

Research paper

Seed viability and optimal germination conditions of *Cedrus atlantica* (Manetti ex Endl.) Carrière

Nesrine Hafidou¹, Zoheir Mehdadi^{2*}, Ali Latreche³, Mohammed Dadach⁴ and Ibrahim Bouchaour⁵

Hafidou, N., Mehdadi, Z., Latreche, A., Dadach, M., Bouchaour, I. 2021. Seed viability and optimal germination conditions of *Cedrus atlantica* (Manetti ex Endl.) Carrière. – Forestry Studies | Metsanduslikud Uurimused 75, 188–201, ISSN 1406-9954. Journal homepage: <http://mi.emu.ee/forestry.studies>

Abstract. Our work is part of the *ex situ* conservation of the Atlas cedar (*Cedrus atlantica* (Manetti ex Endl.) Carrière), a threatened and endemic Pinaceae of the Maghreb. Our objectives are to evaluate the viability of seeds from three Algerian cedar groves ecotypes (Thienet El Had, Chelia, Chrea) by the 2,3,5 triphenyltetrazolium chloride (TZ) test and to elucidate the effect of continuous temperatures (15°C, 20°C, 25°C) on their germinative capacity. Moreover, the effects of some physicochemical pretreatments in breaking dormancy and improving germinability were estimated on the seeds of the cedar grove of Thienet El Had. In addition, in the same ecotype, the water stress, as simulated by using the PEG₆₀₀₀ (0 to -2.20 bar), was induced upon seed germination. Results of the TZ test showed variability in seeds' viability among the three ecotypes with rates of 60, 85 and 90% in those of Chelia, Chrea and Thienet El Had, respectively. However, germination tests revealed the existence of partial dormancy in all tested seeds. Dramatic differences in the degree of dormancy were noted among the three provenances investigated. Untreated seeds of the three ecotypes exhibited maximum germination at 20°C: Thienet El Had (52%), Chelia (32%) and Chrea (7%). The final germination percentage was as high as 76% in seeds soaked in hot water (80°C) for 5 min followed by 56% in seeds treated with GA₃ (150 ppm). The increase in water stress regularly decreases germination with a tolerance threshold corresponding to -2.2 bar.

Key words: *Cedrus atlantica*, germination, seed viability, temperature, physicochemical treatments, water stress.

Authors' addresses: ^{1,2,3}Laboratory of Plant Biodiversity: Conservation and Valorization, Faculty of Natural Sciences and Life, Djillali Liabes University, BP 89, 22000 Sidi Bel Abbes, Algeria; ⁴Bejaia University, Faculty of Natural and Life Sciences, Ecology and Environment Research Laboratory, Targua Ouzemour Road, 06000 Bejaia, Algeria; ⁵Academy of Créteil, 4 Rue Georges Enesco, 94000 Créteil, France; *e-mail: mehdadiz@yahoo.fr

Introduction

Atlas cedar (*Cedrus atlantica* (Manetti ex Endl.) Carrière) is an endemic Pinaceae of the Maghreb of great socio-economical and ecological value (M'hirit & Maghnoij,

1997). In Algeria, *C. atlantica* is estimated occupying an area of 2089 km² (Arar *et al.*, 2020). The Atlas cedar is classified as an endangered species carrying the status of a quite rare plant (Yahi *et al.*, 2008; Touati *et al.*, 2021) and protected in Algeria by the

DOI: 10.2478/fsmu-2021-0020



© 2021 by the authors. Licensee Estonian University of Life Sciences, Tartu, Estonia. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<http://creativecommons.org/licenses/by/4.0/>).

Executive Decree No. 12-03 (J.O.R.A, 2012).

Cedrus atlantica is encountered in mountains of the Tellian and Saharan Atlas (from which its name has been derived, Atlas cedar), where it has a scattered distribution controlled mainly by climatic variables and edaphic traits (Quézel, 1998).

Currently, *C. atlantica* forests are subject to the harmful effects of global warming (Chenchouni *et al.*, 2008; Cheddadi *et al.*, 2017). According to the climate warming scenarios' modulation, the Algerian cedarwood forests are expected to decline to 80.6% by 2070 with an altitudinal shift towards higher elevations and a disappearance at low altitudes (Arar *et al.*, 2020).

In the Maghreb, the Atlas cedar is, in addition, under the threat of uncontrolled exploitation along with the phenomenon of dieback. The combined action of dieback factors as well as anthropogenic pressure (M'hirit & Benzyane, 2006) has resulted in the fragility and disruption in the equilibrium of cedar groves in some localities (Ezzahiri *et al.*, 1994). These latter authors have emphasized that both challenges have generated the disturbance of several physiological and biological mechanisms particularly that of natural resilience. The low regeneration ability is rather exaggerated by climatic changes in the Mediterranean region in recent decades (Bentouati & Bariteau, 2006; Bentouati, 2008; Addar *et al.*, 2016), in particular the increase in temperatures due to global warming and the decrease in precipitation resulting in a water deficit especially in summer which means that the young seedlings of *C. atlantica* cannot establish during this period (Linares *et al.*, 2011).

The dieback problem in cedar groves is owing to multiple causes (M'hirit & Benzyane, 2006; Naggar, 2013), such as drought (Kherchouche *et al.*, 2013; Abel-Schaad *et al.*, 2018; Arar *et al.*, 2020) and parasite attacks by some xylophages belonging mainly to the subfamilies of Buprestidae and Scolytinae (Talbi & Bouhraoua, 2015). This drought is affecting the distribution of

populations of many species, such as the Atlas cedar, which are becoming vulnerable and subject to local extinction (Arar *et al.*, 2020).

The preservation of the remaining Algerian cedar patches urgently requires appropriate scientific studies and the establishment of a relevant restoration project as short-term measures. The evaluation of the viability of seeds and their germination physiology are among the areas of research to be developed, knowing that germination is a crucial step in the establishment of plants in their natural environment.

Germination is often prevented by seed dormancy which is a physiological state of a viable seed that prevents it from germinating till the emergence of favorable environmental conditions. Underestimating seed dormancy and germination requirements when planning a conservation project can lead to high levels (> 90%) of plant establishment failure and seed wastage (Commander *et al.*, 2013; James *et al.*, 2013).

To overcome the seed dormancy of a large number of forest species and improve their germination performance, several types of pretreatments have been applied (Baskin & Baskin, 2014). These pretreatments may be applied before, during or after the seed storage period, and correspond to the methods by which seed dormancy can be released (Debroux *et al.*, 1998). Various studies have shown that cold stratification is a useful pretreatment to stimulate seed germination in different Pinaceae species (Cooke *et al.*, 2002; Ghildiyal *et al.*, 2009). However, some studies found that stratification did not affect seed germination (Nelson, 2015; Shen & Cho, 2021). For example, eight weeks of cold stratification (prechilling at 5°C) is sufficient to significantly improve seed germination of *Cedrus brevifolia* (Hook. f.) A. Henry (90%) compared with untreated seeds (23%) (Daskalakou *et al.*, 2015). The exogenous application of gibberellic acid (GA₃) is another commonly used treatment to promote germination in physiologically dormant seeds

(Kucera *et al.*, 2005). Seed treatments with hot water had been described to improve germination in physically dormant seeds through uplifting water and O₂ permeability of the weakened seed shell (Aydin & Uzun, 2001). Proper pretreatment makes the seeds ready to germinate when all the necessary conditions are met.

In the context of *C. atlantica* conservation, and the given lack of data in its seed germination features, we have undertaken this work in order to fill the knowledge gap regarding the effect of temperature and water stress on seed germination in this species. Some selected physicochemical pretreatments were also tested on promoting seed emergence. The data obtained in this study might contribute to managing an urgent restoration project for this endemic.

Materials and methods

Biological material

The seeds were collected from mature cones belonging to healthy selected trees of *C. atlantica* evolving in three Algerian national parks: namely Thienet El Had, Chrea and Chelia (Table 1). The cedar seeds are sub-triangular, winged, reddish brown to light brown (Figure 1). The seeds were stored in a paper bag and kept in dry conditions until their use.

Preparation of seeds

For all germination tests, only healthy seeds were selected and then dewinged, sterilized with sodium hypochlorite (5%) for 5 min and rinsed with distilled water.



Figure 1. *Cedrus atlantica* (Manetti ex Endl.) Carrière.

a: aerial part; b: seeds; c: longitudinal sections of seeds stained with 2,3,5 triphenyltetrazolium chloride (1–6: viable seeds; 7–9: non-viable seeds); Em = embryo; Mp = micropyle; Ra = radicle; Sc = seed coat. The bar corresponds to 1 cm.

Table 1. Provenances and characteristics of the used seeds.

Provenances	The harvest year	Age of seeds at the time of use	Bioclimate	GPS coordinates
Thienet El Had (Province of Tissemsilt)	2017	2 years	Sub-humid and humid	35°51'56"N 01°55'30"E
Chrea (Province of Blida)	2017	2 years	Perhumid, humid and sub-humid	36°21'36.122"N 02°49'4.184"E
Chelia (Province of Khenchela)	2017	2 years	Sub-humid and humid	35°19'48.5"N 6°39'01.2"E

Seed viability test

The viability assessment of the seeds was determined using the TZ viability test (Ferradous *et al.*, 2017). For each provenance, 25 seeds with 4 replications were soaked in distilled water for 24 hours at 30°C to soften their coats. They were then cut longitudinally into two equal parts and then placed in an incubator in a solution of TZ 1% for 24 hours at 30°C. The dye excess was removed by several rinses with distilled water, then the seeds were examined under a binocular magnifying glass to assess their viability.

Search for optimum thermal germination conditions

Due to the lack of information on the germination of *C. atlantica* seeds, preliminary tests to determine the optimum temperature for germination have been done. The seeds were germinated in the dark in a Memmert type germinator set at three continuous temperatures (15°C, 20°C and 25°C) in glass Petri dishes, lined with two layers of filter paper (Whatman 2) moistened with distilled water. For each test, 100 seeds were split up into four batches of 25 seeds each for the three provenances. The counting of germinated seeds was done daily. The breakthrough of the coats by the radicle was retained as a germination criterion (Calone *et al.*, 2020).

Effect of some pretreatments on improving germination performance

Pretreatments recognized for breaking the integumentary and embryonic dormancy of seeds and therefore improving their

germination capacity were tested. Beforehand, all the seeds were soaked in distilled water for 24 hours at 20°C. The submersion of seeds in distilled water was combined with one of the following pretreatments:

- Soaking in hydrogen peroxide (H₂O₂) at 2% and 4% (Lebrun, 1970),
- Soaking in gibberellic acid (GA₃) at different concentrations of 50 ppm, 100 ppm, 150 ppm, and 200 ppm (Chetouani *et al.*, 2017).
- Stratification: the seeds were placed in Petri dishes containing filter paper moistened with distilled water and placed at 5°C for 1 month (Heller *et al.*, 1990).
- Soaking in hot water at 80°C for 5 min (Jaouadi *et al.*, 2010).

These pretreatments were carried out only on the most viable seeds, originating from the cedar grove of Thienet El Had. The seeds were placed to germinate at 20°C (optimum germination temperature defined from our preliminary tests).

Effect of water stress on seed germination

The effect of water stress was tested, too, on the seeds of the cedar grove of Thienet El Had. Germination tests were carried out under different levels of water potential by using polyethylene glycol with a molar mass of 6000 (PEG₆₀₀₀) in the dark in a controlled incubator set at a continuous temperature of 20°C.

The different used concentrations of PEG₆₀₀₀ are: 0 (control: distilled water), 0.5, 1, 2, 4, 8 and 12%, having water potentials

of 0, -0.04, -0.08, -0.17, -0.42, -1.16, -2.20 bar, respectively. The different water potentials were determined according to the equation of Michel & Kaufman (1973):

$$\Psi = - (1.18 \times 10^{-2}) C - (1.18 \times 10^{-4}) C^2 + (2.67 \times 10^{-4}) CT + (8.39 \times 10^{-7}) C^2 T. \quad (1)$$

Where: Ψ : water potential (bars), C : PEG concentration (g.L^{-1}), T : temperature ($^{\circ}\text{C}$)

Result expression and data treatment

Germination test results were expressed by the final germination percentage (FGP), the latency time (LT) and the velocity coefficient or germination rate (VC). The velocity coefficient was expressed as follows (Jones & Sanders, 1987):

$$VC = \frac{(N1 + N2 + N3 \dots + Nn)}{(N1T1 + N2T2 + N3T3 \dots + NnTn)} \times 100. \quad (2)$$

Where: N is the number of germinated seeds at day T , and T is the number of counting days. In order to understand the physiological significance of the germination behavior. in the studied seeds, we have determined the germination kinetics, reflecting the evolution of the cumulative germination percentages as a function of days.

SPSS (version 20.0, SPSS Inc., Chicago, Illinois, USA) software was used to check the effect of provenance (ecotype) and temperature on various seed germination parameters (FGP, VC and LT) with two-way controlled analysis of variance (ANOVA II). A one-way analysis of variance (ANOVA I) was used, as well, to assess the effect of the different pretreatments and water stress on seed germination. The comparison of the means (two-by-two) of different germination parameters were carried out by the Duncan test. Simple linear regression analysis was used to highlight the relationship between the different water potentials tested on the germination attributes by calculating the coefficient of determination (R^2).

Results

Controlling seed viability by the TZ test

The results obtained show that a high via-

bility rate characterized the Thienet El Had seeds ($90 \pm 8.2\%$) and those of Chrea ($85 \pm 7.6\%$). A lower rate ($60 \pm 5.7\%$) characterized the Chelia seeds. According to Rao *et al.* (2006), the tissues of viable seeds are stained red and those of non-viable ones kept their original staining (Figure 1 c).

Effect of temperature on seed germination

Regardless of the temperature tested, the germination curve showed three phases (Figure 2):

- Latency phase: corresponds to the time necessary for the radicle to pierce the seed coats (the lag time),
- Acceleration phase: corresponds to an exponential increase in the number of germinated seeds,
- Stationary phase: corresponds to the cessation of germination.

The results of the preliminary germination tests established on the untreated seeds showed that the highest germination rates were noted at 20°C for the three provenances. At this temperature, Thienet El Had and Chelia seeds gave the best germination rates, with 52% and 32%, respectively. Only 7% of germination was assessed in Chrea ecotype seeds (Figure 3). The Figure 3 illustrates the variations in FGP and VC of the seeds as a function of the different temperatures. ANOVA II showed a highly

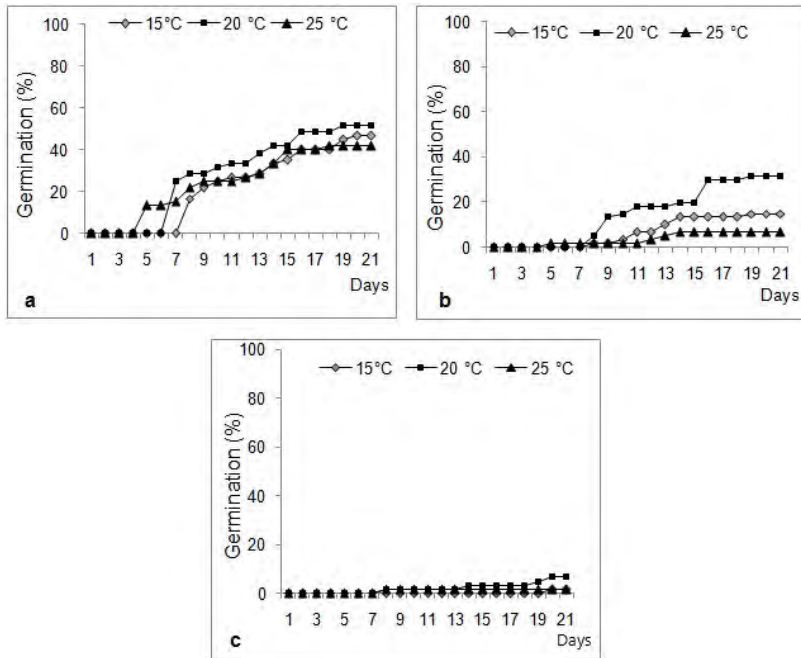


Figure 2. Cumulative germination percentages of *Cedrus atlantica* seeds as a function of temperatures and provenances. a: Thienet El Had; b: Chelia; c: Chrea.

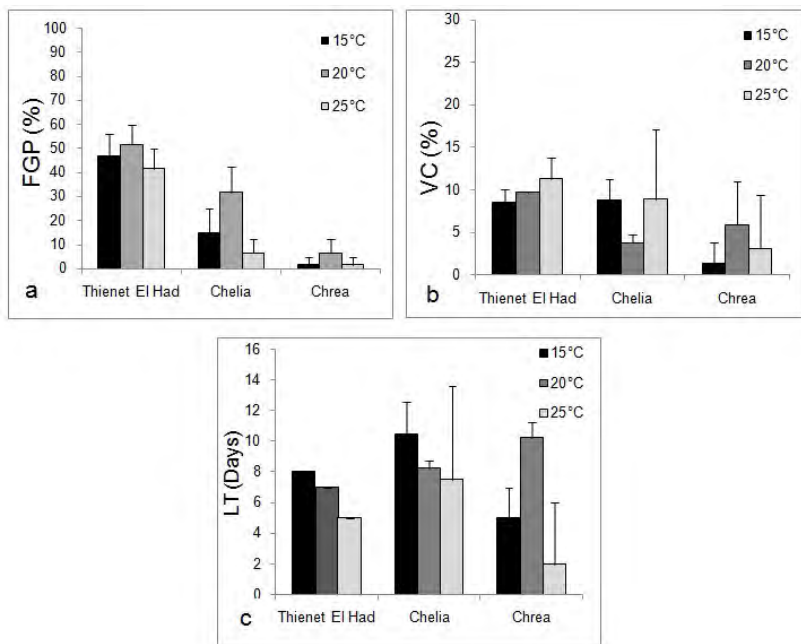


Figure 3. Effect of temperature on the final germination percentage (FGP), velocity coefficient (VC) and latency time (LT) in *Cedrus atlantica* seeds of the three provenances.

significant effect of temperature ($F= 5.9$; $p < 0.01$) and provenance ($F= 62.8$; $p < 0.001$) on FGP of seeds in all provenances. However, a miss of interaction between these two factors on germination was noted ($F= 1.2$; $p > 0.05$). ANOVA II also showed a

highly significant effect of provenance ($F= 8.02$; $p < 0.01$) on VC. On the other side, a non-significant effect ($p > 0.05$) of temperature and the interaction between temperature $\times$ provenance on VC (with $F= 0.65$ and 0.55 , respectively) was noted (Table 2).

Table 2. Effect of temperature and provenance on the final germination percentage (FGP), velocity coefficient (VC) and latency time (LT) in *Cedrus atlantica* seeds of the three provenances (mean $\pm$ SE, $n= 4$).

	Source of variation	DL	Sums of squares	Mean squares	F	p
FGP	Temperature	2	1106.005	553.002	5.946	0.007
	Provenance	2	11682.538	5841.269	62.806	< 0.0001
	Temperature*Provenance	4	456.643	114.161	1.227	0.323
VC	Temperature	2	0.002	0.001	0.650	0.530
	Provenance	2	0.028	0.014	8.025	0.002
	Temperature*Provenance	4	0.004	0.001	0.546	0.703
LT	Temperature	2	91.556	45.778	1.830	0.180
	Provenance	2	56.722	28.361	1.134	0.337
	Temperature*Provenance	4	86.111	21.528	0.860	0.500

DL: degree of liberty; F: Fisher Snedecor variable; p: probability; SE: standard deviation.
In bold: a statistically significant difference at $p < 0.05$.

The latency time of *C. atlantica* seeds was varied according to the temperature tested and the provenance. The shortest latency time was noted at 25°C for the three provenances (ranged from 2 to 7 days) and the longest latency time was noted at 15°C for Thienet El Had (8 days) and Chelia (11 days) and for Chrea at 20°C (10 days) (Figure 3). Despite the variation in LT values, no statistically significant differences ($p > 0.05$) were recorded with temperature, provenance and their interaction (Table 2).

Effect of some pretreatments on seed germination

Table 3 highlights the values of different germination patterns (FGP, VC and LT) in seeds of Thienet El Had provenance that have undergone different pretreatments. As compared to the control (untreated seeds where FGP = 52% and VC = 9.7%), it appeared that soaking seeds in hot water is the treatment that remarkably improved

the final germination percentage (FGP = 77%) and the velocity coefficient (VC = 50.8%). The lowest value of FGP (27%) is recorded in hydrogen peroxide treatment (H₂O₂ at 4%). A significant statistical difference was noted among all studied germination attributes (FGP, VC and LT) at $p < 0.05$. The LT varied among treatments. Interestingly, all pretreatments used have shortened the LT (enhancing seed emergence speed) to only 3 days except the soaking in hot water, the LT was reduced yet at 2 days.

Effect of water stress on seed germination

Enhancing the level of PEG6000 has a detrimental effect on FGP and VC (Figure 4). It was observed that the increment in water deficiency (decreasing the water potential level) in the medium dramatically decreased the values of FGP and VC. Beyond 120 g.L⁻¹ of PEG6000 (-2.20 bar) the germina-

Table 3: Effect of treatments used on the final germination percentage (FGP), velocity coefficient (VC) and latency time (LT) in seeds of *Cedrus atlantica* of Thienet El Had provenance (mean $\pm$ SE, $n = 4$).

	FGP (%)	VC (%)	LT (days)
Control	52 $\pm$ 4.2 ^b	09.7 $\pm$ 0.1 ^a	7 ^a
H ₂ O ₂ (2%)	45 $\pm$ 12.6 ^{ab}	28.3 $\pm$ 3.9 ^c	3 ^b
H ₂ O ₂ (4%)	27 $\pm$ 5.4 ^a	27.9 $\pm$ 3.2 ^c	3 ^b
GA ₃ (50 ppm)	47 $\pm$ 2.7 ^{ab}	19.1 $\pm$ 1.0 ^b	3 ^b
GA ₃ (100 ppm)	50 $\pm$ 5.8 ^{ab}	17.9 $\pm$ 1.5 ^b	3 ^b
GA ₃ (150 ppm)	58 $\pm$ 7.4 ^{bc}	25.6 $\pm$ 1.5 ^c	3 ^b
GA ₃ (200 ppm)	50 $\pm$ 8.8 ^{ab}	17.6 $\pm$ 0.4 ^b	3 ^b
Cold stratification (5°C)	46 $\pm$ 7.7 ^{ab}	18.8 $\pm$ 1.5 ^b	3 ^b
Hot water (80 °C)	77 $\pm$ 6.9 ^c	50.8 $\pm$ 2.2 ^d	2 ^c

H₂O₂: hydrogen peroxide; GA₃: Gibberellic acid.

Different letters indicate significant differences ($p < 0.05$).

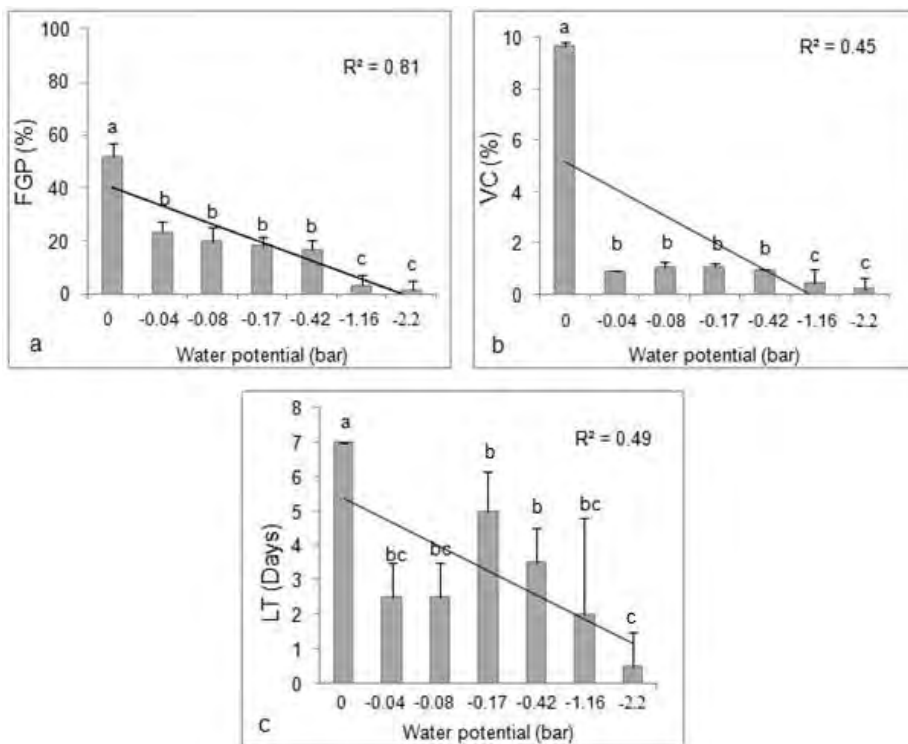


Figure 4. Effect of the different water potentials on the final germination percentage (FGP), velocity coefficient (VC) and latency time (LT) in seeds of *Cedrus atlantica* of Thienet El Had provenance. Different letters indicate significant difference between means ($p < 0.05$).

tion was completely ceased. The effect of different water potentials was confirmed by analysis of variance ($p < 0.05$). Latency times vary from one concentration of PEG6000 to another. The shortest TL (1 day) was recorded at -2.20 bar. However, the longest TL (7 days) was observed at the control (Figure 4).

Linear regression analysis showed a high correlation between FGP and the induced water potentials ($R^2 = 0.81$), while the correlation is moderate with VC ($R^2 = 0.45$) and LT ($R^2 = 0.49$).

Discussion

Our study has showed that the seed viability rate of *C. atlantica*, assessed by the TZ test, differs from one provenance to another. This rate is important in the seeds of the cedar groves of Chrea and Thienet El Had (85% and 90%, respectively); while an average of viability with just 60% characterized the seeds of the Chelia cedar grove. The performance of the TZ test in assessing the physiological quality of seeds, in particular their viability rate, has been confirmed in the works of França-Neto & Krzyzanowski (2019) and Salazar-Mercado *et al.* (2020). The reducing reaction of the TZ salt solution under the action of dehydrogenase enzymes produces triphenylformazan, which shows a carmine red coloration in the living tissues of the seeds (França-Neto & Krzyzanowski, 2019). Completely stained seeds are viable; those that are not colored are not viable; while those which are partially colored will produce either normal or abnormal seedlings (Rao *et al.*, 2006).

The need to restore the natural unpredictable habitats has set forth a rise in demand for seedlings pertaining to native forest species. Most of these species are spread via seeds and the success in seedling formation depends on the knowledge about the germination process of each utilized seed (Rego *et al.*, 2009). This knowledge is missing in Atlas cedar, although

it is available for two other cedars: *Cedrus libani* A. Rich. (Dirik, 2000) and *C. brevifolia* (Daskalakou *et al.*, 2015). Our results showed that temperature and provenance have a highly significant effect on the germination capacity of seeds. However, analysis of variance revealed an insignificant interaction between temperature and provenance for the variables of three germination parameters (FGP, VC and LT). The VC seemed to be influenced only by the provenance factor. Furthermore, no effect of the two above-mentioned factors was demonstrated for the latency time. The best FGPs were noted at 20°C in the seeds of the three provenances. In fact, these FGPs were variable and remained low to medium with 7%, 32% and 52% for Chrea, Chelia and Thienet El Had, respectively. This heterogeneity in FGPs can be explained by genetic effects, harvesting conditions, treatments and storage conditions of seeds (Dirik, 2000).

Simultaneously, this author has obtained the same results on the cedar of Lebanon (*C. libani*) of Turkish origin. Indeed, he has noted that incubating *C. libani* seeds at 20°C, the germinability ranged from 36.2% to 63.4% depending on provenances. On the other hand, the highest germination percentage (80%) was obtained at 15°C in *C. brevifolia* seeds, while at 25°C almost full suppression of germination was observed (Daskalakou *et al.*, 2015). The optimum temperature for germination in Atlas cedar is matched in nature with early spring, when climatic conditions are favorable for seedling survival, development and growth.

By comparing the results of the TZ test with those of the FGP, it appeared that many seeds of *C. atlantica*, although they were viable, failed to germinate. This prompted us to study the effect of some pretreatments on improving the germination performance of the most viable *C. atlantica* seeds from the cedar grove of Thienet El Had. In this context, the results obtained showed a significant effect of the

physical and chemical pretreatments on seed germination behavior. As compared to the control, the treatment of the seeds with hot water (80°C) and with GA₃ 150 ppm improved the germination capacity. We found that hot water allowed the softening of the seed coats and the elimination of the mucilage contained in them, which probably interfered with their germination. The work of Velempini *et al.* (2003) and Mohammadi *et al.* (2012) in the effect of pretreatments on seed germination of *Cochorus olitorius* (L.) and *Abelmoschus esculentus* (L.) Moench, respectively, showed that soaking the seeds of these two species in hot water at 80°C for 10 min and at 75°C for 5 min was the most effective treatment to improve germination. The beneficial effect of hot water treatment (60°C) on seed germination of *Cupressus atlantica* Gaussen has been also revealed by Arjouni *et al.* (2013). According to Budy *et al.* (1986), hot water is a form of thermal scarification which allows the removal of integumentary dormancy by causing cracks in the seed coats without altering the anatomy of the micropyle. These cracks allow water to percolate in order to trigger germination (Pritchard, 2000). The physiological role of GA₃ as a promoter of seed germination has been reported by several authors in a wide range of plant species (Baskin & Baskin, 2004; Rogis *et al.*, 2004; Sharma *et al.*, 2020; El Hamdaoui *et al.*, 2021). The favorable effect of GA₃ and submersion in distilled water has also been demonstrated on *Pinus gerardiana* Wall. ex D. Don. (Kumar *et al.*, 2014). It was noted in the work of Kabar (1998) that the advantageous effect of GA₃ on seed germination of *Pinus brutia* Ten. and *Thuja orientalis* L. is explained by its ability to modulate the inhibitory effect of abscisic acid (ABA). The hormonal cross talk of GA with ABA has been well-documented in seeds of many plant species (Seo *et al.*, 2009). In addition, soaking in distilled water allows the dissolution of germination-inhibiting substances, such as polyphenols in the seed covering (Bessam *et al.*, 2010).

When the water potential decreases in the medium, the FGP and VC values weakened steadily and significantly ($p < 0.05$), especially at -1.16 and -2.20 bar; while the LT became shorter. Compared to many species which are tolerant to water stress and able to germinate at water potentials exceeding -8 bar, such as *Salsola drummondii* Ulbr. (Elnaggar *et al.*, 2019) and *Thymus fontenesii* Boiss. & Reut. (Dadach & Mehdadi, 2021), *C. atlantica* seeds from Thienet El Had are water stress-sensitive species since their germination was completely inhibited beyond -2.20 bar. Similarly, the depressive effect of water stress, especially on FGP and VC has also been highlighted in some Mediterranean species belonging to different botanical families (Dadach & Mehdadi, 2018; Hamdini *et al.*, 2021; El Hamdaoui *et al.*, 2021). Consistently, Dirik (2000) on Cedar of Lebanon (*C. libani*) and Tilki & Dirik (2007) on *P. brutia* have found that in general the germination rate varies according to seed provenance and decreased steeply with increasing water stress in the soil. The decrease in FGP and CV values is a consequence of the reduction in the intensity of water uptake by the seeds due to the water deficit. This can inhibit respiratory pathways and enzymatic activity, causing oxidative stress, which stimulate the accumulation of various toxic by-products, such as reactive oxygen species (ROS). If the production of ROS overwhelms the scavenging capacity of cell, that can lead to lipids, proteins and carbohydrates denaturation (Rasheed *et al.*, 2019), thus as a result the restriction of radicle emergence and seedling formation.

Conclusion

From the obtained results, it appeared that the viability of *C. atlantica* seeds varies according to the three studied provenances, with a moderate to high rate. However, their germination percentages were low to moderate at the optimal temperature of

20°C. This is certainly due to the seed coat dormancy combined with the potential existence of germination inhibitor substances that preclude seeds to emerge. Seeds' germinative performance could be improved remarkably by treatment with hot water. Such easy-to-use pretreatment could be utilized to enhance the production of seedlings to rescue the remaining threatened natural populations of

C. atlantica. The seed dormancy and the sensitivity of *C. atlantica* seeds to the water potential drop at the germination stage are assumed to be the limiting factors for a successful natural regeneration of this species.

Regarding the confirmed dreadful effect of water stress on seed emergence of *C. atlantica*, we recommend planting seedlings in habitats with high annual precipitation levels. We assume rains will mitigate the impact of global warming and hence increase the survival chances of this species in the field. As a complement to this work, it would be interesting to further elucidate, for supporting both *in situ* and *ex situ* conservation projects, the potentiality of adaptation and survival under water stress of several genotypes of Atlas cedar during their early establishment stages.

Acknowledgements. We thank the General Direction of Scientific Research and Technological Development (Ministry of Higher Education and Scientific Research, Algeria) for their financial support for the realization of this work. We also thank the managers of the national parks of Thienet El Had, Chrea and Chelia for their collaboration.

References

Abel-Schaad, D., Iriarte, E., López-Sáez, J.-A., Pérez-Díaz, S., Sabariego Ruiz, S., Cheddadi, R., Alba-Sánchez, F. 2018. Are *Cedrus atlantica* forests in the Rif Mountains of Morocco heading towards local extinction? - The Holocene, 28(6), 1023-1037.

Addar, A., Khedache Z., Righi, H., Dahmani-Megrerouche, M. 2016. Monitoring the early stages of Cedar natural regeneration in some stations of Chréa Forest (Blidean Atlas, Algeria). (Suivi de la régénération naturelle du Cèdre de l'Atlas dans les premiers stades de développement dans quelques stations du massif forestier de Chréa (Atlas Blidéen, Algérie)). - Revue d'Ecologie (Terre et Vie), 71(4), 367-384. (In French with English summary).

Arar, A., Nouidjem, Y., Bounar, R., Tabet, S., Kouba, Y. 2020. Modeling of the current and future potential distribution of Atlas cedar (*Cedrus atlantica*) forests revealed shifts in the latitudinal, longitudinal and altitudinal range towards more humid conditions. - Ecological Questions, 31(3), 49-61.

Arjouni, Y., El Alaoui El Fels, M.A., El Mercht, S., Abou Sy, A., El Wahidi, F., Romane, A., Roques, A., Yart, A. 2013. Contribution to the improvement of germination among a forest endemic of Morocco, the Atlas cypress. (Contribution à l'amélioration de la germination chez une endémique forestière du Maroc, le cyprès de l'Atlas). - Ecologia Mediterranea, 39(1), 201-208. (In French with English summary).

Aydin, I., Uzun, F. 2001. Effects of some applications on germination rate of Gelemen clover seeds gathered from natural vegetation in Samsun. - Pakistan Journal of Biological Sciences, 4(2), 181-183.

Baskin, C.C., Baskin, J.M. 2014. Seeds: Ecology, Biogeography, and Evolution of Dormancy and Germination. Second Edition. San Diego, Academic Press. 150-162.

Baskin, J.M., Baskin, C.C. 2004. A classification system for seed dormancy. - Seed Science Research, 14(1), 1-16.

Bentouati, A. 2008. Status of the Atlas cedars in the Aurès Mountains (North Africa). (La situation du cèdre de l'Atlas dans les Aurès (Algérie)). - Forêt Méditerranéenne, 29(2), 203-208. (In French with English summary).

Bentouati, A., Bariteau, M. 2006. Reflections on the dying off of Atlas cedars in the Aurès region of Algeria. (Réflexions sur le dépérissement du Cèdre de l'Atlas des Aurès (Algérie)). - Forêt Méditerranéenne, 27(4), 317-322. (In French with English summary).

Bessam, F.Z., Mehdadi, Z., Bessam, M.H., Marouf, A. 2010. Effects of some physical pretreatments on improving the germination performance of *Stipa tenacissima* L. and characterization of inhibitory substances. (Effets de quelques prétraitements physiques sur l'amélioration des performances germinatives de *Stipa tenacissima* L. et caractérisation des substances inhibitrices). - Acta Botanica Gallica, 157(2), 349-360. <https://doi.org/10.1080/12538078.2010.10516212>. (In French with English summary).

- Budy, J., Evans, R., Young, J. 1986. Understanding seed handling for germination. – Volunteers in Technical Assistance (VITA), 1–12.
- Calone, R., Sanoubar, R., Noli, E., Barbanti, L. 2020. Assessing *Salicornia europaea* tolerance to salinity at seed germination stage. – Agriculture, 10(2), 29. <https://doi.org/10.3390/agriculture10020029>.
- Cheddadi, R., Henrot, A.-J., François, L., Boyer, F., Bush, M., Carré, M., Coissac, E., De Oliveira, P.E., Ficetola, F., Hambuckers, A., Huang, K., Lézine, A.-M., Nourelbait, M., Rhoujjati, A., Taberlet, P., Sarmiento, F., Abel-Schaad, D., Alba-Sánchez, F., Zheng, Z. 2017. Microrefugia, climate change, and conservation of *Cedrus atlantica* in the Rif Mountains, Morocco. – Frontiers in Ecology and Evolution, 5, 114.
- Chenouchi, H., Abdelkrim, S.B., Athmane, B. 2008. The deterioration of the Atlas Cedar (*Cedrus atlantica*) in Algeria. – Proceedings of the International Conference “Adaptation of Forests and Forest Management to Changing Climate with Emphasis on Forest Health: A Review of Science, Policies, and Practices”, Sweden, 25–28 Aug. 2008. Umeå, 51.
- Chetouani, M., Mzabri, I., Aamar, A., Boukroute, A., Kouddane, N., Berrichi, A., 2017. Effect of gibberellic acid (AG3) on the germination of seeds of *Thymus satureioides* L. and *Lavandula dentata*. – Journal of Materials and Environmental Sciences, 8(3), 942–948.
- Commander, L.E., Rokich, D.P., Renton, M., Dixon, K.W., Merriitt, D.J. 2013. Optimising seed broadcasting and greenstock planting for restoration in the Australian arid zone. – Journal of Arid Environments, 88, 226–235.
- Cooke, J., Cooke, B., Gifford, D. 2002. Loblolly pine seed dormancy: constraints to germination. – New Forests, 23(3), 239–256.
- Dadach, M., Mehdadi, Z. 2018. Germination responses of *Ballota hirsuta* seeds under conditions of temperature, salinity and water stress. – Hellenic Plant Protection Journal, 11(1), 34–39.
- Dadach, M., Mehdadi, Z. 2021. Drought tolerance of three ethnomedicinal shrubs evaluated based on their seed germination rates at different drought levels induced by using polyethylene glycol (PEG6000). – Folia Oecologica, 48(1), 49–54.
- Daskalakou, E.N., Thanos, C.A., Georgiou, K. 2015. Seed biology, reproductive phenology and conservation of *Cedrus brevifolia*, a threatened endemic tree of Cyprus. – Seed Science and Technology, 43, 378–389.
- Debroux, L., Mbolo, M., Delvingt, W., Amougou, A. 1998. Moabi and mukulungu regeneration in Cameroun. Management prospects. (Régénération du Moabi et du Mukulungu au Cameroun. Perspectives pour l'aménagement). – Bois et Forêts des Tropiques, 255, 5–17. (In French).
- Dirik, H., 2000. Effect of osmotic water stress on germination of Lebanon cedar (*Cedrus libani* A. Rich.) seeds from the provenances of Turkey. – Annals of Forest Science, 57(4), 361–367.
- El Hamdaoui, A., Mechqoq, H., El Yaagoubi, M., Bouglad, A., Hallouti, A., El Mousadik, A., El Aouad, N., Ait Ben Aoumar, A., Msanda, F. 2021. Effect of pretreatment, temperature, gibberellin (GA3), salt and water stress on germination of *Lavandula mairei* Humbert. – Journal of Applied Research on Medicinal and Aromatic Plants, 24, 100314.
- Elnaggar, A., El-Keblawy, A., Mosa, K.A., Navarro, T. 2019. Adaptive drought tolerance during germination of *Salsola drummondii* seeds from saline and nonsaline habitats of the arid Arabian deserts. – Botany, 97, 123–133.
- Ezzahiri, M., Belghazi, B., Bahmad, M. 1994. Evaluation of natural regeneration of Atlas cedar in some enclosed parts of middle Atlas (Morocco). (Bilan de la régénération naturelle de la cédraie dans les parcelles clôturées du Moyen Atlas (Maroc)). – Annales de la Recherche Forestière du Maroc, 1(27), 259–268. (In French).
- Ferradous, A., Lamhamedi, M.S., Ouhammou, A., Alifriqui, M. 2017. Operational implementation of the tetrazolium viability test in argan seed (*Argania spinosa*) stored for several years. (Mise en application opérationnelle du test de viabilité au tétrazolium chez les semences d'arganier (*Argania spinosa*) stockées pendant plusieurs années). – Canadian Journal of Forest Research, 47(9), 1286–1292. (In French with English summary).
- França-Neto, J.B., Krzyzanowski, F.C. 2019. Tetrazolium: an important test for physiological seed quality evaluation. – Journal of Seed Science, 41(3), 359–366.
- Ghildiyal, S.K., Sharma, C.M., Gairola, S. 2009. Effect of cold stratification on the germination of seeds of chirpine (*Pinus roxburghii* Sargent) from Indian Himalayan region. – Nature and Science, 7(8), 36–43.
- Hamdini, A., Mehdadi, Z., Dadach, M., Latreche, A. 2021. Effect of abiotic stresses on germination behaviour of *Ruta chalepensis*. – Botanica, 27(1), 23–33.
- Heller, R., Esnault, R., Lance, C. 1990. Plant Physiology. 2. Development. (Physiologie Végétale. 2. Développement). Paris, Masson. 266 pp. (In French).
- James, J.J., Sheley, R.L., Erickson, T., Rollins, K.S., Taylor, M.H., Dixon, K.W. 2013. A systems approach to restoring degraded drylands. – Journal of Applied Ecology, 50(3), 730–739. <https://doi.org/10.1111/1365-2664.12090>.
- Jaouadi, W., Hamrouni, L., Souayah, N., Larbi Khouja, M. 2010. Study of *Acacia tortilis* seed germination under different abiotic constraints. (Étude de la germination des graines d'*Acacia tortilis* sous différentes contraintes abiotiques). – Biotechnologie, Agronomie, Société et Environnement, 14(4), 643–652. (In French with English summary).

- Jones, K.W., Sanders, D.C. 1987. The influence of soaking pepper seed in water or potassium salt solutions on germination at three temperatures. – Journal of Seed Technology, 11(1), 97–102.
- J.O.R.A., 2012. Executive decree of January 18, 2012, completing the list of uncultivated and protected plant species. (Décret exécutif du 18 janvier 2012, complétant la liste des espèces végétales non cultivées et protégées). – Journal Officiel de la République Algérienne, 3-12/12 du 18-01-2012. 12–38. (In French).
- Kabar, K. 1998. Comparative effects of kinetin, benzyladenine, and gibberellic acid on abscisic acid inhibited seed germination and seedling growth of red pine and arbor vitae. – Turkish Journal of Botany, 22, 1–6.
- Kherchouche, D., Kalla, M., Gutiérrez, E., Briki, A., Hamchi, A. 2013. Drought and dieback of Atlas cedar (*Cedrus atlantica* Manetti) in the Belezma mountains (Algeria). (La sécheresse et le dépérissement du cèdre de l'Atlas (*Cedrus atlantica* Manetti) dans le massif du Belezma (Algérie)). – Sécheresse, 24, 129–37. (In French with English summary).
- Kucera, B., Cohn, M.A., Leubner-Metzger, G. 2005. Plant hormone interactions during seed dormancy release and germination. – Seed Science Research, 15(4), 281–307.
- Kumar, R., Shamet, G.S., Mehta, H., Alam, N.M., Tomar, J.M.S., Chaturvedi, O.P., Khajuria, N. 2014. Influence of gibberellic acid and temperature on seed germination in chilgoza pine (*Pinus gerardiana* Wall.). – Indian Journal of Plant Physiology, 19(4), 363–367.
- Lebrun, C. 1970. Pretreatment of Douglas fir seeds with hydrogen peroxide. (Prétraitement des graines de Douglas à l'eau oxygénée). – Revue Forestière Française, 22(4), 473–476. (In French).
- Linares, J.C., Taïqui, L., Camarero, J.J. 2011. Increasing drought sensitivity and decline of Atlas cedar (*Cedrus atlantica*) in the Moroccan Middle Atlas forests. – Forests, 2(3), 777–796. <https://doi.org/10.3390/f2030777>.
- M'hirit, O., Benzyane, M. 2006. The Atlas Cedar. A Memory of Time. (Le Cèdre de l'Atlas. Mémoire du Temps). Sprimont, Editions Mardaga. 288 pp. (In French).
- M'hirit, O., Maghnouj, M. 1997. Strategy for the conservation of forest genetic resources in Morocco. (Stratégie de conservation des ressources génétiques forestières au Maroc). – Birouk, A., Rejdali, M. (eds.). Ressources Phytogénétiques et Développement durable. Rabat, Maroc, Actes Editions, 123–138. (In French).
- Michel, B.E., Kaufmann, M.R. 1973. The osmotic potential of polyethylene glycol 6000. – Plant Physiology, 51(5), 914–916.
- Mohammadi, G., Khah, E.M., Jalali Honarmand, S., Shirkhani, A., Shabani, G. 2012. Effects of seedhardness breaking techniques on okra (*Abelmoschus esculentus* L.) germination. – International Journal of Agriculture and Crop Sciences, 4(6), 264–273.
- Naggar, M. 2013. Regeneration of the cedar in the central Middle Atlas range (Morocco). (La régénération du cèdre dans le moyen Atlas central au Maroc). – Forêt Méditerranéenne, 34(1), 25–34. (In French with English summary).
- Nelson, S.O. 2015. Seed treatment applications. – Nelson, S.O. (ed.). Dielectric Properties of Agricultural Materials and their Applications. London, San Diego, Academic Press, 57–69.
- Pritchard, H.W. 2000. Baskin, C.C., Baskin J.M. 1998. Seeds. Ecology, Biogeography, and Evolution of Dormancy and Germination. – Annals of Botany, 86(3), 705–707. <https://doi.org/10.1006/anbo.2000.1238>.
- Quézel, P. 1998. Cedars and cedar forests on the Mediterranean rim: bioclimatic and phytogeographic significance. (Cèdres et cédraies du pourtour méditerranéen: signification bioclimatique et phytogéographique). – Forêt Méditerranéenne, 19(3), 243–260. (In French with English summary).
- Rao, N.K., Hanson, J., Dulloo, M.E., Ghosh, K., Nowell, D., Larinde, M. 2006. Manuel de manipulation des semences dans les banques de gènes. – Manuels pour les banques de gènes N° 8. Rome, Biodiversity International, 165 pp. (In French).
- Rasheed, A., Rasool, S.G., Gul, B., Khan, M.A., Hameed, A. 2019. Reactive oxygen species production and scavenging during seed germination of halophytes. – Hasanuzzaman, M., Nahar, K., Öztürk, M. (eds.). Ecophysiology, Abiotic Stress Responses and Utilization of Halophytes. Singapore, Springer, 63–81.
- Rego, S.S., Nogueira, A.C., Kuniyoshi, Y.S.; dos Santos, Á.F. 2009. Germination of seeds of *Blepharocalyx salicifolius* (HBK) Berg. in different substrates and conditions of temperatures, light and moisture. (Germinação de sementes de *Blepharocalyx salicifolius* (H.B.K.) Berg. em diferentes substratos e condições de temperaturas, luz e umidade). – Revista Brasileira de Sementes, 31(2), 212–220. (In Portuguese with English summary).
- Rogis, C., Gibson, L.R., Knapp, A.D., Horton, R. 2004. Enhancing germination of eastern gamagrass seed with stratification and gibberellic acid. – Crop Science, 44, 549–552.
- Salazar-Mercado, S.A., Quintero-Caleño, J.D., Rojas-Suárez, J.P. 2020. Determining the viability of *Capsicum annuum* L. seeds using the tetrazolium test. (Determinación de la viabilidad de semillas de *Capsicum annuum* L. usando la prueba de tetrazolio). – Aibi Revista de Investigación, Administración e Ingeniería, 8(3), 7–12. (In Spanish with English summary).

- Seo, M., Nambara, E., Choi, G., Yamaguchi, S. 2009. Interaction of light and hormone signals in germinating seeds. – *Plant Molecular Biology*, 69(4), 463–472.
- Sharma, L., Reddy, B.M., Chatterjee, M., Dhawan, S., Pai, V. 2020. Influence of mechanical scarification and gibberellic acid on seed germination and seedling performance in *Pinus gerardiana* Wall. – *International Journal of Current Microbiology and Applied Sciences*, 9(4), 1356–1365.
- Shen, X., Cho, M.-J. 2021. Factors affecting seed germination and establishment of an efficient germination method in sugar pine (*Pinus lambertiana* Dougl.). – *HortScience*, 56(3), 299–304. <https://doi.org/10.21273/HORTSCI15562-20>.
- Talbi, Y., Bouhraoua, R.T. 2015. Xylophagous complex associated with decline of Atlas cedar in the Belezma mountains (Algeria). (Complexe xylophage associé au dépérissement du cèdre de l'Atlas au Bélezma (Algérie)). – *Lebanese Science Journal*, 16(1), 97–106. (In French with English summary).
- Tilki, F., Dirik, H. 2007. Seed germination of three provenances of *Pinus brutia* (Ten.) as influenced by stratification, temperature and water stress. – *Journal of Environmental Biology*, 28(1), 133–136.
- Touati, L., Hamel, T., Meddad-Hamza, A., de Bélair, G. 2021. Analysis of rare and endemic flora in northeastern Algeria: the case of the wilaya of Souk Ahras. – *Bulletin de la Société Royale des Sciences de Liège*, 90, 213–240.
- Velempini, P., Riddoch, I., Batisani, N. 2003. Seed treatments for enhancing germination of wild Okra (*Corchorus olitorius*). – *Experimental Agriculture*, 39(4), 441–447.
- Yahi, N., Djellouli, Y., de Foucault, B. 2008. Floristic and biogeographical diversity of cedar forests in Algeria. (Diversités floristique et biogéographique des cédraies d'Algérie). – *Acta Botanica Gallica*, 155(3), 389–402. (In French with English summary).

Received December 01, 2021, accepted December 31, 2021