Botanica
ISSN 2538-8657
https://doi.org/10.35513/Botlit.2026.1.4
2026, 32(1): 37–53

Original research

Effect of storage conditions on the germination of *Mentha* × *rotundifolia* seeds from western Algeria

Celia Bentaleb[1], Fatima Zohra Bendimered-Mouri[1]*, Zoheir Mehdadi[1], Ali Latreche[1], Khadidja Smahi[1]

[1] Djillali Liabès University, Life and Nature Sciences Faculty, Laboratory of Plant Biodiversity: Conservation and Valorisation, 22000, Sidi Bel Abbès, Algeria
*Corresponding author. E-mail: mourifz@gmail.com

Received: 21 September 2025. Accepted: 5 February 2026. Published online: 10 March 2026.

Abstract

Bentaleb C., Bendimered-Mouri F.Z., Mehdadi Z., Latreche A., Smahi K., 2026: Effect of storage conditions on the germination of *Mentha* × *rotundifolia* seeds from western Algeria. – Botanica, 32(1): 37–53. https://doi.org/10.35513/Botlit.2026.1.4

This study focuses on the *ex situ* conservation of *Mentha* × *rotundifolia* (L.) Huds. (Lamiaceae), a spontaneous aromatic and medicinal plant from western Algeria. It has been little studied from a physiological and reproductive perspective. It requires conservation attention, as its regeneration in the wild is difficult due to anthropogenic pressures such as overgrazing, trampling, uprooting and habitat disturbance. This study aimed to determine the effect of different temperature regimes on seed viability during short-term storage in a seed bank. After determining the optimal temperature and light conditions for post-harvest germination of the seeds, three samples were stored separately at -20°C, 5°C, and room temperature (20°C to 25°C) and their viability was assessed after 6-, 14-, and 18-month storage. The germination results showed that the seeds were positively photoblastic, required a post-maturation period of at least three months, and had an optimum germination temperature of 30°C. Storage conditions (temperature and duration) significantly affected seed viability. The best germination rate (100%) was obtained after 6 months' storage at 5°C. For longer storage, the recommended temperature is –20°C. This study should contribute to a better understanding of the conservation conditions for this species and provide essential information for improved management of this a regionally threatened plant.

Keywords: e*x situ* conservation, *ex situ* seed bank, germinative performance, Lamiaceae, seed viability, storage duration, storage temperature.

INTRODUCTION

The genus *Mentha* L. belongs to the Lamiaceae family and contains around 42 species with a cosmopolitan distribution across all agro-climatic conditions. This genus is widely known for its essential oils, valued at over 400 million US dollars (Bounatirou et al., 2007). *Mentha* ×*rotundifolia* belongs to the tribe *Mentheae* within the *Nepetoideae* subfamily (Harley et al., 2004) and is a taxonomically ambiguous species within the genus. Earlier studies described it as a natural hybrid between *Mentha longifolia* (L.) Huds. and *Mentha suaveolens* Ehrh. (Kokkini & Papageorgiou, 1988; Lorenzo et al., 2002; Denslow and Poindexter, 2009). Recent molecular analyses have confirmed this interpretation, supporting its hybrid origin and taxonomic affinity with *Mentha suaveolens* (Olofsson et al., 2024). It is a perennial plant

frequently found along pathways, in ditches and in other damp places. It grows in semiarid and humid bioclimates with warm and temperate variations, across the Mediterranean basin, in America and in Western Asia (Derwich et al., 2010). It thrives in moderately humid soils, rich in organic matter, and with a slightly alkaline pH (Brahmi et al., 2022).

Mentha ×*rotundifolia* is traditionally used as a painkiller with antiseptic and anti-inflammatory agents to treat wounds and infections (Riahi et al., 2013). It has antioxidant, anti-tyrosinase, anti-acetylcholinesterase and insecticidal activities (Yahia et al., 2019). These properties are mainly attributed to the presence of several phenolic compounds, including rosmarinic acid, the dominant metabolite (Ferdjioui et al., 2019). Several antioxidant therapeutic approaches are currently being studied for the treatment of pathologies linked to oxidative stress, including neurological diseases. Some of its metabolites are presently undergoing clinical trials (Forman & Zhang, 2021).

Alongside studies on the therapeutic properties of aromatic and medicinal species, understanding seed viability and the germination process is vital. Goraï et al. (2011) point out that detailed information on germination patterns is crucial not only for successful cultivation, but also for understanding species establishment and tolerance to abiotic factors, as plant establishment success largely depends on germination. This physiological phenomenon is an essential stage in the life cycle of plants and is characterised by a high degree of spatio-temporal unpredictability. Various environmental factors, including temperature, salinity, light and soil moisture, simultaneously influence this process (Goraï et al., 2011).

Knowledge of seed dormancy and germination is important not only for understanding plant reproduction in its natural habitat, but also for developing effective cultivation methods that can reduce pressure on wild populations while supporting conservation actions and improving the livelihoods of local populations (Phondani et al., 2015). Moreover, in the context of *ex situ* biodiversity conservation strategies, information regarding the storage behaviour of seeds, particularly for uncultivated Mediterranean plant species, remains scarce or entirely lacking (Brits et al., 2015). Rao et al. (2006) have noted that, to preserve seed viability over long periods, basic collections are generally stored at temperatures below 0°C, often between -18 and -20°C. Moreover, seed longevity is not determined solely by temperature. The longevity of seeds is influenced by their initial quality and by the humidity and temperature to which they are exposed during storage (Walters et al., 2005).

In the Sidi Bel Abbès region (northwestern Algeria), the populations of spontaneous plant species, sought after for their virtues, are depleted by overexploitation, overgrazing, trampling and changes in local habitat conditions. Excessive grazing, a key component of the economic and social organisation of rural populations, is a major cause of degradation of natural ecosystems. It affects the dynamics, cover, and diversity of rangelands in the Western Algerian steppe, preventing regeneration and depleting available resources, leading to vegetation cover loss caused by overgrazing (Bencherif et al., 2021) and forest fires (Saidi et al., 2016).

The present study was conducted as part of ongoing efforts to establish a seed bank dedicated to the conservation of the spontaneous flora of western Algeria. It focused on the germination behaviour of several native species, particularly those belonging to the Lamiaceae family. This work provides valuable insights into seed dynamics under different storage conditions and strengthens the scientific foundation for developing effective long-term conservation strategies. This approach is essential for monitoring and managing seed banks.

Mentha × *rotundifolia* is among the most endangered Lamiaceae in the Sidi Bel Abbès region of western Algeria (Baraka, 2008) and requires appropriate conservation measures. To date, only limited research has investigated the storage behaviour and longevity of germination and viability of *Mentha* × *rotundifolia* seeds, and little information is available regarding its natural regeneration. Among the few existing studies, Liu et al. (2012) have examined the effects of specific treatments on seed germination of this species. Additional work has focused on other species within the *Mentha* genus, such as *Mentha* × *piperita* L., *Mentha aquatica* L., *Mentha pulegium* L., exploring their dormancy and germination responses under various thermal regimes (Brändel, 2006; Bendjeddi et al., 2025). However, studies on seed viability and storage longevity within the genus remain scarce, particularly for *Mentha* × *rotundifolia*, for which most available studies have focused on its chemical composition rather

than its physiological and germinative aspects. Notable examples include the works of Seladji et al. (2014), Benomari et al. (2018), Sbai et al. (2020), Yakoubi et al. (2024) and Afrokh et al. (2024).

The objective of this study was to determine the optimal conditions for germination of *Mentha* × *rotundifolia* seeds and to assess their viability after harvesting. The study aimed to evaluate the effects of different storage temperatures and durations on germination performance and the seeds' ability to withstand short-term storage. This approach was essential for the monitoring and management of plant species seeds stored in seed banks (Meddour & Derridj, 2007), enabling optimal storage conditions to be defined for short- or long-term storage. Therefore, we sought to answer the following research questions: (1) How did photoperiod affect the germination response? (2) What was the optimal temperature for seed germination under controlled laboratory conditions? (3) How did storage temperature and duration influence seed viability and germination during short-term *ex situ* conservation?

MATERIALS AND METHODS

Harvesting site

Seeds of *Mentha* × *rotundifolia* were collected in October 2021 from a natural population located in the Ain Thrid region, a municipality of Sidi Bel Abbès province (Western Algeria). The geographical coordinates of the collection site are as follows: 35.26156° N and 0.73547° W, at an altitude of 521 m.

Mature seeds were manually harvested from healthy individuals bearing well-developed infructescences. Random sampling was applied across all accessible fruiting plants to ensure representativeness and reproducibility of the material used for experimentation. Seed collection was carried out during the period of peak fruit maturity to obtain predominantly mature, fully developed seeds from healthy populations (Fig. 1).

This region belongs to a semiarid bioclimatic zone characterised by cold winters and very low average annual precipitation of 174 mm. The soils are predominantly calcareous, poor in humus, and highly

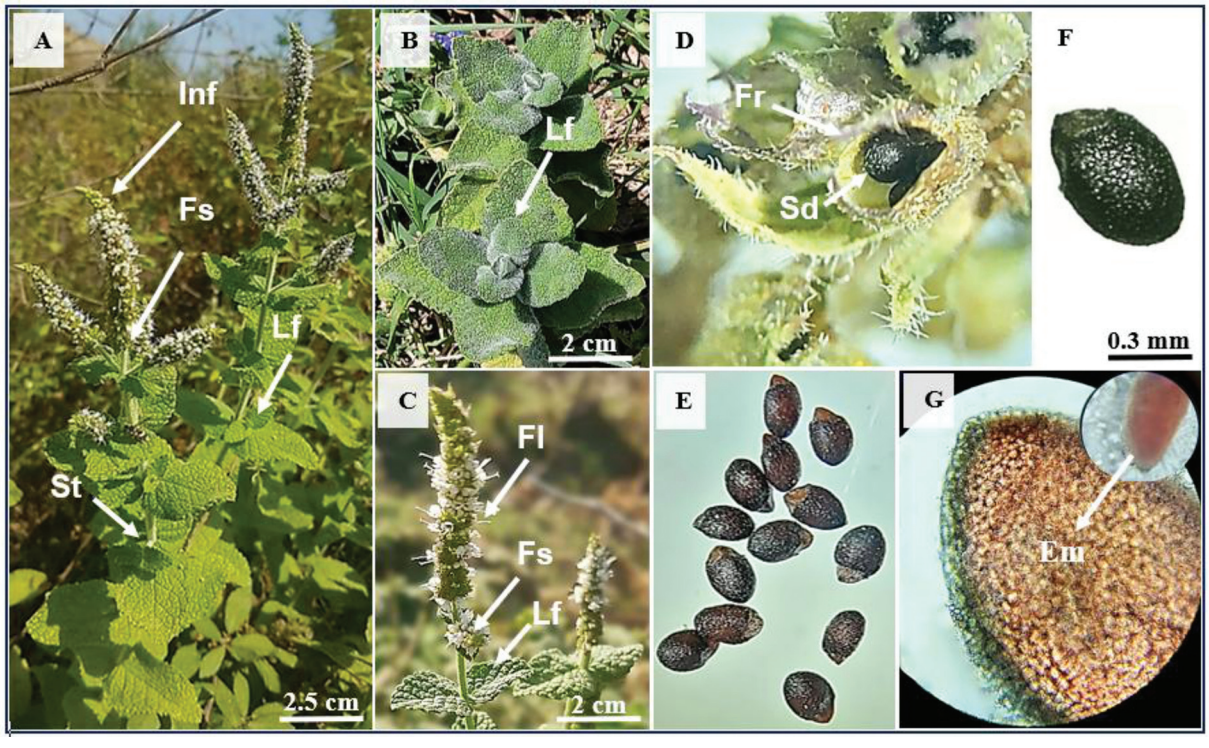

Fig. 1. *Mentha* × *rotundifolia* (**A**) the plant in its natural habitat; (**B**) plant in vegetative state; (**C**) flowering plant; (**D**) fruit tetrakene (× 20); (**E**) seeds (× 20); (**F**) seed (× 30); (**G**) a section of a seed staining with 2,3,5-triphenyltetrazolium (× 40); Em: embryo; Fs: flowering stem; Fl: flowers; F: Fruits; Inf: inflorescence; Lf: leaf; St: stem; Sd: seeds. Photographs by Celia Bentaleb, 2021.

vulnerable to erosion. The vegetation cover is sparse, and traditional extensive sheep farming in this semi-arid region further accentuates soil degradation and vegetation loss, making this ecosystem even more fragile (Bouiadjra et al., 2011).

Morphometric and moisture analysis of seeds

Morphometric measurements were conducted using an *EduBlue Euromex* binocular microscope, equipped with an integrated digital camera. Seed dimensions (length, width and thickness) were measured using the calibrated measurement tool of the *Optika Vision Lite* software (Optika, Italy) at ×30 magnification. Mean values were calculated from measurements of 100 seeds randomly selected from the collected populations.

The moisture content of seeds was determined from a 100-seed sample before and after drying. Seeds were dehydrated in a desiccator containing orange silica gel (Isolab Laborgerate Gmbh, Bahnhofstrasse 1097877 Wertheim, Germany) at laboratory temperature (20–25°C) until a constant weight was reached. The dry weight was measured using a high-precision analytical balance (P.I. Precision Instruments, model FA2004B, readability ± 0.0001 g). Moisture content (MC) was calculated according to ISTA (2021), as the percentage difference between the fresh and dry weights, relative to the fresh weight.

Biochemical test

The biochemical test of viability was performed by applying a standard the tetrazolium (TTC) staining method to a sample of 100 seeds. According to Moore (1985), this test is a rapid and reliable method of determining seed viability, particularly for species with slow or irregular germination. The procedure was carried out in accordance with the (TTC) protocol detailed by Starfinger & Karrer (2016). The seeds were first soaked in distilled water for 24 hours at room temperature to soften the tissues. Each seed was carefully cut longitudinally with a fine blade through the embryo under a binocular magnifying glass to ensure that the embryo was sectioned. The seeds were then immersed in a 1% solution of 2,3,5-triphenyltetrazolium chloride (TTC) in glass containers and incubated in the dark at 30°C for 24 hours in a Memmert oven. After incubation, the seeds were rinsed several times with distilled water to remove any excess stain and then soaked in a lactophenol solution for one to two hours before final observation. Seed tissues showing uniform or partial red staining were considered viable, while those remaining uncoloured were classified as non-viable. The results of this test provide a direct indication of the viability of embryonic tissue in seeds.

Conduct of the germination tests

Before germination, the seeds were disinfected with 10% sodium hypochlorite (NaClO) for five minutes, then rinsed. The entire protocol was carried out under aseptic conditions. Germinated seeds were counted daily.

For all germination experiments, including those evaluating the effect of photoperiod, temperature, and storage conditions, the same experimental design was used. Each treatment was replicated five times, with 10 seeds per replicate.

Germination tests were conducted in 90 mm × 14.2 mm sterile Petri dishes lined with a single layer of PRAT DUMAS (France) filter paper moistened with sterile distilled water. Seeds were moistened daily using a sterile Pasteur pipette to maintain controlled optimal humidity and to prevent excess water accumulation, which could inhibit germination in these small seeds. The test was performed with five replicates of 10 seeds each, for a total of 50 seeds per treatment. Germinated seeds were counted every 24 hours for 40 consecutive days under aseptic conditions, in proximity to a Bunsen burner flame to minimise the risk of contamination. The germination criterion was the breakthrough of the seed coat by the radicle (Calone et al., 2020).

Germination test

Effect of photoperiod on seed germination

The germinative viability test was applied to post-harvest seeds subjected to two light regimes: alternating light and darkness (natural photoperiod of 12/12 hours) and continuous darkness, at a constant temperature of 20°C. This temperature was selected based on previous studies on *Mentha* species, which report optimal germination under moderate temperature (Brändel, 2006).

To assess the effect of light availability on germination and the potential photosensitivity of the species, the seeds were exposed to the natural photoperiod by keeping the exterior door of the Memmert-type incubator open, allowing light to penetrate through the inner transparent door.

Effect of temperatures on seed germination

To assess the effect of temperature on germination, some characteristics of the natural environment were recreated to better understand how seeds respond to different thermal conditions, detect their germinative capacity before storage and identify the favourable germination conditions essential for monitoring them during storage.

Based on preliminary tests that determined the light preference of *Mentha* × *rotundifolia* seeds, germination was performed under the most suitable photoblastic conditions. The experiment evaluated germination responses of the seeds across eight temperature regimes: 5°C, 10°C, 15°C, 20°C, 25°C, 30°C, 35°C, and 40°C to determine the thermal optimum for this physiological process. These temperature treatments followed the standard protocols commonly used in seed germination studies (Bewley & Black, 1994).

Effect of storage conditions on seed germination

The seed storage protocol applied in this study followed the recommendations of Gomez-Campo (1985) and Meddour & Derridj (2007). Cleaned and pre-dried seeds were placed in hermetically sealed borosilicate glass tubes (18 × 150 mm) with metallic screw caps. We added 12 g of silica gel as a desiccant, separated from the seeds by a thin layer of permeable cotton to prevent direct contact.

Before storage, the seeds were pre-dried under ambient laboratory conditions (20–25°C) to reach a stable moisture level suitable for conservation. The prepared seeds were then divided into three samples: the first was stored at room temperature (20–25°C), the second in a refrigerator (5°C), and the third was placed in a freezer (-20°C).

Three subsamples of *Mentha* × *rotundifolia* seeds were thus stored separately under three temperature conditions, with the ambient laboratory temperature serving as a control to provide a natural reference for assessing the effects of cold and freezing storage on seed viability. The first temperature slows down metabolic processes (Harrington & Kozlowski, 1972; Meddour & Derridj, 2007), whereas the second better preserves the long-term viability by limiting cellular deterioration.

Three successive storage periods were considered in this study: 6, 14 and 18 months. For each storage condition, germination tests were conducted at the optimum temperature and light conditions predetermined during preliminary germination tests.

Statistical analysis

Seed vigour, photosensitivity and the effects of thermal and storage conditions on seed viability were determined from curves showing the evolution of germination capacity over time, and by calculating the following germination parameters: germination capacity, velocity coefficient, latency time and mean germination time. The velocity coefficient was calculated according to Kotowski's (1926) formula. The mean germination time, expressed in days, was calculated as the reciprocal of the velocity coefficient multiplied by 100, following the method of Côme (1968).

All statistical analyses were performed using IBM SPSS Statistics for Windows, with the integrated R extension, which enabled PERMANOVA. Due to violations of the normality and homogeneity of variance assumptions, non-parametric tests were employed. The Kruskal-Wallis test was used to assess differences among the groups, and pairwise comparisons were performed using the Mann-Whitney U test, with the significance ($p < 0.05$), to evaluate the effect of the tested conditions on the viability of *Mentha* × *rotundifolia* seeds.

RESULTS

Seed characteristics

The seeds are ovoid, with a glossy, dark-brown surface showing fine wrinkles. The average dimensions measured were 0.64 ± 0.1 mm in length, 0.42 ± 0.1 mm in width and 0.31 ± 0.1 mm in thickness, while the average weight of 100 seeds was 0.0072 g (Fig. 1). The average moisture content was low at 6.94% for a sample of 100 seeds.

Using the 2,3,5-triphenyltetrazolium (TTC) test, 82% of the seeds were found to be viable (Fig. 1). However, germination tests conducted on freshly harvested *Mentha* × *rotundifolia* seeds without any post-maturation period revealed 0% germination.

Effect of photoperiod on germination of *Mentha* × *rotundifolia* seeds

Seeds exposed to natural photoperiod began germinating earlier and showed a progressive rise, which stabilised and remained almost unchanged for the rest of the experiment. In contrast, seeds kept in continuous darkness showed a late onset and a nearly flat germination trajectory throughout the test (Fig. 2).

Seeds subjected to natural photoperiod germinated at an average rate of 32 ± 14.83%, significantly higher ($p < 0.05$) than that obtained for seeds maintained in continuous darkness (germination capacity was 6 ± 5.47%). In comparison, the final germination rates were relatively low (< 40%) across the two conditions tested (Fig. 3 A).

Continuous darkness conditions prolonged the germination process over time ($p < 0.01$), as shown by the higher mean germination time, from 15.67 ± 0.57 days (in the absence of light) to 12.40 ± 0.89 days (in the presence of light). Pairwise comparisons revealed no significant differences in the effects of day and night alternation on the coefficient of velocity or latency time (Fig. 3 B, C). However, slightly more favourable values were observed under natural light for these two parameters, although these differences were not statistically significant.

All the parameters studied revealed positive photosensitivity in the seeds of this species. Light significantly improved germination rate and reduced mean germination time (Fig. 3 A, D). It also induced a non-significant increase in the velocity coefficient and a reduction in latency time, indicating a favourable effect on the overall germination process. Although germination can occur in dark conditions, natural light significantly optimises key parameters and acts as a facilitator.

Effect of temperatures on germination and determination of the thermal optimum

The germination responses of *Mentha* × *rotundifolia* seeds varied with temperature (Fig. 4 and Fig. 5). Seeds sprouted at temperatures ranging from 10 to 35°C (Fig. 4), while germination was absent at 5 and at 40°C.

The germination parameters calculated show significantly different behaviour of seeds subjected to different temperatures (Fig. 5). Germination ca-

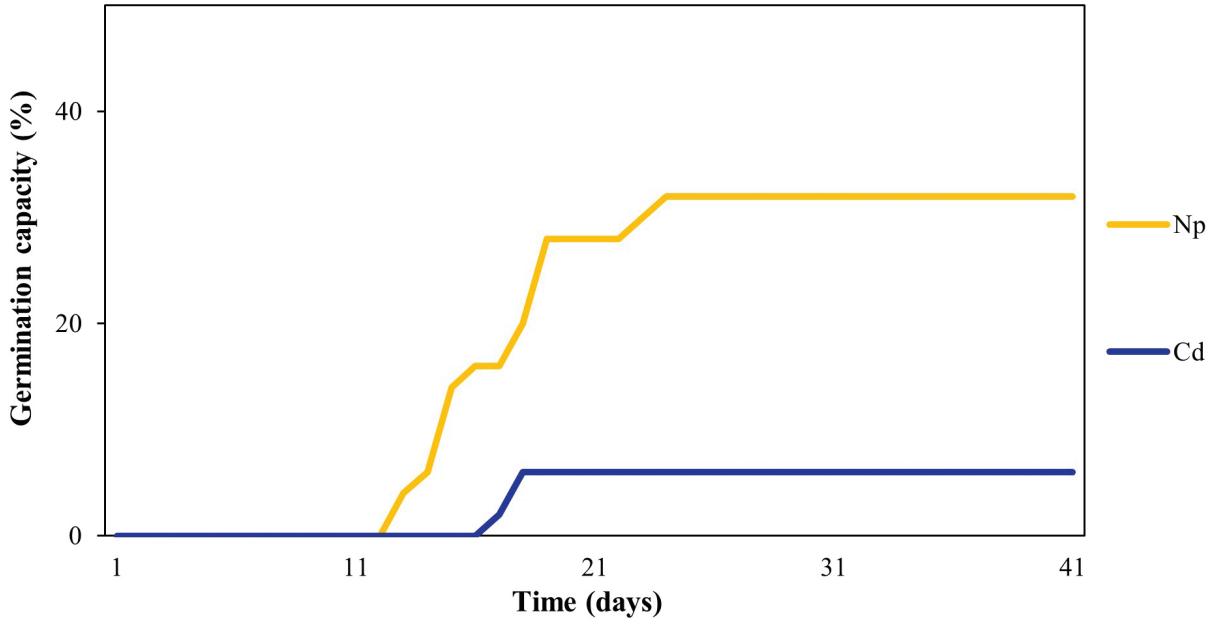

Fig. 2. Germination kinetics of *Mentha* × *rotundifolia* seeds in natural photoperiod (Np) and in continuous darkness (Cd).

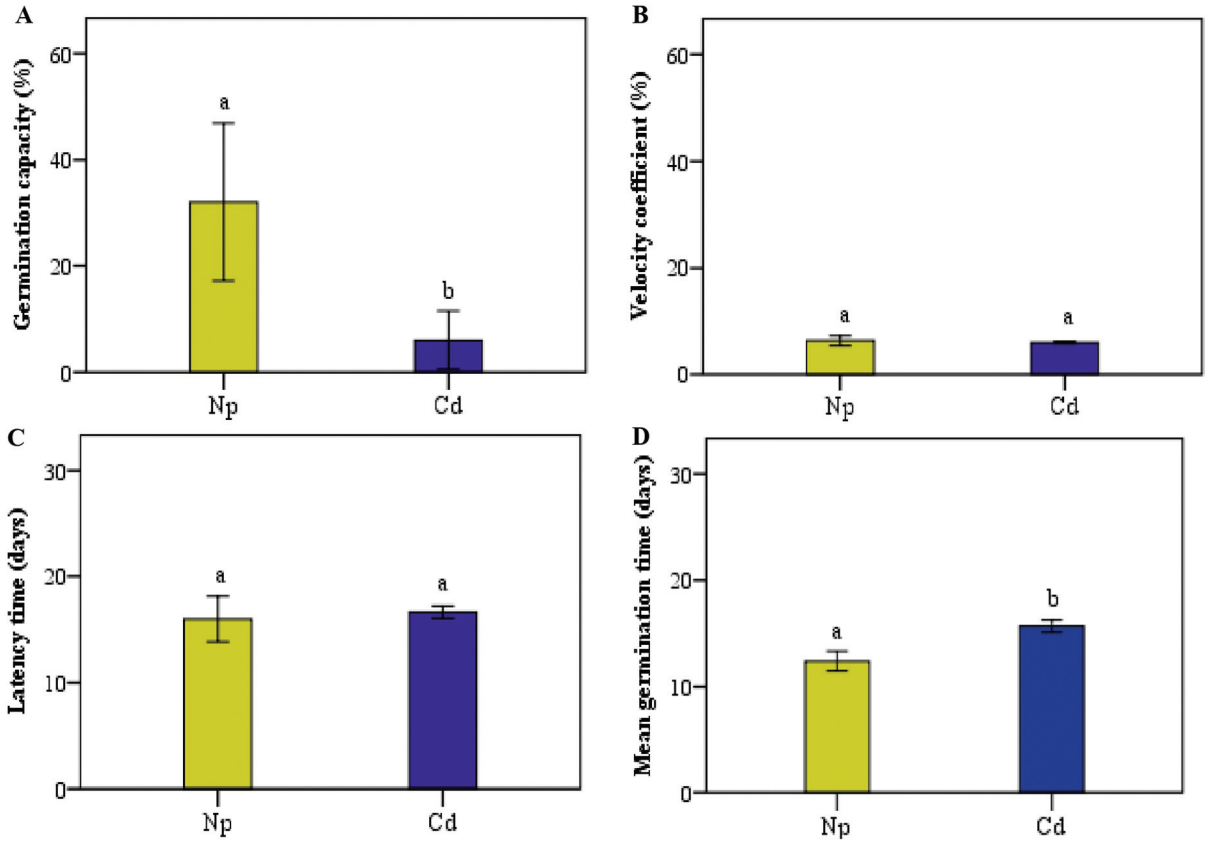

Fig. 3. Results of the calculation of germination parameters of *Mentha* × *rotundifolia* seeds subjected to two light regimes: natural photoperiod (Np) and continuous darkness (Cd). Different letters above bars indicate significant differences according to the results of the Mann-Whitney post hoc test.

pacity increased progressively with temperature, reaching its maximum at 30°C (42 ± 4.47%). Significant differences were observed for germination capacity between 10°C and 25°C (p = 0.029), 10°C and 30°C (p = 0.018), 15°C and 25°C (p = 0.026), and 15°C and 30°C (p = 0.015). A marked decrease was observed at 35°C, which differed significantly from 20°C (p = 0.018), 25°C (p = 0.006), and 30°C (p = 0.005), with an average germination capacity of 18.0 ± 4.47% (Fig. 5 A). At the same time, temperature significantly influenced the velocity coefficient. Significant differences were found in the velocity coefficient between 10°C and higher temperatures (25°C, 30°C, and 35°C; p = 0.009) and between 15°C and 25–35°C (p = 0.009–0.036). Additional differences were observed between 25°C and 30°C (p = 0.009) and between 30 °C and 35°C (p = 0.016). The highest velocity coefficient (12.54 ± 1.59%) was recorded at 30°C. In comparison, the lowest mean value (4.96 ± 1.06%) was recorded at 10°C, indicating that increasing temperature significantly favours increased germination speed, reaching an optimum of 30°C (Fig. 5 B). For each temperature treatment, five replicates of ten seeds were used (n = 50 seeds per treatment).

The lowest values of latency time and mean germination time were recorded at 30°C (3.8 ± 1.3 days and 8.07 ± 0.95 days, respectively), whereas the highest values were noted at 10°C (16.8 ± 3.89 days and 20.85 ± 4.07 days, respectively) (Fig. 5 C, D). These results indicate that increasing the temperature up to 30°C markedly reduces the time required to trigger and complete germination (p = 0.009 for latency time; p = 0.007 for mean germination time). Beyond 30°C, these parameters increased again. This temperature, therefore, appeared to be the most favourable under the tested conditions, as confirmed by the statistical analysis (p < 0.05).

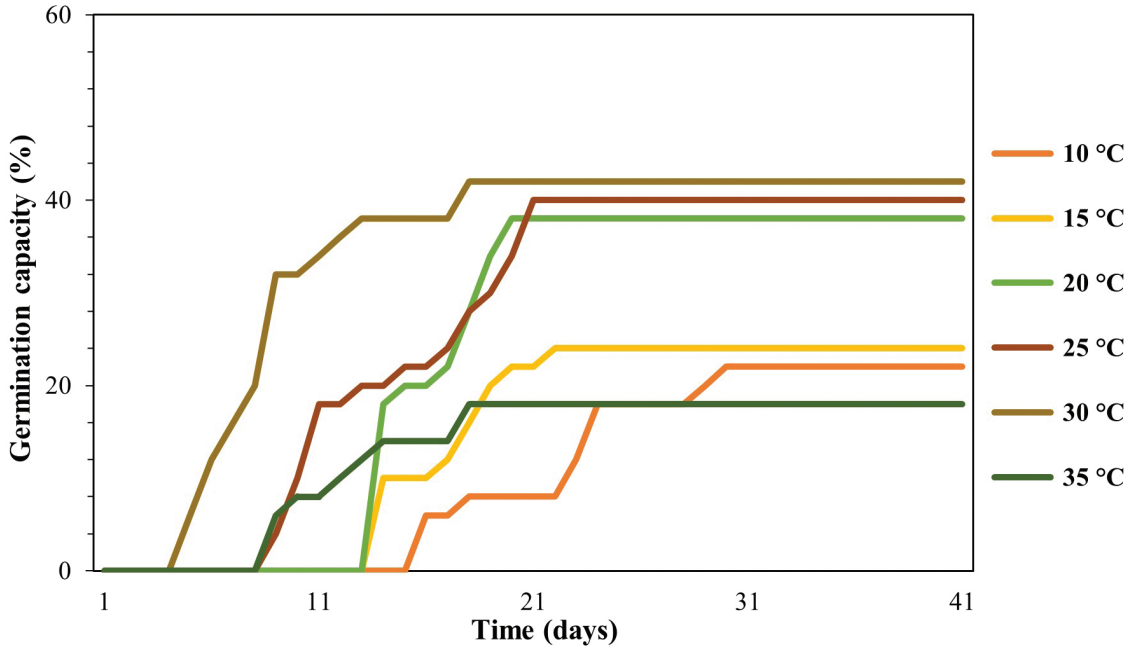

Fig. 4. Germination kinetics of *Mentha* × *rotundifolia* seeds under different temperature conditions (from 10°C to 35°C). No germination occurred at 5°C or 40°C.

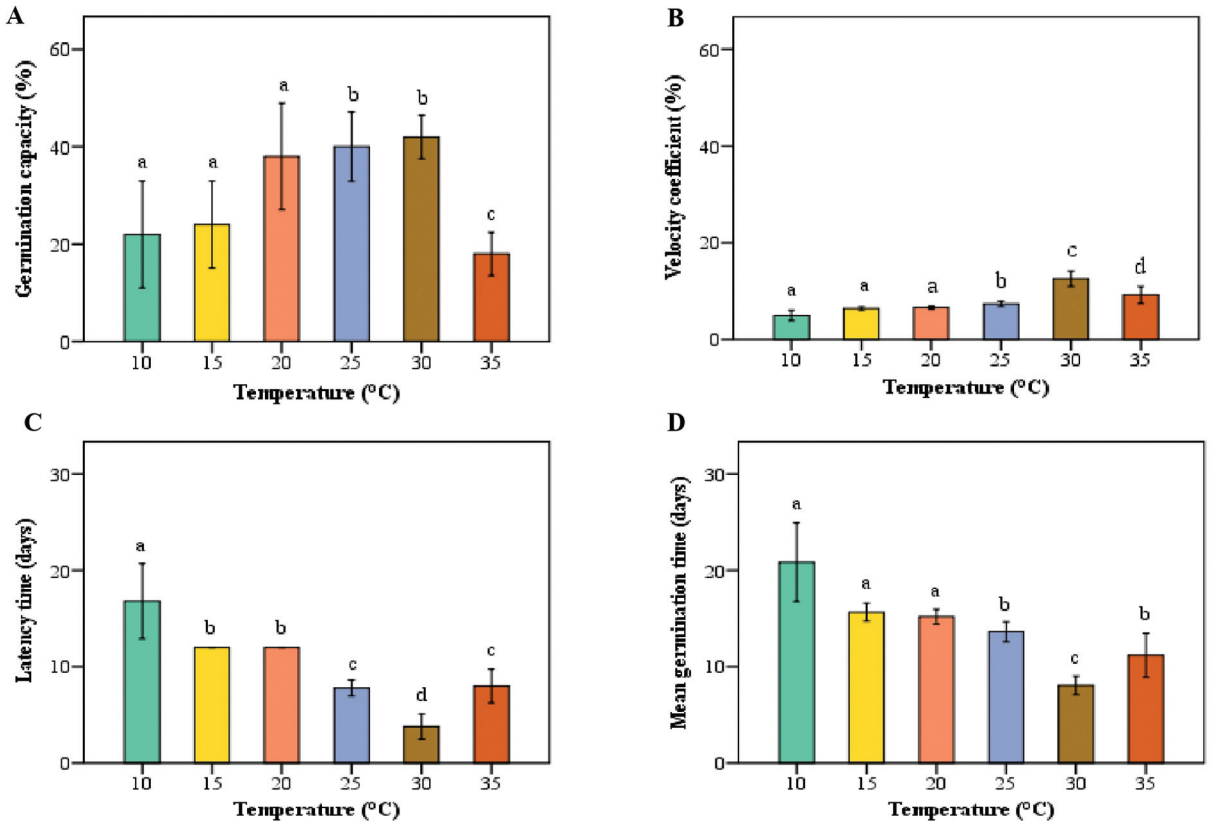

Fig. 5. Results of the calculation of germination parameters of *Mentha* × *rotundifolia* seeds tested at different temperatures (from 10°C to 35°C). Different letters above bars indicate significant differences ($p < 0.05$) according to the results of the Mann-Whitney post hoc test.

Effect of storage conditions on seed viability

To assess the influence of storage temperature and period on seed viability and germination behaviour, a permutational multivariate analysis of variance (PERMANOVA) was performed. These analyses were conducted to better understand how storage conditions affect seed performance and to guide the development of effective strategies for their *ex situ* conservation.

The results of the permutational multivariate analysis of variance revealed that storage temperature significantly affected all measured germination parameters, while storage duration alone generally had no measurable influence (Table 1). The effect of temperature on germination capacity was significant ($p = 0.024$), whereas storage duration did not affect germination ($p = 0.390$). Nevertheless, their interaction was highly significant ($p = 0.001$), indicating that the temperature response varied across storage periods. The influence of temperature was significantly stronger on the velocity coefficient ($p = 0.001$), with storage duration remaining non-significant ($p = 0.781$); the interaction between both factors was again highly significant ($p = 0.001$). Regarding latency time, storage temperature also exerted a pronounced effect ($p = 0.001$), whereas storage duration was not significant ($p = 0.143$). However, their interaction remained significant ($p = 0.001$). Storage temperature had a significant effect on mean germination time ($p = 0.001$); the duration effect was negligible ($p = 0.638$), and the interaction between temperature and duration was significant ($p = 0.001$).

The calculated germination parameters reflect seed viability and the degree of heterogeneity within a sample and between different storage treatments. The initial sample was tested three months after harvest and stored under ambient conditions. The second sample, which also represents seeds kept at ambient temperature, serves as the control. The third and fourth samples include seeds stored at 5°C and -20°C, respectively.

As shown in Table 2, seeds stored at 5°C exhibited the highest germination capacity (germination capacity was 100%, $p < 0.05$*)* after six months. Both 5°C and -20°C significantly influence this parameter during the 6- and 14-month storage periods, with values declining from 100% to 54% at 5°C ($p = 0.005$) and increasing from 52% to 62% at -20°C ($p = 0.156$).

After 18 months, seeds stored at -20°C maintained the highest germination capacity (64%, $p > 0.05$). Seeds from the initial sample germinated at 32%. Seeds kept at the same temperature showed a gradual increase in germination capacity, reaching 36% and 52% after six and 14 months ($p = 0.019$), and 70% after 18 months ($p = 0.011$; $p = 0.041$, respectively). Seeds stored at cold and freezing temperatures showed significantly higher germination capacity than those of the initial sample ($p < 0.05$).

Seeds stored at 5 and -20°C showed a significant decrease in latency time compared to the initial and ambient samples after six months ($p = 0.005$; $p = 0.006$; $p = 0.005$; $p = 0.012$, respectively), and after 14 months ($p = 0.005$; $p = 0.005$; $p = 0.050$; $p = 0.050$, respectively). After 18 months, no sig-

Table1. Results of permutational multivariate analysis of variance (PERMANOVA) showing the effects of storage temperature, storage duration, and their interaction on the germination parameters of *Mentha* × *rotundifolia* seeds. The significance ($p < 0.05$) is indicated in bold. Values of F and R^2 were computed from 999 permutations.

Parameter	Factor	df	F	R^2	*p* value
Germination capacity	Temperature	2	4.39	0.173	0.024
	Duration	2	0.91	0.041	0.390
	Temperature × duration	8	12.30	0.732	0.001
Coefficient of velocity	Temperature	2	20	0.488	0.001
	Duration	2	0.26	0.012	0.781
	Temperature × duration	8	7.38	0.621	0.001
Latency time	Temperature	2	11.41	0.352	0.001
	Duration	2	2.15	0.093	0.143
	Temperature × duration	8	8.36	0.650	0.001
Mean germination time	Temperature	2	22.91	0.522	0.001
	Duration	2	0.43	0.020	0.638
	Temperature × duration	8	9.78	0.685	0.001

Table 2. Germination parameters (germination capacity, coefficient of velocity, latency time and mean germination time) of *Mentha* × *rotundifolia* seeds stored under different temperature conditions and duration. Values are expressed as mean ± standard deviation (SD). Different lowercase letters indicate statistically significant differences in storage temperature at the same storage duration. In contrast, uppercase letters indicate significant differences in storage duration across the same temperature condition (Mann-Whitney post hoc test, $p < 0.05$).

Storage temperature (°C)	Storage duration (months)	Germination capacity (%)	Coefficient of velocity (%)	Latency time (days)	Mean germination time (days)
-20	6	52 ± 4.47^{cA}	28.76 ± 4.49^{cA}	1.2 ± 0.44^{cA}	3.54 ± 0.56^{cA}
-20	14	62 ± 13.03^{bA}	25.34 ± 6.87^{bA}	1 ± 0^{cA}	4.17 ± 1.10^{bA}
-20	18	64 ± 18.16^{bA}	25.88 ± 1.41^{cA}	1.4 ± 0.54^{bA}	3.87 ± 0.21^{cA}
5	6	100 ± 0^{bA}	32.17 ± 5.42^{cA}	1 ± 0^{cA}	3.18 ± 0.54^{cA}
5	14	54 ± 11.40^{bB}	25 ± 2.44^{bB}	1 ± 0^{cA}	4.03 ± 0.41^{bB}
5	18	62 ± 10.95^{bAB}	24.57 ± 6.35^{cAB}	1 ± 0^{bA}	4.24 ± 0.85^{cAB}
Control	6	36 ± 11.40^{aA}	10.82 ± 5.18^{bA}	2.80 ± 0.83^{bA}	10.45 ± 3.23^{bA}
Control	14	52 ± 4.47^{bB}	19.24 ± 9.24^{bB}	1.60 ± 0.54^{bB}	6.18 ± 2.74^{bB}
Control	18	70 ± 14.14^{bC}	14.86 ± 4.61^{bAB}	1.40 ± 0.54^{bAB}	7.27 ± 2.21^{cAB}

nificant differences were observed between ambient, 5°C, and -20°C conditions ($p = 0.134$; $p = 1.000$). The longest LT corresponded to seeds from the first sample, with the highest value recorded at 12.4 days ($p < 0.05$). The best results were obtained after 18 months of storage at 5°C, with a value of one day, followed by seeds stored at -20°C and at room temperature, both showing a latency time of 1.4 days for the same duration (Table 2).

For the coefficient of velocity, seeds stored under cold (5°C) and freezing (-20°C) conditions displayed a markedly higher coefficient of velocity than those kept at ambient temperature and from the initial sample. After six months, significant differences were detected between the initial sample and each storage condition (ambient, 5°C, -20°C; $p = 0.016$; $p = 0.009$; $p = 0.009$), as well as between ambient and low-temperature treatments ($p = 0.009$), while no significant difference occurred between 5°C and -20°C ($p = 0.459$). After 14 months, the initial sample again showed the lowest coefficient of velocity ($p = 0.009$), whereas no significant difference was observed between ambient and cold conditions ($p = 0.175$) or ambient and freezing conditions ($p = 0.175$). After 18 months, significant differences were again found between the initial sample and all storage conditions ($p = 0.009$) and between ambient and 5°C ($p = 0.012$) as well as between ambient and -20°C ($p = 0.009$), while no significant difference occurred between 5°C and -20°C ($p = 0.117$). The highest coefficient of velocity was recorded at 5°C (32.17%), followed by -20°C (28.76%, 25.34%, and 25.88% for 6, 14, and 18 months, respectively).

For the mean germination time, seeds from the initial sample germinated significantly more slowly than all stored lots ($p = 0.016$-0.009). After six months, significant differences were found between the initial sample and all storage temperatures ($p \leq 0.016$) and between ambient (control) and low-temperature treatments ($p = 0.009$), but not between 5°C and -20°C ($p = 0.459$). After 14 months, the initial sample again differed from all stored seeds ($p = 0.009$), while no difference occurred among storage temperatures ($p = 0.175$-0.754). After 18 months, seeds from the initial sample remained significantly slower ($p = 0.009$), and ambient-stored seeds differed from those at 5°C ($p = 0.012$) and -20°C ($p = 0.009$), whereas no difference was observed between 5°C and -20°C ($p = 0.117$).

The results of the calculation of germination kinetics parameters for seeds stored at cold and freezing conditions, and those stored at ambient temperature, showed a significant difference, indicating a reduction in seed viability for seeds stored at ambient conditions compared to those stored at cold conditions ($p < 0.05$).

The best results were obtained with seeds stored at 5°C for short periods and at -20°C for more than 14 months, compared to the other conditions studied ($p < 0.05$). The improvement in germination performance, reflecting enhanced viability, was statistically significant for seeds stored at the lowest temperature for durations exceeding 14 months ($p < 0.05$).

DISCUSSION

The small, ovoid seeds of *Mentha* × *rotundifolia*, characterised by a glossy surface with fine granulation, may reflect an adaptation that facilitates dispersal in their natural habitat. Similarly, a dark brown colour could help protect against UV rays and limit moisture evaporation. The small size provides a reference for comparison with other *Mentha* species or Lamiaceae members. The low average weight indicates dispersal mainly by wind, facilitating the wide distribution of this species.

Our seed observations revealed exalbuminous seeds, a feature that is consistent with the pattern reported for members of the *Nepetoideae* subfamily. The Lamiaceae family exhibits considerable diversity in seed types, which can be either albuminous or exalbuminous depending on the subfamily. For instance, among the 42 genera of *Nepetoideae*, all except *Bystropogon* produce exalbuminous seeds, whereas the *Lamioideae* subfamily predominantly includes species with albuminous seeds, with *Amethystea* being a notable exception (Zoz & Litvinenko, 1979). Seeds are exalbuminous (Fig. 1), meaning that the endosperm is absent or greatly reduced, and the nutritional reserves are stored primarily in the cotyledons of the embryo. This allows direct nutrient transfer to the growing tissues during germination (Morot-Gaudry et al., 2021). This clearly confirms that *Mentha* × *rotundifolia* belongs to the exalbuminous group within the Lamiaceae.

The Royal Botanic Gardens Kew (2020) recommends maintaining seed moisture content between 3% and 7%, which is considered optimal for long-term storage. In our study, the seeds reached a moisture content of 6.94%, which falls within this recommended range. Combined with their hygroscopic behaviour, this supports their classification as orthodox seeds. Seeds were dried in glass desiccators containing silica gel immediately after cleaning until reaching this moisture range before storage. Excessive humidity may promote mould and microbial growth, while overly low moisture levels can lead to excessive dehydration, reducing seed viability. As stated by Nadarajan et al. (2023), Harrington's Thumb Rules (1972) indicate that appropriate drying improves seed longevity by 1% for every reduction in seed moisture content to equilibrium. The ability of orthodox seeds to withstand long-term storage is a major advantage for seed banks and *ex situ* conservation programmes. Studies have shown that the longevity of orthodox seeds can be increased by reducing their moisture content and storage temperature, with a doubling of their lifespan for every 1–2% reduction in their moisture content (Corbineau, 2024).

Freshly harvested *Mentha* × *rotundifolia* seeds did not germinate, indicating the presence of innate dormancy or physiological immaturity of the embryo that could be overcome by a post-maturation treatment. Post-maturation is a process of storing harvested seeds for varying periods at ambient temperatures (Baskin & Baskin, 1998). Seeds kept for three months in ambient conditions germinated with relatively low vigour. Thus, those subjected to a natural photoperiod regime germinated at 32%, while those subjected to continuous obscuration germinated at only 6%. These results highlighted the importance of the post-maturation period to trigger the physiological processes necessary for seed germination in this species. The results of this study agree with those of Baskin & Baskin (2014), who have reported that seeds of species in the Lamiaceae family generally exhibit shallow physiological dormancy. According to Bewley & Black (1994), dormancy allows for time-spread germination by making the elimination of dormancy dependent on an environmental factor that is itself time-spread. The seeds of *Mentha* × *rotundifolia* studied are in dormancy or physiological immaturity and require a post-maturation period of several months. In addition, it is reported that the seeds of the genus *Mentha* are mainly characterised by physiological dormancy (Baskin & Baskin, 2004; Brändel, 2006; Elhindi et al., 2016; Lopez del Egdigo et al., 2019). Physiological dormancy is the most common type of dormancy, characterised by the influence of environmental and hormonal factors that prevent immediate seed germination even under favourable conditions (Baskin & Baskin, 2014).

The results obtained highlight the crucial role of natural photoperiod in the germination process of *Mentha* × *rotundifolia* seeds. This explains why this species is adapted to an alternating regime of light and darkness rather than continuous darkness, which is vital in preparing it to regulate essential subsequent biological processes such as photosynthesis and growth. The results obtained corroborate the findings of several previous studies on this subject.

According to Quail (2010), the seeds of many plants that require light to initiate germination contain a quantity of phytochrome (photoreceptor) in an inactive form, which becomes active when the seeds absorb light rays. In contrast, seeds capable of germinating without light have been reported to already possess enough active phytochrome (Quail, 2010). Therefore, the need for light observed in our study confirms that our seeds are positively photoblastic. This finding is further supported by Liu et al. (2012), who have reported a significantly higher germination rate for *Mentha* × *rotundifolia* seeds under white light than under total darkness. This dependence on light is consistent with the low nutrient reserves in the seed (absence of endosperm), making rapid post-germination photosynthesis essential for seedling survival. In line with this, previous studies by Milberg et al. (2000) and Pearson et al. (2002) have shown that small seeds depend on light to germinate because they have limited internal reserves, which require rapid activation of photosynthesis after germination.

According to Probert (2000), the ideal temperature range for germination is generally determined by the thermal conditions in the habitat. In our study, seed germination occurred between 10°C and 35°C, an amplitude comparable to that reported for several Mediterranean arid and semiarid species of the Lamiaceae family, such as *Lavandula dentata* L., *Teucrium gnaphalodes* L'Hér., *Thymbra capitata* (L.) Cav. and *Thymus hyemalis* Lange (Kadis & Georghiou, 2010), and *Salvia sclarea* L., which germinates seeds between 10°C and 30°C (Côme, 1993). Beyond this thermal range, a distinct optimum was identified at 30°C under light conditions. These findings are consistent with those of Liu et al. (2012), who have also reported 30°C as the most favourable germination temperature for *Mentha* × *rotundifolia*, reinforcing the ecological relevance of this thermal preference. Similarly, *Hyptis marrubioides* Epling (Lamiaceae) exhibited optimal germination at the same temperature under comparable light conditions (Sales et al., 2011). It is known that optimal thermal intervals are related to the geological origin of species and promote their adaptation and regeneration in semiarid Mediterranean climate.

Seed longevity is strongly influenced by initial quality, moisture levels, and temperature to extend seed life during storage. Numerous studies have demonstrated that seed longevity increases when moisture is reduced and storage temperature is lowered, underscoring the importance of properly managing these factors to optimise seed conservation (Walters et al., 2010; Nadarajan et al., 2023). According to Rao et al. (2006), maintaining seed viability over long periods requires storage temperatures below 0°C, often between -18°C and -20°C. According to the observations of Harrington & Kozlowski (1972), temperature and humidity are indeed determinants of seed conservation, and they found that every a 5°C reduction in storage temperature doubles the lifespan of seeds. Hay et al. (2023) have emphasised that maintaining seed viability and vigour over extended periods requires strict control of moisture content, usually between 3% and 7 %, in combination with cold storage in airtight containers. These conditions ensure that the seeds remain below the critical water activity threshold that triggers biochemical deterioration. The synergy between low humidity and cold temperature is therefore essential for slowing down the rate of viability loss during *ex situ* storage systems (Hay & Probert, 2013).

For Lamiaceae species, such as *Mentha aquatica*, *Lycopus europaeus* L., and *Stachys palustris* L., Brändel (2006) has shown that dormancy release occurs at temperatures below 12°C, indicating clear physiological tolerance to cold. This supports the suitability of low-temperature regimes (from -18°C to -20°C) for the long-term preservation of *Mentha* seeds in seed banks. The present findings thus align with these previous reports, reinforcing the view that sub-zero storage environments are optimal for maintaining the germinative potential and physiological integrity of Lamiaceae seeds over prolonged periods.

Ikeda et al. (1960) have demonstrated that to preserve the germination capacity of *Mentha* × *piperita* seeds from Japan, they must be kept at low temperatures and under drying conditions. These findings guided the selection of storage conditions for the present study. The viability of *Mentha* × *rotundifolia* seeds was maintained during the three storage periods considered in this study. Several studies have examined the germination potential of seeds of certain Lamiaceae species stored for varying durations, and their results corroborate our findings. This is the case for *Salvia officinalis* L. and *Mentha spicata* L., where seeds maintained high germination rates for 18

months of storage under controlled conditions (Ellis et al., 1985). In this study, a significant improvement in the viability of *Mentha* × *rotundifolia* seeds was observed after six months of storage at 5°C, with the final rate reaching 100%. Among the temperatures evaluated in this study, 5°C stands out as a particularly advantageous storage option for short-term storage. This rate decreased significantly after 18 months of storage to an average value of 62%. This storage temperature could be reserved for short-term use, when rapid, high germination is required. Walters et al. (2005) have demonstrated that refrigeration temperatures can preserve seed viability in the short term. An extended shelf life can lead to cell membrane degradation and affect physiological processes, eventually reducing viability. These results confirm that low-temperature storage effectively preserved germination velocity and maintained seed vigour compared to ambient conditions.

The results of our study suggest that freezing temperatures can not only preserve but also improve seed viability over extended periods. This observation is supported by the research of Pritchard & Dickie (2003), who have demonstrated that freezing temperatures can slow down the metabolic and enzymatic processes responsible for seed degradation, thereby improving conservation of viability. Similarly, Walters et al. (2010) have revealed that low-temperature storage delays cellular degradation by slowing enzymatic and metabolic processes responsible for seed ageing, while also protecting the integrity of RNA and DNA. In our study, the seeds preserved at -20°C exhibited the best overall stability, suggesting that freezing temperatures not only maintain but also enhance long-term viability compared to cold or ambient conditions. Conversely, storage at room temperature likely accelerated lipid peroxidation and membrane disruption (Corbineau et al., 2024). While some studies have reported that long-term storage at room temperature can enhance seed viability, the relationship between temperature and seed dormancy remains complex. Low temperatures are often associated with alleviation of dormancy (Bouwmeester & Karssen, 1993a, 1993b), but in some species, they can also induce dormancy. Conversely, room temperature or higher temperatures may induce dormancy in certain cases, while promoting its release in others (Brändel, 2004; Brändel & Schutz, 2005).

Seeds stored at -20°C maintained relatively stable germination rates, reaching 64% after 18 months of storage, although the observed variations were not statistically significant ($p > 0.05$). In parallel, this study demonstrated that storage at ambient temperature can enhance germination rates, depending on the duration of storage. A significant improvement was observed between six and 14 months, and between six and 18 months ($p < 0.05$). Interestingly, seeds stored at ambient temperature (control) showed the highest germination rate after 18 months, suggesting that, under certain conditions, room temperature may favour long-term viability in *Mentha* × *rotundifolia.* These findings are consistent with those reported by Ikeda et al. (1960), who have demonstrated the effectiveness of low-temperature storage combined with a drying agent in preserving the germination capacity of *Mentha* × *piperita* seeds for over a year. However, the same authors have also observed that this capacity is almost entirely lost within a year when the seeds are stored in dry air at room temperature. The germination capacity of *Mentha* × *rotundifolia* seeds was maintained and even improved after one and a half years of storage in dry air, without a drying agent, at ambient temperature.

CONCLUSION

The present study provides essential baseline information on the biological response of *Mentha* × *rotundifolia* seeds under controlled conditions, contributing to a better understanding of the species' regeneration potential in *ex situ* conservation programmes.

Storage temperature significantly affected all measured germination parameters, whereas storage duration alone had no measurable influence. The best germination performance was obtained under cold and freezing conditions, which maintained high vigour, and short germination times even after 18 months of storage. In contrast, seeds kept under ambient conditions germinated more slowly and exhibited reduced vigour, despite occasionally higher germination percentages. These results confirm that low-temperature storage is the most suitable strategy to preserve seed viability and physiological integrity over time.

This study showed promising results in maintaining and even enhancing the germinative performance

of *Mentha* × *rotundifolia* seeds during storage. Future research should consider extending the storage duration to better assess seed longevity and ensure long-term conservation efficiency.

ACKNOWLEDGEMENTS

We thank the Directorate General for Scientific Research of the Ministry of Higher Education and Scientific Research (Algeria) and the director and members of the teams at the Plant Biodiversity: Conservation and Development Laboratory of the faculty of Natural and Life Sciences (University of Sidi Bel Abbès, Algeria), for the material and financial support required to carry out this work. This work is part of a national research project on the socio-economic impact of the creation and management of a seed bank for the conservation of plant taxa in western Algeria (contract N° 398 du 20/12/2020). We also thank Dr S. Bouazza of the faculty of Natural and Life Science at the University of Sidi Bel Abbès (Algeria) for his advice on the statistical analysis of the data in this study.

Author contributions. All authors prepared the material and performed sampling. Experimental and data analyses were carried out by C.B. and F.Z.B.-M. The first draft of the manuscript was written by C.B., revised and corrected by F.Z.B.-M. All the authors read and approved the final version of the manuscript.

REFERENCES

Afrokh M., El Mehrach K., Chatoui K., Bihi M.A., Sadki H., Zarrouk A., Tahrouch S., 2024: Quality criteria, chemical composition and antimicrobial activity of the essential oil of *Mentha suaveolens* Ehrh. – Heliyon, 10(7): e28125. https://doi.org/10.1016/j.heliyon.2024.e28125

Baskin C.C., Baskin J.M., 1998: Seeds: Ecology, Biogeography, and Evolution of Dormancy and Germination. San Diego.

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

Baskin C.C., Baskin J.M., 2014: Seeds: Ecology, Biogeography, and Evolution of Dormancy and Germination (2nd ed.). San Diego, London.

Baraka D., 2008: Inventaire et caractérisation des plantes médicinales de Djebel Tessala (Willaya de Sidi Bel Abbés). Magister dissertation on Biology, Djilali Liabès, Sidi Bel Abbés University, Algeria.

Bencherif S., Dahmani M.B., Burgas D., Manzano P., 2021: Current social and rangeland access trends among pastoralists in the Western Algerian steppe. – Land, 10(7): 674. https://doi.org/10.3390/land10070674

Bendjeddi A., Bakhy K., Abdelmoumen H., El Yadini M., Homrani Bakali A., 2025: Comparative study of seeds biometric and germinative characteristics of four Moroccan wild *Mentha* species. – Vegetos. https://doi.org/10.1007/s42535-025-01349-0

Benomari F.Z., Andreu V., Kotarba J., Dib M.E.A., Bertrand C., Muselli A., Djabou N., 2018: Essential oils from Algerian species of *Mentha* as new bio-control agents against phytopathogen strains. – Environmental Science and Pollution Research, 25: 29889–29900. https://doi.org/10.1007/s11356-017-9991-4

Bewley J.D., Black M.,1994: Dormancy and the control of germination. – In: Bewley J.D., Black M. (eds), Seeds: Physiology of Development and Germination (2nd ed.): 199–271. New York. https://doi.org/10.1007/978-1-4899-1002-8_5

Bouiadjra S.E.B., El-Zerey W., Ait Bella O., 2011: Étude diachronique des changements du couvert végétal dans un écosystème montagneux par télédétection spatiale: cas des monts du Tessala. – Journal of Applied Remote Sensing and Geographic Information Systems, 5: 211–225. https://doi.org/10.4000/physio-geo.2048

Bounatirou S., Simitis S., Miguel M.G., Faleiro L., Rejeb M.N., Neffati M., Costa M.M., Figueiredo A.C., Barroso J.G., Pedro L.G., 2007: Chemical composition, antioxidant and antibacterial activities of the essential oils isolated from Tunisian *Thymus capitatus* Hoff. et Link. – Food Chemistry, 105(1): 146–155. https://doi.org/10.1016/j.foodchem.2007.03.059

Bouwmeester H.J., Karssen C.M., 1993a: The effect of environmental conditions on the annual dormancy pattern of seeds of *Spergula arvensis* L. – Canadian Journal of Botany, 71(1): 64–73. https://doi.org/10.1139/b93-008

Bouwmeester H.J., Karssen C.M., 1993b: Annual changes in dormancy and germination in seeds of *Sisymbrium officinale* (L.)

Scop. – New Phytologist, 124(1): 179–191. https://doi.org/10.1111/j.1469-8137.1993.tb03808.x

Brahmi F., Lounis N., Mebarakou S., Guendouze N., Yalaoui- Guellal D., Madani K., Boulekbache-Makhlouf L., Duez P., 2022: Impact of growth sites on the phenolic contents and antioxidant activities of three Algerian Mentha species (*M. pulegium* L., *M. rotundifolia* (L.) Huds., and *M. spicata* L.). – Frontiers in Pharmacology, 13: 886337. https://doi.org/10.3389/fphar.2022.886337

Brändel M., 2004: Dormancy and germination of heteromorphic achenes of *Bidens frondosa*. – Flora-Morphology, Distribution, Functional Ecology of Plants, 199(3): 228–233. https://doi.org/10.1078/0367-2530-00150

Brändel M., Schutz W., 2005: Temperature effects on dormancy levels and germination in temperate forest sedges (*Carex*). – Plant Ecology, 176: 245–261. https://www.jstor.org/stable/20051356

Brändel M., 2006: Effect of temperature on dormancy and germination in three species in the Lamiaceae occurring in northern wetlands. – Wetlands Ecology and Management, 14(1) 11–28. https://doi.org/10.1007/s11273-005-1548-5

Brits G.J., Brown N.A.C., Calitz F.J., Van Staden J., 2015: Effects of storage under low temperature, room temperature, and in the soil on viability and vigour of *Leucospermum cordifolium* (Proteaceae) seeds. – South African Journal of Botany, 97: 1–8. https://doi.org/10.1016/j.sajb.2014.11.003

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

Côme D., 1968: The germination of seeds: a review. – Proceedings of the International Seed Testing Association, 33: 301–316.

Côme D., 1993: Germination of seeds of some aromatic plants. – In: Identification, Preservation, Adaptation, and Cultivation of Selected Aromatic and Medicinal Plants Suitable for Marginal Lands of the Mediterranean Region: 111–126. Chania

Corbineau F., 2024: The effects of storage conditions on seed deterioration and ageing: how to improve seed longevity. – Seeds, 3(1): 56–75. https://doi.org/10.3390/seeds3010005

Denslow M.W., Poindexter D.B., 2009: *Mentha suaveolens* and *Mentha* × *rotundifolia* in North Carolina: a clarification of distribution and taxonomic identity. – Journal of the Botanical Research Institute of Texas, 3(1): 383–389.

Derwich E., Benziane Z., Boukir A., 2010: Antibacterial activity and chemical composition of the leaf essential oil of *Mentha rotundifolia* from Morocco. – Electronic Journal of Environmental, Agricultural and Food Chemistry, 9(1): 19–28.

Elhindi K.M., Dewir Y.H., Asrar A.W., Abdel-Salam E., Sharaf El-Din A., Ali M., 2016: Improvement of seed germination in three medicinal plant growth regulators. – HortScience, 51(7): 887–891. https://doi.org/10.21273/HORTSCI.51.7.887

Ellis R.H., Hong T.D., Roberts E.H., 1985: Handbook of Seed Technology for Gene Banks. Handbooks for Genebanks. No. 3. Volume II. Compendium of Specific Germination Information and Test Recommendations. International Board for Plant Genetic Resources. Rome.

Ferdjioui S., Belhattab R., Al-Zoubi R.M., 2019: Chemical composition and antioxidant effect of *Mentha rotundifolia* extracts. – Pharmacognosy Journal, 11(3): 521–526. https://doi.org/10.5530/pj.2019.11.83

Forman H.J., Zhang H., 2021: Targeting oxidative stress in disease: promise and limitations of antioxidant therapy. – Nature Reviews Drug Discovery, 20(9): 689–709. https://doi.org/10.1038/s41573-021-00233-1

Gomez-Campo C., 1985: Seed banks as an emergency conservation strategy. – In: Gomez-Campo C. (ed.), Plant Conservation in the Mediterranean Area: 237–247. Dordrecht.

Goraï M., Gasmi H., Neffati M., 2011: Factors influencing seed germination of medicinal plant *Salvia aegyptiaca* L. (Lamiaceae). – Saudi Journal of Biological Sciences, 18(3): 255–260. https://doi.org/10.1016/j.sjbs.2011.01.004

Hay F.R., Probert R.J., 2013: Advances in seed conservation of wild plant species: a review of recent research. – Conservation Physiology, 1(1): cot030. https://doi.org/10.1093/conphys/cot030

Hay F.R., Razaei S., Wolkis D., McGill C., 2023: Determination and control of seed moisture. – Seed Science and Technology, 51(2): 267–285. https://doi.org/10.15258/sst.2023.51.2.11

Harley R.M., Atkins S., Budantsev A.L., Cantino P.D., Conn B.J., Grayer R.J., Harley M.M.,

2004: *Labiatae*. – In: Kubitzki K. (ed.), The Families and Genera of Vascular Plants, 7 (1): 167–275. Berlin.

Harrington J.F., Kozlowski T.T., 1972: Seed storage and longevity. – In: Kozlowski T.T. (ed.), Seed Biology, 3: 145–245. New York.

Ikeda N., Udo S., Saisho I., Minakata S.,1960: Studies on the storage of mint seed. – Scientific Reports of the Faculty of Agriculture, Okayama University, Japan, 16(1): 1–5.

International Seed-Testing Association (ISTA), 2021: International Rules for Seed Testing. – Seed Science and Technology, Supplement, Zürich, International Seed Testing Association.

Kadis C., Georghiou K., 2010: Seed dispersal and germination behaviour of three threatened endemic Labiates of Cyprus. – Plant Species Biology, 25(1): 77–84. https://doi.org/10.1111/j.1442-1984.2009.00265.x

Kokkini S., Papageorgiou V.P., 1988: Constituents of essential oils from *Mentha* × *rotundifolia* growing wild in Greece. – Planta Medica, 54(2): 166–167. https://doi.org/10.1055/s-2006-962381

Kotowski F., 1926: Temperature relations to germination of vegetable seeds. – Proceedings of the American Society for Horticultural Science, 23: 176–184.

Liu J.C., Gong W.H., Xiang F., Yang J.X., Fu Z.R., Peng X.M., 2012: Effects of different treatments on seed germination of *Mentha rotundifolia*. – Chinese Traditional and Herbal Drugs, 43(5): 1000–1002.

Lopez del Egdigo L., Toorop P.E., Lanfermeijer F.C., 2019: Seed enhancing treatments: comparative analysis of germination characteristics of 23 key herbaceous species used in European restoration programmes. – Plant Biology, 21(3): 397–407. https://doi.org/10.1111/plb.12937

Lorenzo D., Paz D., Dellacassa E., Davies P., Vila R., Canigueral S., 2002: Essential oils of *Mentha pulegium* and *Mentha rotundifolia* from Uruguay. – Brazilian Archives of Biology and Technology, 45(4): 519–524. https://doi.org/10.1590/S1516-89132002000600016

Meddour R., Derridj A., 2007: Les banques de semences : une stratégie de conservation *ex situ* des plantes et endémiques. – Revue Campus, 5: 71–79.

Milberg P., Andersson L., Thompson K., 2000: Large-seeded spices are less dependent on light for germination than small-seeded ones. – Seed Science Research, 10(1): 99–104. http://doi.org/10.1017/S0960258500000118

Moore R.P., 1985: Handbook on tetrazolium testing. Zürich, International Seed Testing Association.

Morot-Gaudry J.-F., Lepiniec L., Prat R., Gévaudant F., Laufs P., Masclaux-Daubresse C., Parcy F., 2021: Biologie végétale: croissance et développement (4th ed.). Paris.

Nadarajan J., Walters C., Pritchard H.W., Ballesteros D., Colville L., 2023: Seed longevity: progress and a conceptual framework. – Plants, 12: 471. https://doi.org/10.3390/plants12030471

Olofsson J.K., Tyler T., Dunning L.T., Hjertson M., Rühling Å., Hansen A.J., 2024: Morphological and genetic evidence suggest gene flow among native and naturalized mint species. – American Journal of Botany, 111(2): e16280. https://doi.org/10.1002/ajb2.16280

Pearson T.R., Burslem D.F., Mullins C.E., 2002: Germination ecology of neotropical pioneers: interacting effects of environmental conditions and seed size. – Ecology, 83(10): 2798–2807. https://doi.org/10.1890/00129658(2002)083[2798: GEONPI]2.0.CO;2

Phondani P.C., Bhatt A., Elsarrag E., Alhorr Y.M., 2015: Seed Germination and growth performance of *Aerva javanica* (Burm. f.) Juss. ex-Schult. – Journal of Applied Research on Medicinal and Aromatic Plants, 2(4): 195–199. https://doi.org/10.1016/j.jarmap.2015.08.002

Pritchard H.W., Dickie J.B., 2003: Predicting seed longevity: the use and abuse of seed viability equations. – In: Smith R.D., Dickie J.B., Linington S.H., Pritchard H.W., Probert R.J. (eds), Seed conservation: turning science into Practice: 653–722. Kew.

Probert R.J., 2000: The role of temperature in the regulation of seed dormancy and germination. – In: Fenner M. (ed.), Seeds: Ecology of Regeneration in Plant Communities (2nd ed.): 261–292. Wallingford. https://doi.org/10.1079/9780851994321.0261

Quail P., 2010: Phytochromes. – Current Biology, 20(12): R504–R507. https://doi.org/10.1016/j.cub.2010.04.014

Rao N.K., Hanson J., Dulloo M.E., Ghosh K., No-

well D., Larinde M., 2006: Manual of Seed Handling in Genebanks. 8. Handbooks for Genebanks. Rome.

Riahi L., Elferchichi M., Ghazghazi H., Jebali J., Ziadi S., Aouadhi C., Milki A., 2013: Phytochemistry, antioxidant and antimicrobial activities of the essential oils of *Mentha rotundifolia* L. in Tunisia. – Industrial Crops and Products, 49: 883–889. https://doi.org/10.1016/j.indcrop.2013.06.032

Royal Botanic Gardens, Kew, 2020: Seed conservation standards for Millennium Seed Bank Partnership collections. Royal Botanic Gardens, Kew.

Saidi B., Latreche A., Hakemi Z., Mehdadi Z., Bouker A., 2016: Dynamique post-perturbation (post-incendie ou post-surpâturage) des communautés végétales dans les monts Tessala, ouest algérien. – Ecologia Méditerranea, 42(2): 41–49. https://doi.org/10.3406/ecmed.2016.1990

Sales J.F., Pinto J.E.B.P., de Oliveira J.A., Botrel P.P., Silva F.G., Correa R.M., 2011: The germination of *Hyptis marrubioides* seeds under different temperatures and light conditions. – Acta Scientiarum. Agronomy, 33(4): 709–713. https://doi.org/10.4025/actasciagron.v33i4.4611

Sbai A., Ouhssine M., Chaouch A., Oubihi A., El Hartiti H., Rhaim N., Talbi F.Z., 2020. Chemical composition and antimicrobial activities of the essential oil of *Mentha rotundifolia.* – International Journal of Engineering Research & Technology, 9(8): 955–958.

Seladji M., Belmekki N., Bekhechi C., Bendimerad N., 2014: Antioxidant and antimicrobial activity of aqueous and methanolic extracts of *Mentha rotundifolia* L. from Algeria. – International Journal of Pharmaceutical Science Review and Research, 26(1): 228–234.

Starfinger U., Karrer G., 2016: A standard protocol for testing viability with the triphenyl tetrazolium chloride (TTC) test. – Julius-Kühn-Archiv, 455: 65–72. https://doi.org/10.5073/jka.2016.455.18

Walters C.L., Wheeler M., Grotenhuis J.M., 2005: Longevity of Seeds Stored in a Genebank: Species Characteristics. – Seed Science Research, 15(1): 1–20. https://doi.org/10.1079/SSR2004195

Walters C., Ballesteros D., Vertucci V.A., 2010: Structural mechanics of seed deterioration: standing the test of time. – Plant Science, 179: 565–573. https://doi.org/10.1016/j.plantsci.2010.06.016.

Yakoubi R., Benouchenne D., Bendjedid S., Megateli S., Sadok T.H., Gali L., Azri O., 2024: Chemical constituents and *in vitro* biological activities of *Mentha rotundifolia*'s essential oils extracted by ultrasound-assisted hydrodistillation compared to conventional hydrodistillation. – Biomass Conversion and Biorefinery, 15(4): 5267–5279. https://doi.org/10.1007/s13399-024-05336-4

Yahia I.B.H., Jaouadi R., Trimech R., Boussaid M., Zaouali Y., 2019: Variation of chemical composition and antioxidant activity of essential oils of *Mentha* × *rotundifolia* (L.) Huds. (Lamiaceae) collected from different bioclimatic areas of Tunisia. – Biochemical Systematics and Ecology, 84: 8–16. https://doi.org/10.1016/j.bse.2019.03.001

Zoz I.G., Litvinenko V.I., 1979: [On the division of the family Lamiaceae Juss. into natural groups]. – Botanical Journal, 64(7): 989–997.

CB https://orcid.org/0009-0005-4879-6882
FZB-M https://orcid.org/0000-0001-9000-4774
ZM https://orcid.org/0000-0002-1664-9424
AL https://orcid.org/0000-0003-1262-1464
KS https://orcid.org/0009-0008-2364-3187