Effect of alternating temperature and seeds storage duration on the germination of *Thymus satureioides*.

Ouahzizi B.[1], ElBouny H. [1], Sellam K. [1], Alem C. [1] and Homrani Bakali A. [2]

abdelmonaim.homranibakali@inra.ma

1: Laboratory of Bioactive, Health and Environment, Faculty of Sciences & Techniques, Moulay Ismail University, Errachidia, Morocco.

2: Regional Center of Agricultural Research of Errachidia, National Institute of Agricultural Research, Avenue Ennasr, BP 415 Rabat Principale, 10090 Rabat, Morocco.

Abstract

Thymus satureioides Cosson is an endemic chamaephyte to Morocco and Algeria. It belongs to the *Lamiaceae* family and commonly used in traditional medicine. Its over-exploitation threatens its sustainability in many ecosystems in Morocco. This study aims to explore the effect of environmental factors (temperature and storage) on the germination capacity of the seeds of this species in order to contribute to contribute to the rehabilitation of degraded ecosystems of *T. satureioides*. Accordingly, seeds collected from natural plant populations were subjected to six alternating temperatures (40/25, 35/20, 30/15, 20/10, 15/5 and 10/0 °C with a 14/10 hr. thermoperiod) and three storage duration (3 years, 5 years and 8 years) under ambient laboratory conditions. The results indicate that the alternating temperature and the seed storage duration have a significant effect on the germination parameters according to the test of Student Newman and Keuls (SNK). The highest germination percentage 93% was recorded for the alternating temperature of 15/05 °C, while high alternating temperature 40/25 °C strongly inhibited the seed germination. The 20/10 °C temperature regime was the best in terms of higher capacity and speed germination as indicated by the germination index (7.7 %/day for 20/10 °C versus 0.5 %/day for 10/0 °C). Furthermore, these results show that seed stored for longer duration delayed germination and decreased the germination capacity and rate. Seeds stored at room condition retain seeds viability up to 3 years. However, after 5 and 8 years of storing, the germination percentage was significantly reduced (75.0 and 64.5% respectively), which also suggests a reduction of seed viability.

Keywords: *Dwarf shrub*, Germination, Temperature, Storage, Longevity, Rehabilitation.

Influence des températures alternées et la durée de stockage sur la germination des semences de *Thymus satureioides*.

Résumé

Thymus satureioides Cosson est une chaméphyte endémique du Maroc et de l'Algérie. Elle appartient à la famille des Lamiacées et couramment utilisée en médecine traditionnelle. Sa surexploitation menace sa durabilité dans de nombreux écosystèmes au Maroc. Cette étude vise à tester l'effet des facteurs environnementaux (température et stockage) sur la capacité de germination des graines de cette espèce en vue de contribuer à la réhabilitation des écosystèmes dégradés de *T. satureioides*. Ainsi, les semences récoltées à partir de populations naturelles ont été soumises à six températures alternées (40/25, 35/20, 30/15, 20/10, 15/5 et 10/0 °C avec une thermopériode de 14/10 h) et trois durées de stockage (3, 5 et 8 ans) dans les conditions du laboratoire. Les résultats montrent que la température alternée et la durée de stockage des graines ont un effet significatif sur la germination (Student Newman et Keuls). Le taux de germination le plus élevé (93 %) est enregistré pour la température alternée de 15/5 °C. Alors qu'aucune germination n'est observée pour la température alternée de 40/25°C. Le régime de température 20/10 °C reste le meilleur en termes de capacité et de vitesse de germination comme indiqué par l'indice de germination (7,7 %/jour pour 20/10 °C contre 0,5 %/jour pour 10/0 °C). Ces résultats montrent également qu'une durée de conservation plus longue des graines, entraîne un retard de germination et diminue la capacité et la vitesse de cette dernière. Les graines stockées dans des conditions ambiantes conservent leur viabilité jusqu'au-delà de 3 ans. Cependant, après 5 et 8 ans de stockage, le taux de germination baisse significativement (75,0 et 64,5% respectivement) suggérant également une réduction de la longévité des semences.

Mots-clés : *Ligneux bas*, Germination, Température, Stockage, Longévité, Réhabilitation.

African & Mediterranean AGRICULTURAL JOURNAL AL AWAMIA

Ouahzizi B. et al. (2022). AFRIMED AJ –Al Awamia (135). p. 149-164

تأثير درجات الحرارة المتناوبة ومدة التخزين على إنبات بذور الزعيترة *Thymus satureioides*

إبراهيم وهزيزي، حمزة البوني، خالد سلام، شكيب عالم وعبد المنعم الحمراني البقالي

الملخص

يعتبر الزعتر البري أو الزعيترة *Thymus satureioides* Cosson من النباتات المعمرة والمتوطنة بالمغرب والجزائر. ينتمي إلى عائلة الشفويات ويستعمل لأغراض طبية. الإفراط في استغلاله يهدد استدامته في العديد من النظم البيئية في المغرب.

يهدف هذا البحث إلى استكشاف تأثير درجة الحرارة والتخزين على قدرة إنبات بذور هذا النوع والمساهمة في إعادة تأهيل النظم البيئية المتدهورة من *T. satureioides*. وفقًا لذلك، تعرضت البذور التي تم جمعها من مجموعات النباتات الطبيعية لست درجات حرارة متناوبة (25/40، 20/35، 15/30، 10/20، 5/15 و0/10 درجة مئوية مع فترة حرارية 8/14 ساعة) وثلاثة فترات التخزين (3 سنوات و5 سنوات و8 سنوات) في ظروف المختبر.

أظهرت النتائج أن درجة الحرارة المتناوبة ومدة تخزين البذور كان لهما تأثير مهم على معاملات الإنبات وفقًا لاختبار الإحصاء SNK. تم تسجيل أعلى نسبة إنبات 93٪ لدرجة الحرارة المتناوبة 5/15 درجة مئوية، بينما لم يسجل أي إنبات لدرجة الحرارة المتناوبة 25/40 درجة مئوية. كما أن نظام الحرارة 10/20 درجة مئوية هو الأفضل من حيث القدرة وسرعة الإنبات كما هو مبين في مؤشر الإنبات (7.7٪ / يوم لـ 10/20 ° C مقابل 0.5٪ / يوم لـ 0/10 ° C). أظهرت النتائج أيضًا أن تخزين البذور لمدة أطول أدى إلى تأخر الإنبات، كما أنه قلل من قدرة وسرعة الإنبات. تحتفظ البذور المخزنة في الظروف المحيطة بقدرتها على الإنبات لمدة تفوقً 3 سنوات. بعد 5 و8 سنوات من التخزين، تنخفض نسبة الإنبات بشكل مهم (75.0 و64.5 ٪ على التوالي) مما يشير إلى انخفاض عمر البذور.

الكلمات المفتاحية: شجيرة صغيرة، الإنبات، الحرارة، التخزين، طول العمر، إعادة تأهيل.

Introduction

Thymus satureioides Cosson is a dwarf shrub, spontaneous plant, endemic to Morocco and Algeria, adapted to hot and dry climate of the Mediterranean region. It is called *Azoukenni* or *Azokni* or *Tazoukennit* in Berber, and *Zaitra* or *Zaâtar* in Arabic. It is used as a spice in several foods and as a treatment for inflammatory diseases in traditional medicine (Bellakhdar et al., 1991). Due to the economic value of its essential oil and its use as a condiment by herbalists, it has become one of the most commercialized plants in Morocco.

Nevertheless, due to uncontrolled and excessive exploitation for medicinal purposes and the overgrazing, the production, density and the recovery of *T. satureioides* are reduced in many steppes of Morocco. This species is threatened with extinction in certain regions of Morocco. Therefore, it is necessary to restore the steppe rangelands based on *Thymus* (Ouaddich and Bouzoubaa, 2016). This would ensure its rational use, and its rehabilitation can be done in-situ or ex-situ using different techniques.

Germination is a complex process controlled by several factors, either environmental (availability of humidity, light intensity, and duration of temperature) or biological (species, size, viability, and dormancy of seeds) (Bewley and Black 1994; Steckel *et al.*, 2004). Information on the requirements and characteristics of seed germination is therefore crucial for seed dispersal and thus for rangeland restoration and rehabilitation.

Temperature is the most important factor affecting the maximum germination percentage and rate of germination (Phartyal *et al.,* 2003). Many studies have shown the effect of temperature on the germination of *Thymus* species (Nadjafi *et al.,* 2009; Abbad *et al.,* 2011; Tolyat *et al.,* 2014; Yadegari, 2018).

Alternating temperatures have been proposed to be more favorable for germination than constant temperatures because seeds are subject to frequent variations under natural conditions (Baskin and Baskin 2014).

Similarly, the storage period is the main factor affecting seed germination (Conversa and Elia, 2009). Storage at room temperature often leads to low seed germination, seed deterioration and loss of viability, which are natural phenomena during storage (Nasreen *et al.,* 2000).

In Morocco, no studies have been conducted on the germination of *T. satureioides.* Thus, the control of the germination conditions would contribute to the development of propagation and production methods for this plant and, ultimately, to the rehabilitation of degraded steppes dominated by *T. satureioides*.

Consequently, the main hypotheses of this work are (i) *T. satureioides* could germinate in wide range of temperatures and (ii) seeds of this species could maintain their viability and germinability when stored for mid and long periods of time.

To verify these hypotheses, we have set, as objective to test the effect of different alternating temperature regimes and seed storage duration on the germination response of *T. satureioides* seeds.

Material and Methods

Seed collection and storage

Fresh seeds of *T. satureioides* were collected from the region of Ait Hani in 2020, located in Tinghir province in the southeast of Morocco (31.75 North, 5.48 West, altitude 1992m). Old seeds were harvested in 2012, 2015 and 2017 from Experimental Station of Errachdia (31.92 North, 4.44 West, altitude 1040m). After harvest, seeds were stored dry in paper bags under laboratory room conditions. Temperature was ranging between 12 and 22 °C before the beginning of the germination test. In the laboratory, the inflorescences were shaken manually to detach the seeds, and then the selection of the seeds was done using a stereo-microscope (45x) to detect the good seeds.

Germination Experiments

Germination tests started at the beginning of September 2020. The study was carried out under ambient laboratory conditions and inside a controlled incubator during 8 months (till April 2021) in the Experimental Station of Errachidia (INRA-Errachidia).

First, seeds of *T. satureioides* were selected and disinfected with 5% diluted hypochlorous acid for 5 minutes. Then, they were washed thoroughly 3 times with distilled water. Finally, they were placed on moistened filter papers in 15 cm diameter Petri dishes.

Four dishes per treatment (50 seeds per dish) were placed in a controlled incubator according to a randomized complete block design. The photoperiod was 14h of light and of 10h dark. Seeds were considered germinated when the rootlets exceeded 1 mm in length. Petri dishes are moistened with the same amount of water every two to three days depending on the temperature regime. This process was carried out for 30 days for each treatment.

Seed temperature treatment

Five temperature regimes were defined to simulate the average day-night temperature of each season: R1: (10/0 °C), R2: (15/05 °C), R3: (20/10 °C), R4: (30/15 °C), R5: (35/20 °C) and R6: (40/25°C). For each temperature, the petri dishes were arranged in a randomized complete block design.

Seed storage treatment

Mature seeds (S1=8 years, S2=5 years, S3=3 years, and S4=control) were harvested in 2012, 2015, 2017, and 2020 respectively. Each year, samples were taken from individuals (plants) from the same area. The seeds of different storage duration were incubated in a randomized complete block design at the alternating temperature of 15/05 °C.

Germination parameters

Final germination percentage or germination capacity is the most important and used parameter, but this parameter alone is not sufficient for reporting results because it does not give an idea on the time, spread, speed, uniformity and synchrony of germination (one lot of seed may have germinated well before the other, but both attained the same final germination percentage). These parameters are important for ecologists because it is possible to predict the degree of successful of a species based on the capacity of harvest seed to spread germination through the time (Ranal and Santana, 2006).

Final Germination Percentage (FGP), Mean Germination Time (MGT), Germination Index (GI) or speed of germination, part germination time ((T_{25}, T_{50}, T_{75}, T_{90}) and Coefficient of Variation of the germination Time (CVt) were determined for each seed treatment to estimate the germination capacity and rate using the following formulas:

$$\text{FPG (\%)} = \frac{N_g}{N_t} \times 100$$ (ISTA, 2015).

Where, N_g is the number of germinated seeds and N_t is the total number of seeds.

$$\text{MGT (day)} = \sum_{i=1}^{k} \frac{NiTi}{\sum_{i=1}^{k} N_i}$$ (Ranal and Santana, 2006).

Where, T_i is the time from the start of the experiment to the i^{th} interval, N_i is the number of germinated seeds in the i^{th} time interval, and k is the total number of time intervals.

$$\text{GI (\%/day)} = \sum_{i=1}^{k} \frac{Ni}{Ti}$$ (Ranal and Santana, 2006).

Where, T_i is the time from the start of the experiment to the i^{th} interval, N_i is the number of germinated seeds in the i^{th}, time interval, and k is the total number of time intervals.

$$CV_t = \sqrt{\frac{St^2}{MGT}},$$ (Ranal and Santana, 2006).

Where, St^2 is the variance of germination time and MGT is the mean germination time.

Finally, the part time of germination Tx, where x is the time to reach x% of final germination, for example the formula of the median germination time (T_{50}, time to reach 50% of final germination) is as follow:

$$T_{50}(day) = Ti + \frac{(\frac{N+1}{2} - N_i)(T_j - T_i)}{N_j - N_i}$$ (Coolbear *et al.,* 1984).

N is the final number of germinated seeds, and Ni and Nj are the total number of seeds germinated in adjacent counts at time Ti and Tj respectively, when Ni < N+1/2 < Nj.

Data Analysis

Data were analyzed using SPSS 18 (IBM Corporation, 2010). The data were processed using one-way ANOVA analysis of variance for the test of temperature regimes and the seed storage duration. Means were compared using the Student Newman and Keuls test (SNK), and significance was determined at 95% confidence limits.

Results

Effect of alternating temperature on seed germination

The alternating temperature regimes had a significant effect according to SNK test on Final Germination Percentage, Mean Germination Time, Germination Index, and Coefficient of Variation of germination time (Tab. 1).

Germination occurred across a relatively wide range of temperatures, the highest final germination percentage was registered for the seeds incubated at three regimes (15-05°C (93%), 20-10°C (85%) and 30-15 °C (85.5%). On the other hand, germination ceased at the temperature regime 40-25 °C and was very low for the temperature regime 10-0 °C. Moreover, high and low temperature significantly delayed seed germination (Fig. 1). Indeed, we need 15 days at the temperature regime 10-0 °C versus 3 days at the temperature regime 20-10 °C to reach 10% of the final germination (Fig. 1). Thus, the most rapid germination was registered on the temperature regime 20-10 °C as shown by the table 1. Likewise, MGT that is a measurement of the average length of time required for maximum seed germination (Bewley and Black, 1994) showed a slower germination process for the temperature regime of 10/0 °C. On the other hand, the temperature regimes of 20/10 °C and 30/15 °C exhibited a faster process of germination.

Additionally, the germination index that is mixed parameter, which reflects the percentage of germination per day and germination speed, so higher GI values, indicates higher and faster germination. The result show also that the regimes of temperature 20/10 °C and 35/20 °C were the fastest ones while the temperature regime 10/0°C was the lowest one (Tab. 1).

The germination also expressed by the coefficient of variation of the germination time (CVt) precisely describes the uniformity of germination during the whole period of germination. A higher CVt was registered for the temperature regime of 30/15°C (69.3 ± 5), indicating more irregular germination compared to other regimes. This coefficient (CVt) is significantly influenced by the increase of temperature regime resulting in more irregular germination process.

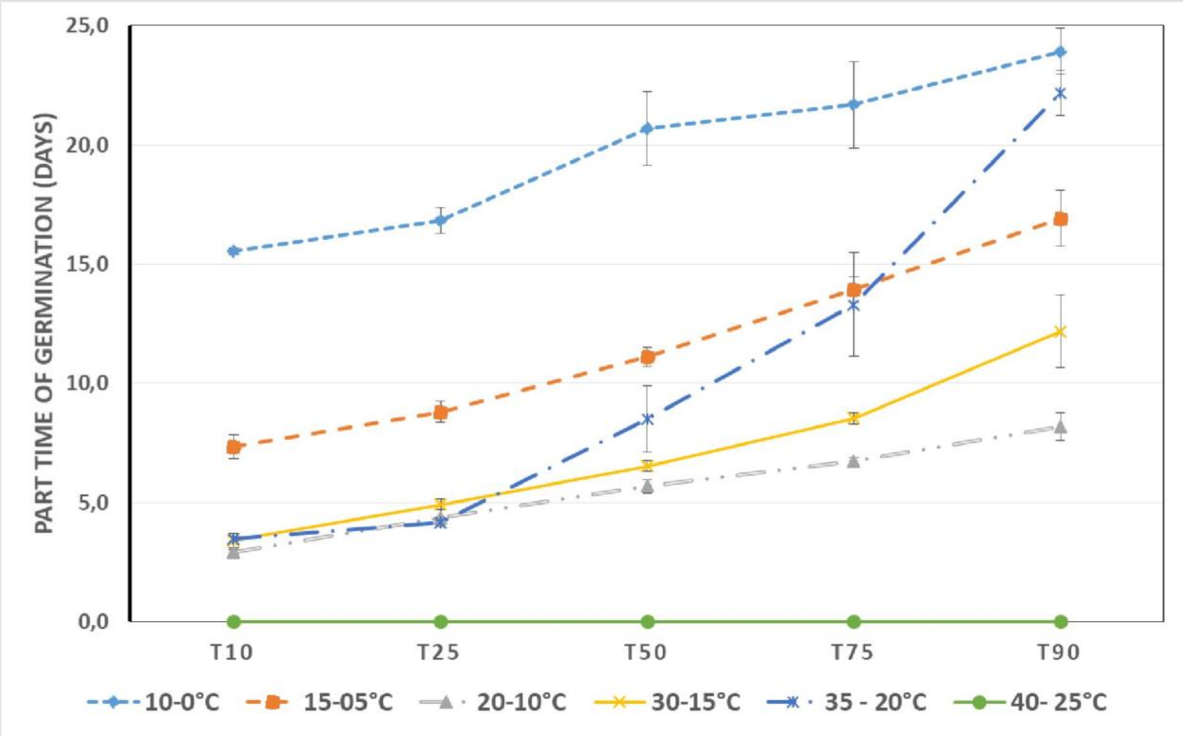

Figure 1. Effect of temperature regime on the germination time of a given percentage (from 10% to 90%). Bars represent Standard Error (±SE).

Table 1. Effect of temperature on germination parameters of *T. satureioides* in different temperature regimes.

Temperatures	FGP (%)	MGT (day)	GI (%/day)	CV_t
10-0°C	19.0 ± 05.3^{b}	20.1 ± 2.0^{d}	0.5 ± 0.2^{a}	15.6 ± 2.9^{b}
15-5°C	93.0 ± 03.5^{d}	12.1 ± 1.0^{c}	4.4 ± 0.5^{b}	31.8 ± 3.9^{c}
20-10°C	85.0 ± 17.9^{d}	06.2 ± 0.4^{b}	7.7 ± 1.2^{c}	34.1 ± 5.5^{c}
30-15°C	85.5 ± 13.4^{d}	07.9 ± 0.9^{b}	6.7 ± 1.0^{c}	52.5 ± 7.6^{d}
35-20°C	60.0 ± 16.2^{c}	10.7 ± 1.4^{c}	4.3 ± 1.3^{b}	69.3 ± 5.0^{e}
40-25°C	00.0 ± 00.0^{a}	00.0 ± 0.0^{a}	0.0 ± 0.0^{a}	00.0 ± 0.0^{a}

FGP: final germination percentage; MGT: mean germination time (day); GI: germination index (%/day); CV_t: coefficient of variation of the germination time. The means ± standard deviation with different letters are significantly different according to the SNK test at p<0.05.

Effect of seed storage duration

The results (Tab. 2) indicate that seed storage duration had a significant effect on germination of *T. satureioides*. Table 2 reveals that fresh seeds (<1 years, control) had a higher FGP (93%) but not significantly different from those stored for 3 years (91.5%). After that period, FGPs were significantly lower with long storage. Lowest significant FGPs are shown in seeds stored for 5 and 8 years (78 and 64.5% respectively). This indicates that seeds start to lose their viability after 5 years in room condition of Errachidia.

The figure 2 show that the storing period affects slightly the kinetic of germination. In fact, at the beginning, fresh seeds (< 1 year) have germinated slowly compared to other storing periods, but at the end, fresh seeds and those stored for 3 years and 5 years germinated faster than seeds stored for 8 years. This result is also approved by MGT (Tab. 2). The GI show that the duration of seed storage negatively affects the capacity and speed of germination. Nevertheless, the CVt showed an opposite positive effect of the storage duration by improving the uniformity of germination in comparison with the fresh seeds (Tab. 2).

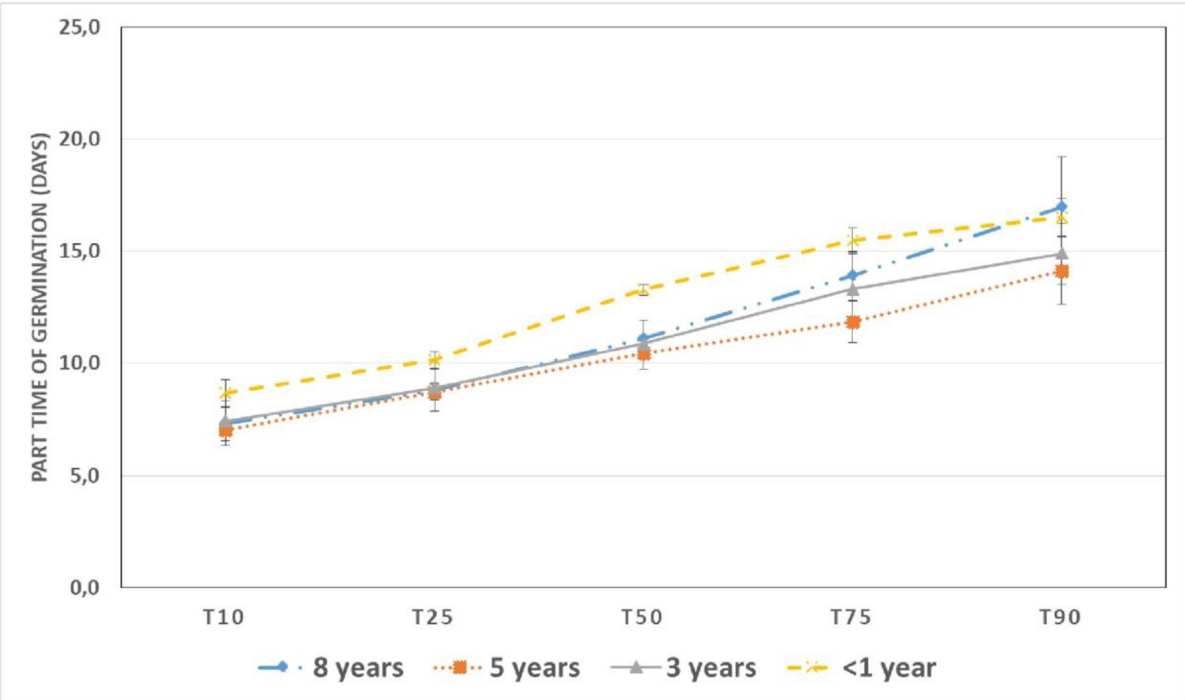

Figure 2. Effect of storage duration on the germination time of a given percentage (from 10% to 90%). Bars represent Standard error (±SE).

Table 2. Effect of storage duration on germination parameters of *T. satueiroides*.

Storage duration	FGP (%)	MGT (day)	GI (%/day)	CVt
< 1 year (control)	93.0±3.5[c]	12.1±1.0[a]	4.3±0.5[c]	31.8±3.9[a]
3 years	91.5±4.1[c]	11.0±0.5[a]	4.5±0.2[c]	25.3±4.1[ab]
5 years	78.0±5.7[b]	11.6±0.7[a]	3.6±0.3[b]	26.8±4.0[ab]
8 years	64.5±7.5[a]	13.4±0.5[b]	2.6±0.4[a]	24.3±1.3[b]

FGP: final germination percentage; MGT: mean germination time; GI: index of germination velocity; CVt: coefficient of variation of the germination time. The means ± standard deviation with different letters are significantly different according to the SNK test at p<0.05.

Discussion

Effect of alternating temperature on seed germination

The study of the effect of temperature on seeds germination of *T. satureioides* revealed that this factor is highly important, especially for higher and lower temperatures. The establishment of plants in arid regions is often limited by the temperature even though the conditions of humidity are favorable. In general, higher proportion of germinated seeds was recorded for three temperatures regimes (15-05°C, 20-10°C and 30-15°C) which could indicate a wider plant thermo-tolerance. Increasing and decreasing temperatures affect not only seed germination, but also affect the germination process. Sometimes, high or low temperatures also result in secondary dormancy, known as thermos-dormancy (Huo *et al.*, 2013). Previous investigations on *Thymus vulgaris* and *Thymus daenensis* showed quite similar results as the highest final germination percentage was registered at a temperature regime of 25/15 °C (Yadegari, 2018). Furthermore, the maximum final germination percentage for *Thymus maroccanus* was recorded at temperature of 15 °C (Abbad *et al.,* 2011). Nadjafi (2009) reported that the optimal temperature for *T. daenensis subsp. daenensis* seeds was 20°C. Thanos *et al.* (1995) defined this temperature range (15 to 20°C) as the optimum temperature for germination of many thyme species. In addition, the optimal temperature of seed germination differs within the same family, genus and even for the same species (Baskin and Baskin, 1998).

The results demonstrate that the germination of *T. satureioides* is quite good in several temperatures (15-05°C, 20-10°C and 30-15°C) which could justify their great elasticity and distribution in the Moroccan territories. Such information is useful to identify suitable geographical regions for plant establishment. In addition, the ability of this plant to germinate in almost all the temperatures regime (except 40-25 °C) indicates the potential of establishment in areas with different thermal conditions. Alternating temperature regime has been reported to be one of the most important methods of breaking seed dormancy, and is often more favorable to germination than constant temperatures (Baskin and Baskin, 2004; Yogeesha *et al.,* 2006).

Effect of seed storage duration

The present investigation, testing seeds for mid and long-term storage, confirms that the storage duration significantly affects the seed germination capacity as also indicated by Montaño-Arias (2021). Nevertheless, this attribute depends on the species, Wang *et al.* (2010) reported an increase in seed germination with the increase of seed storage time for other species. The viability of seeds of *T. satureioides* at room condition could last at least 8 years, which indicate a greater longevity of seeds of this species. However, this ability to germinate is decreasing over time, requiring always the utilization of fresh seeds or seeds that not exceed 3 years when stocked at room conditions. Unfavorable storage environmental conditions especially the increase of temperature and relative humidity might deteriorate the seeds resulting the decrease of seed vigor and germination (Mariucci *et al.*, 2018). The seeds of most species of plants could be stored for long periods in dry cold conditions and then germinated. The potential of seeds to stay dormant is highly useful for ex-situ conservation as it allowed by freezing. Ex-situ preservation requires controlling the moisture content of seeds and keeping them at low temperatures (FAO/IPGRI, 1994). Indeed, in the Mediterranean flora, especially vascular flora, seed storage may be good tool for the restoration and conservation of a number of rare and endangered species (Demonty *et al.,* 2014). Moreover, the oxidation of seeds by reactive oxygen species is the major responsible factor for much of the damage that is done during storage (Bailly, 2004; Kranner *et al.,* 2010). Since seeds enter dormancy in seed bank storage, ageing processes are slowed, and variability in seed longevity between species can be due to the properties of the glassy state (Walters *et al.,* 2010).

Conclusion and additional research

This study demonstrates that temperature is a determining factor for the germination capacity and process of *T. satureioides.* The results suggest that *this species* germinates quite very well over a wide range of moderate temperatures (15/05 °C, 20/10 °C, and 35/20 °C) indicating a wide thermo-tolerance plasticity. Additionally, the regime 20/10 °C was the best one in achieving germination in short time. High temperatures (40-25 °C) strongly inhibit the germination of *T. satureioides.* Storage duration under room conditions negatively affects the ability and speed of seeds to germinate. After 8 years of storage at room conditions, seed are still alive but the germination capacity was significantly decreased to 64.5%.

This study provides several information on the germination and viability of *T. satureioides* seeds, these data certainly serve as part of the domestication and the preservation of this endangered taxon. However, several complementary studies could be carried out in order to control all steps of storing seeds, multiplication and establishment of the species. As perspectives, more investigation should be done for determining the factors of the seed deterioration during storage (is it temperature dependent?). The study of the effects of salinity and drought stress on the germination will permit to identify more suitable geographical regions for plant establishment. In addition, the test of the vigor and the growing conditions of seedlings according to different temperatures should be accomplished. As a final point, the definition of geographical regions and the optimal conditions for transplanting seedlings should be explored.

Funding

This work was supported by CNRST and INRA-Errachidia.

References

Abbad A., Belaqziz B., Bekkouche K. and Markouk M. (2011). Influence of temperature and water potential on laboratory germination of two Moroccan endemic thymes: *Thymus maroccanus* Ball. and *Thymus broussonetii* Boiss. *Afr. J. Agric. Res*. 6. p. 4740-4745.

Baskin J.M. and Baskin C.C. (1998). Seeds: Ecology, Biogeography, and Evolution of Dormancy and Germination. Academic Press, San Diego, CA, USA.

Baskin J.M. and Baskin C.C. (2004). A classification system for seed dormancy. *Seed Sci. Res*.14. p. 1-16.

Baskin C.C. and Baskin J.M. (2014). Seeds: Ecology, Biogeography, and Evolution of Dormancy and Germination. Academic Press, San Diego, CA, USA.

Bailly C. (2004). Active oxygen species and antioxidants in seed biology. *Seed Sci. Res*.14. p. 93-107.

Bellakhdar J., Claisse R., Fleurentin J. and Younos C. (1991). Repertory of standard herbal drugs in the Moroccan pharmacopoeia. *J. Ethnopharmacol.* 35. p. 123-143.

Bewley D.J. and Black M. (1994). Seeds: Physiology of Development and Germination. Plenum Press, New York.

Conversa G. & Elia A. (2009). Effect of seed age, stratification, and soaking on germination of wild asparagus (*Asparagus acutifolius* L.). *Scientia horticulturae*, 119(3). p. 241-245.

Coolbear P., Francis A., and Grierson D. (1984). The effect of low temperature pre-sowing treatment on the germination performance and membrane integrity of artificially aged tomato seeds. *Journal of Experimental Botany* 35. p. 1609-1617.

Demonty E., Dixon L. and Fort N. (2014). Collection de graines : Conservation et germination des plantes patrimoniales de Provence-Alpes-Côte d'Azur. Conservatoire botanique national alpin, Gap; Conservatoire botanique national méditerranéen de Porquerolles, Hyères, 152p.

FAO/IPGRI. (1994). Gene bank standards. Rome: Food and Agriculture Organization of the United Nations and International Plant Genetic Resources Institute.

IBM Corporation (2010). SPSS Statistics for Windows. Version 18.0. IBM Corp., Armonk, NY.

ISTA. (2015). Chapter 5: The germination test. International Rules for Seed Testing. International Seed Testing Association, Zurich, Switzerland. p. 5-56.

Kranner I., Minibayeva F., Beckett R.P. and Seal C.E. (2010). What is stress? Concepts, definitions and applications in seed science. *New Phytol*. 188. p. 655-673.

Huo H. Dahal P. Kunusoth K. McCallum C. Bradford K. (2013) Expression of 9-cis-EPOXYCAROTENOID DIOXYGENASE4 is essential for thermoinhibition of lettuce seed germination but not for seed development or stress tolerance. *Plant Cell.* 25. p. 884–900.

Mariucci G.E.G., Suzukawa A.K., Braccini A.L., Scapim C.A., Lima L.H. da S., Angelotti P. and Marteli D.C.V. (2018). Physiological potential of maize seeds submitted to different treatments and storage periods. *Journal of Seed Science*, 40(1). p. 60-66.

Montaño-Arias S. A., Zavaleta-Mancera, H. A., Camargo-Ricalde S. L. and Grether R. (2021). Effect of seed age on germinaton, seedling survival and growth of *Mimosa luisana* (Leguminosae). *Trees*. 35(1), p. 231-239.

Nadjafi F., Tabrizi L., Shabahang J. and Mahdavi Damghani A.M. (2009). Cardinal germination temperatures of some medicinal plant species. *Seed Sci. Technol.* 31. p. 156-163.

Nasreen S., Khan B.R. and Mohmad A.S. (2000). The effect of storage temperature, storage period and seed moisture content on seed viability of soya bean, *Pak. J. Biol. Sci*. 12. p. 2003-2004.

Ouaddich B. and Bouzoubaa Z. (2016). Elaboration d'une stratégie de promotion de la chaine de valeur Thym dans le bassin versant d'Aoulouz et dans la forêt de Mesguina Région Souss-Massa. In « Programme d'Adaptation au Changement Climatique et Valorisation de la Biodiversité/ Protocole de Nagoya (ACCN) ». 75p.

Phartyal S.S., Thapliyal R.C., Nayal J.S. and Joshi G. (2003). Seed storage physiology of Himalayan elm (Ulmuswallichiana): an endangered tree species of tropical highlands. *Seed Sci. Technol*. 31 p. 651-658.

Ranal M.A. and Santana D.G. (2006). How and why to measure the germination process? *Rev. Bras. Bot*. 29. p. 1-11.

Steckel L.E., Sprague C., Stoller E.W. and Wax L. (2004). Temperature effects on germination of nine *Amaranthus* species. *Weed Sci.* 52. p. 217-221.

Thanos C.A., Costas C., Kadis C.C. and Skarou F. (1995). Ecophysiology of germination in the aromatic plants thyme, savory and oregano (*Labiatae*). *Seed Sci. Res*. 5. p. 161-170.

Tolyat M.A., Tavakkol A.R., Jahansoz M.R., Nadjafi F. and Naghdibadi H.A. (2014). Determination of cardinal germination temperatures of two ecotypes of *Thymus daenensis subsp. Daenensis*. *Seed Sci. Technol.* 42. p. 28-35.

Walters C., Ballesteros D. and Vertucci V.A. (2010). Structural mechanics of seed deterioration: Standing the test of time. *Plant Sci.* 6. p. 565-573.

Wang J. H., Baskin C. C., Chen W., and Du G. Z. (2010) Variation in seed germination between populations of five sub-alpine woody species from eastern Qinghai-Tibet Plateau following dry storage at low temperatures. *Ecological Research*. 25(1). p. 195-203.

Yadegari M. (2018). Effects of the environmental characters on germination properties of seeds of *Thymus daenensis* and *T. vulgaris*. *J. Agric. Sci*. *Belgrade* 63 (4). p. 343-354.

Yogeesha H.S., Upreti K.K., Padmini K., Bhanuprakash K. and Murti G.S.R. (2006). Mechanism of seed dormancy in eggplant (*Solanumm elongena L.).* Seed Sci. Technol. 34. p. 319-325.