruits (k, l).
Photos: K. Boubetra

hulled Seeds (T7), dehulled Seeds (T8).

2.4. Sowing and Germination in nursery

The seeding has been carried out in nursery of the experimental station of Baraki (INRF). The treated seeds have been placed in WM containers containing a substrate composed of $\frac{1}{2}$ topsoil, $\frac{1}{4}$ sand and $\frac{1}{4}$ organic manure.

Germination rate (GR %) was calculated according to the following formula: $GR (\%) = (n/N) \times 100$, where n is the number of seeds that germinated during the experiment and N is the total number of seeds subjected to the germination test. The germination rate expresses the percentage of seeds that successfully germinated under the experimental conditions and is used as an indicator of seed viability and treatment effectiveness. The results obtained were subjected to analysis of variance (ANOVA) at a probability level of 5% using the Statistica Edition 12.0.

3. Results and Discussion

3.1. Botanical description

Pistacia atlantica Desf. is called in arabic 'Al bottm, or 'El buttum al atlassi' to distinguish it to *Pistacia terebinthus* or *Pistacia palaestina* which are also called sometimes El buttum (El Zerey-Belaskri, 2019). Dioecious species, strong and vigorous, reaching up to 15–20 m in height (Figure 3a,b). The trunk of mature tree is woody with a striated bark, and can exceed 1 m in diameter (Figure 3c). It has a strong root system which allowed it to occupy arid lands. The leaves are deciduous, pale green, composed of one to eight leaflet pair and a terminal leaflet (Figure 3 d,e). Tree loses leaves in December. Flowering (March) occurs before vegetative development and characterized by a clustered inflorescence with a petal flowers. Generally, the male flowers tend to before female. The male flowers are large and arranged in clustered in a terminal disposition, apetalous and pale yellow in color (Figure 3f). Five stamens are developed with short filaments fused at the base. The female flowers are apetalous and grouped in panicles (Figure 3g), the perianths show a morphological variability. the perigynous flowers carry tricarpellous, unilocular ovaries. The fruit is drupe, Small, oblique to round,

yellowish-brown (Figure 3i,j), sometimes turning reddish when ripe (Figure 3k), it reaches maturity in September, while having a dark green color. The single seed is ovate and yellow.

3.2. Effect of pretreatments on the seed germination

The following histograms showing mean seed germination rates of Atlas pistachio from different provenances based on ANOVA analysis.

The germination rate of untreated seeds (control T0) showed a marked difference between the eight provenances. The highest germination rate had been observed in the provenance of Beni Snous with 33.63%. Ain Ben Khelil and Messad showed more or less high germination rate (24.48% and 20.68%) respectively.

In contrast, the provenances of M'Doukal and Ras El Miad recorded very low germination rates with 2.53% and 1.79% respectively (Figure 4). The pretreatments of sulfuric acid (50%) (T2), gibberellic acid (1,000 ppm) (T3), and cold stratification of dehulled seeds for 45 days (T8), have a marked positive effect, particularly, on the provenance of Beni Snous which showed the highest germination rates with 64.3%, 52%, and 69.3% respectively (Figure 5).

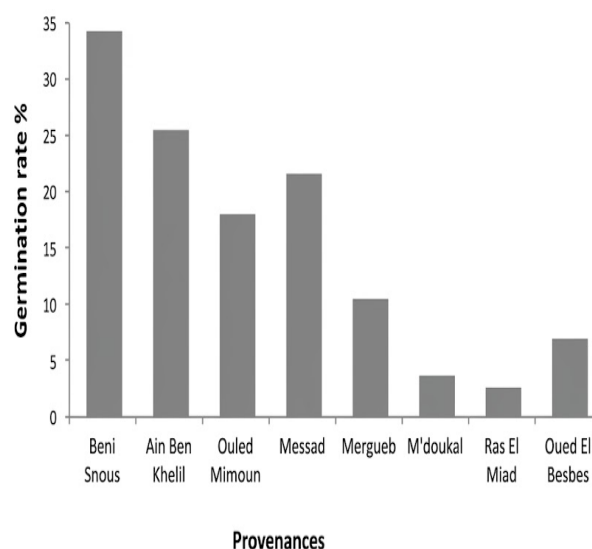


Figure 4. Seed germination rate of Atlas pistachio (*Pistacia atlantica*) from different provenances in Algeria

Seeds from the provenance of Ain Ben Khelil showed also a high germination rates only the cold stratification of dehulled seeds for 45 days (T8) with 54.2%. Whereas, seeds from Ouled Mimoun provenance achieved germination rate exceeding 50% only with gibberellic acid (1,000 ppm) (T3) (48.5%). For the provenance of Messad, all the pre-treatments have been enhanced the germination potential of seeds (23.4% to 41.7%) compared to the control which recorded 20.68%. This improvement could be linked to the specific characteristics of seeds from this provenance.

The rest of the provenances, the various pre-treatments applied to the seeds exhibit a variable response in the germination rates (lower than the control) (12% to 28.3%). The pretreatment with concentrated sulfuric acid did not significantly improve seed germination compared with the control. This lack of a positive effect may be attributed to

potential damage to the embryonic tissues caused by the corrosive action of sulfuric acid (Figure 5).

In this study, the germination rate of untreated seeds varied between provenances. The highest germination rates were recorded in the provenances of Beni Snous, Ben Khelil, and Messad. In general, seed germination in species of the genus *Pistacia* is low and is closely related to seed origin (Aleta et al., 1997; İsfendiyaroğlu and Özeker, 2001).

The pretreatments of sulfuric acid (50%) (T2), gibberellic acid (1,000 ppm) (T3), and cold stratification of dehulled seeds for 45 days (T8), have a positive effect on germination seeds particularly for the provenances of Benis nous, Ain Ben khelil and Messad. According to Chen et al. (2022), the germination of *Pistacia atlantica* seeds requires a period of cold stratification, which can increase the germination rate to up 90%.

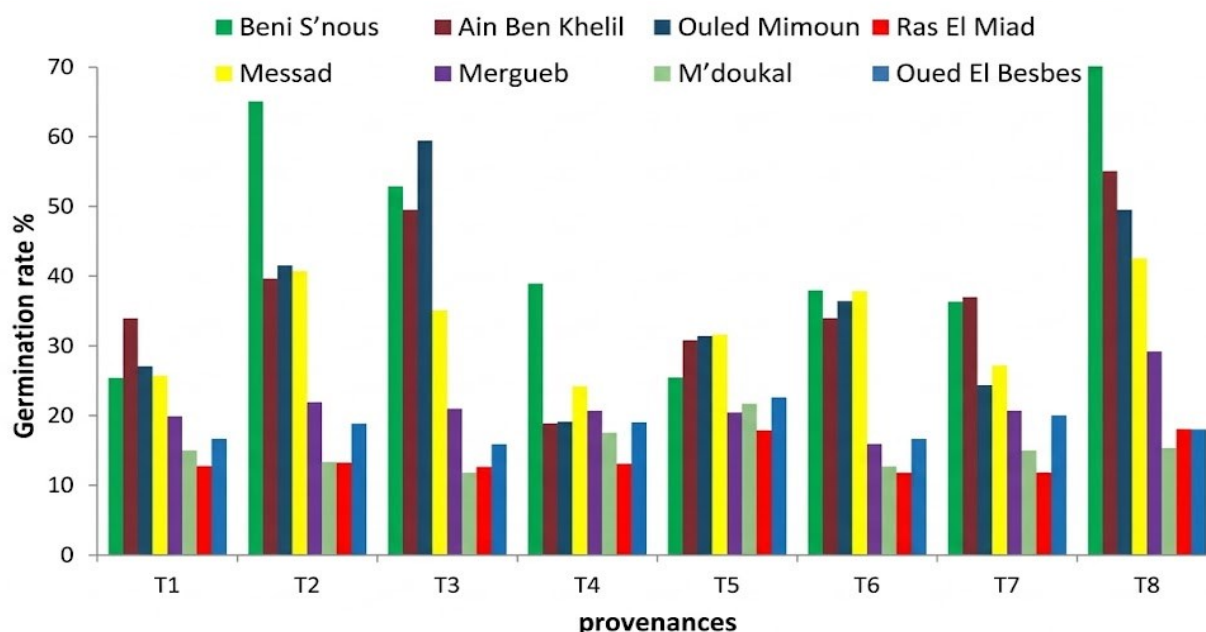


Figure 5. Seed germination rate according to provenance and different pretreatments

Acid sulfuric 96% (T1), diluted to 50% (T2), Gibberellic 1,000 ppm (T3) and 500 ppm (T4), cold stratification at 5°C hulled Seeds (30 days), hulled Seeds (T5), dehulled Seeds (T6), cold stratification at 5°C (45 days) hulled Seeds (T7), dehulled seeds (T8)

The hormonal pretreatment with gibberellic acid has also been appears to be a beneficial for improving the seed germination in this species (Ayfer and Serr, 1961; Crane and Forde, 1974; Chebouti-Meziou et al. (2014), showed that the seeds of Atlas pistachio (Djelfa provenance) exhibit a high germination rate (70%) following several days of cold stratification. The observed differences in germination rates could be related to the year of seed collection.

In contrast, Oukara et al. (2017), reported that the highest germination rate subjected to cold stratification at 4 ± 1 °C for 30 days combined with mechanical scarification. Whereas, Frutos and Barone, (1988); Vargas and Romero (1998), revealed that seeds germinate very well after cold and moist stratification (2–4 °C) for two months.

However, the results obtained showed that there is marked difference in germination rate of seeds depending the provenances. Germination rates obtained (with or without treatments) showed that higher values only than for seeds from the Beni Snous, Ain Ben Khelil, and Messad. According to Chebouti-Meziou et al. (2014), the germination rate was also influenced by the origin of the seeds. Wang (1987), noted that stratification time and seed genotype are two factors that influence germination.

The seed dormancy in *Pistacia atlantica* is probably due to structures surrounding the embryo and a phenolic compound produced in fruits (Baskin and Baskin, 1998; Bewley and Black, 1994). Whereas, Côme, 1970, attributed it to tegumentary origin.

4. CONCLUSION

In Algeria, the Atlas pistachio is widely distributed in semi-arid and arid regions, particularly in steppe ecosystems, where it can be playing a crucial role as a natural barrier against desertification. Therefore, its propagation and conservation are essential for supporting the sustainable development in these regions.

Our results indicate that seed germination varies significantly depending on seed origin (provenance) and the type of pretreatment applied. The use of sulfuric acid (50%), gibberellic acid (1,000 ppm), and thermal stratification of dehulled

seeds for 45 days proved to be the most effective treatments for overcoming seed dormancy.

This species deserves greater attention, as it is well adapted to semi-arid and arid climates and holds considerable ecological and socio-economic importance.

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