

Seed germination behavior of *Teucrium santae* Quézel & Simonneau: a vulnerable and endemic Lamiaceae (Northwest Algeria)

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Abstract

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As part of the ex-situ conservation of the rare plant species *Teucrium santae* (Lamiaceae), which is native to Algeria flora, we conducted this study under controlled conditions to determine the optimal circumstances for the germination of its seeds in terms of light, temperature, and water stress. The seeds showed a double dormancy that could be overcome using scarification with sulfuric acid combined with soaking in Gibberellic acid at 1,500 ppm. The seed's photosensitivity was tested afterward and found to be indifferent. The highest final germination percentage (75%) was obtained at a temperature of 20 °C. The temperature has no significant effect on the velocity coefficient, unlike the initial germination day and the mean germination time which decrease with increasing temperature. On the opposite of the velocity coefficient, water stress results in a tremendous depressive effect on the final germination percentage, initial germination time and mean germination time. The value of –1.2 MPa constitutes the water potential beyond which germination becomes impossible.

Keywords

conservation, germination, temperature, *Teucrium santae*, viability, water stress

Introduction

The Mediterranean region is considered one of world's most exceptional biodiversity center (MEDAIL, 2019). The IUCN lists this region as a conservation priority due to its high levels of endemism (IUCN, 2022). The Mediterranean Basin, as a center of biodiversity, is the second largest in the world and ranks third in terms of plant species diversity (COWLING et al., 2005). This basin is home to no less than 25,000 species of vascular plants, of which about 5,500 are endemic (ALVARADO-LOPEZ and FARRIS, 2022). However, several factors threaten this floristic diversity such as habitat fragmentation, climate change, pol-

lution, drought, introduction of invasive alien species and overexploitation which are responsible for the decline of Mediterranean biodiversity (UNDERWOOD et al., 2009). In this context, more than 25% of the 6,000 Mediterranean species (plants and animal species) that the IUCN has assessed since 2006 are in danger of extinction.

Cold and humid winters followed by hot and dry summers characterize the Mediterranean climate and the North African region in particular (TASSIN, 2012). The current annual average temperature has increased by about 1.5 °C compared to that recorded during the pre-industrial period (1880–1899). The trend, which is 0.03 °C per year (CRAMER et al., 2018), will inevitably influence climate

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stability and water resources accordingly.

Temperature influences most plant processes like photosynthesis, transpiration, respiration, germination, and flowering (KOGER et al., 2004). The rise in temperature favors evaporation processes, which consequently lead to the aridity of the soils (KIDRON and KRONENFELD, 2015). Water stress occurs when the ambient temperature is high, and the soil moisture and relative air humidity are low (LIPIEC et al., 2013). Water stress strongly affects seed germination, but the intensity of the response depends on the species (LI et al., 2013).

Due to its geographical location and its pedoclimatic diversity, the overall Algerian biodiversity has about 4,185 species, (of which 3,152 are spermatophytes) according to the report of the Algerian Ministry of Spatial Planning and Environment (2015), 1,734 of them are rare. Of these plant species, DOBIGNARD and CHATELAIN (2010–2013) show the number of endemic species to be around 290, MEDDOUR and JUR (2023) reported that 248 of them are vascular plants.

The Tessala mountains, located in Algeria's north-west, are one of the several reliefs and mountain massifs

that form the country. They are known for their significant floristic richness which has been the subject of numerous studies and surveys (BOUZIDI et al., 2012; BOUTERFAS et al., 2013; FERTOUT et al., 2016). Nevertheless, the impact of anthropozoogenic action and global warming have a negative influence on this specific richness, which means that many species are now threatened in their natural habitats (SAIDI et al., 2016; CHERIFI et al., 2021).

In the context of endangered species conservation, we have undertaken a study on a phytotaxa characterizing the floristic composition of the Tessala Mountains, which is *Teucrium santae* (Quézel & Simonneau ex Greuter & Burdet, 1985), this species belonging to the Lamiaceae family is distributed mainly in North-West Africa, and is endemic to Algeria (EL OUALIDI et al., 2012). In addition, it is described as extremely rare by QUÉZEL and SANTA (1962–1963), and is classified among the vulnerable species of Algeria (Official Journal of the Algerian Republic N° 3 of January 18, 2012; MAROUF, 2021).

Species of the genus *Teucrium* have been used since ancient times as traditional medicine and to prepare tea with choleric and antiseptic properties (ROEDE et al.,

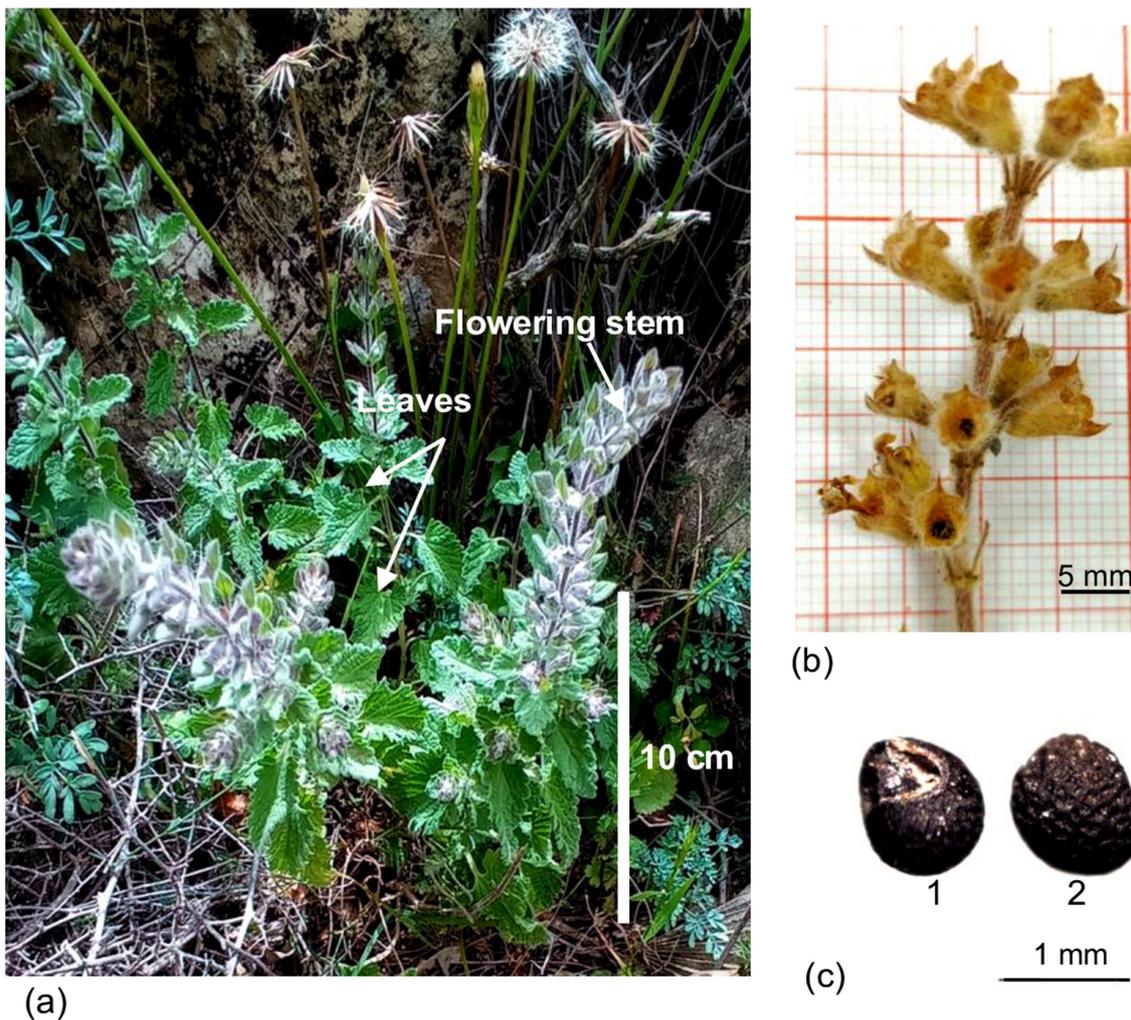


Fig. 1. *Teucriumsantae*. (a) The plant *T. santae* in natural habitat; (b) Fertilized flowers; (c) Seeds (Gr ×30); 1, ventral side; 2, dorsal side. Photo by Meriem Bentekhici and Zoheir Mehdadi.

2010). Additionally, they have been used as diuretics, diaphoretics, antiseptics, antipyretics, antispasmodics, antiulcers, antirheumatics, antibacterials, antioxidants, hypoglycemics and antiappetizers for many years (RUITERS et al., 2016). These species are known to be rich in phenolic compounds with high biological activity (YIN et al., 2009).

The study has been undertaken to advance knowledge of this species germination properties. It is crucial to determine whether the seeds are dormant or not, and if so, what type of dormancy is affecting them and what pretreatments are permitting its release, in order to facilitate the production of new plants by sowing to reinforce the natural populations, therefore, enable further research and studies concerning their physical and chemical features. As part of the *ex-situ* conservation of this species, our work consists of evaluating the viability of its seeds, highlighting the physico-chemical pre-treatments allowing to break their dormancy, and studying their germination behavior by emphasizing the optimal conditions for germination in terms of light, temperature and water stress.

Materials and methods

Harvest location, description and preparation of seeds

The mature seeds of *Teucrium santae* were collected on July 30, 2021 in Tessala Mountain (located in northwest Algeria). The geographical coordinates of the collection site are: latitude: 35°16.089'N, longitude: 000°46.807'W, altitude: 886 m.

The seeds were stored in paper bags at ambient laboratory temperature. For germination tests, we only sorted healthy and intact seeds which were then sterilized in 1% sodium hypochlorite for 10 minutes and then rinsed with distilled water to remove traces of chloride. Each germination test involved 80 seeds distributed over four batches of 20 seeds (four replications).

The *T. santae* seeds are dark brown, ovoid to kidney-shaped, with a hard, reticulate seed coat. The average length and width of 10 seeds are respectively: 1.41 ± 0.07 and 1.26 ± 0.11 mm (Fig. 1).

Preliminary germination tests

Due to the absence of studies about the germination behavior of *T. santae* seeds and in order to verify their viability, we carried out preliminary germination tests in an incubator type Memmert IPP 200 at a constant humidity and a constant temperature of 20 °C, with a photoperiod of (14h light/10h darkness) and/or continuous darkness. The experiments were conducted in May, when daylight lasts 14 hours (TIME AND DATE, 2023). The seeds were exposed to the natural photoperiod by keeping the exterior door of the incubator open to allow the penetration of light via the inner transparent door which was closed. Germination assays were undertaken in Petri dishes of 90 mm diameter

and 14.2 mm height lined with a double layer of standard filter paper moistened with distilled water.

This preliminary test revealed that *T. santae* seeds are unable to germinate, which is what prompted us to test their viability using the Tetrazolium colorimetric test.

Seed's viability testing

Seed viability was assessed by the 2,3,5-Triphenyltetrazolium chloride colorimetric test (FERRADOUS et al., 2017). This technique comprehends soaking the seeds in distilled water for 24 hours at 30 °C before cutting them longitudinally into two equal parts passing through the embryo, and incubating them afterward for 24 hours at 30 °C in a solution of 2,3,5- Triphenyltetrazolium chloride 1%. Viable seeds are coloured red, in contrast with the non-viable ones which remain unstained (STARFINGER and KARRER, 2016).

Dormancy breaking pre-treatments

The seed's high viability rate (see results section) indicates that they are dormant.

In order to dispose of this inability to germinate and to release this dormancy, different sets of seeds were subjected to several physico-chemical pre-treatments and are then germinated at 20 °C in continuous darkness. The pre-treatments applied to each group of seeds are:

- Soaking in distilled water for 72 hours (ÖNOL and YILDERIM, 2021);
- Chemical scarification by sulfuric acid (96%) for 5 minutes (PINTO et al., 2021);
- Soaking in gibberellic acid (GA₃) 1,500 ppm for 72 hours (ÖNOL and YILDERIM, 2021);
- Chemical scarification by sulfuric acid (96%) for 5 minutes associated with soaking in GA₃ 1,500 ppm for 72 hours (FARAHANI and HAJIBARAT, 2014).

Of all these pre-treatments, solely the seeds pre-treated with chemical scarification with sulfuric acid (96%) for 5 minutes, followed by soaking in GA₃ 1,500 ppm for 72 hours, could germinate.

To this end, the effects of light, temperature, and water stress experiments were applied to seeds that were previously subjected to this pre-treatment.

Effect of temperature and water stress on germination

The germination tests were carried out in Petri dishes lined with filter paper soaked in distilled water, in continuous darkness, in an incubator regulated at different constant temperatures: 5, 10, 15, 20, 25, 30, 35, and 40 °C.

The effect of water stress on germination was conducted at 20 °C under different water potentials obtained from different concentrations of polyethylene glycol (PEG₆₀₀₀) (MICHEL and KAUFMANN, 1973). The water potentials tested are 0 (control), -0.2, -0.4, -0.6, -0.8, -1, and -1.2 MPa. These germination tests were performed in Petri dishes, containing filter paper moistened with the different PEG solutions.

Table 1. Effect of light and dark on the FGP, VC, IGD and MGT of *Teucrium santae* seeds

Photoperiod	FGP (%)	VC (%)	IGD (days)	MGT (days)
Light 14h / dark 10h	75 ± 12.90	29.00 ± 10.48	1.25 ± 0.50	3.68 ± 1.42
Continuous darkness	75 ± 10.00	25.75 ± 11.70	1.75 ± 0.50	4.25 ± 1.70
F value	0 ^{ns}	0.171 ^{ns}	2 ^{ns}	0.268 ^{ns}

Mean value ± Standard deviation. FGP, final germination percentage; VC, velocity coefficient; IGD, initial germination day; MGT, mean germination time; F, Snedecor Fisher variable; ns, non significant at p > 0.05.

Expression of results and statistical analyses

For each germination test, the number of germinated seeds is recorded every 24 hours. A seed is considered germinated when the radicle breaks through the seed coat (SONG and CHOI, 2019).

The germination kinetics is represented by curves indicating the evolution of the cumulative germination percentage as function of time. Based on these curves, we determined the final germination percentage (FGP) and the initial germination day (IGD). The velocity coefficient, or germination speed (VC), was also evaluated.

The FGP of the seeds was calculated using the following formula:

$$FGP (\%) = \frac{\text{Number of germinated seeds}}{\text{Total number of seeds}} \times 100.$$

The VC was calculated by the following formula given by MAGUIRE (1962):

$$VC = \frac{N1+N2+N3.....Nn}{N1t1+N2t2+N3t3.....NnTn} \times 100,$$

where N1 is the number of seeds germinated at first day (t1); N2 is number of seeds germinated at the second day (t2);NnTn.

The MGT was calculated using AL-MUDARIS (1998) formula:

$$MGT (\%) = \frac{1}{VC} \times 100.$$

The effect of light, darkness, temperature and wa-

ter stress on germination parameters was verified by a one-way analysis of variance ANOVA using IBM SPSS software version 22. The pairwise comparison of the means was carried out by the Tukey test.

The regression coefficient (R²) was calculated to emphasize the existing relationship between the water potential and the germination parameters. This coefficient indicates to what extent the value of the dependent variable (germination parameters) varies with the variation of the independent one (water stress). The greater the correlation, the closer the R² is to 1, and vice versa (OZER, 1985).

Results

Seed's viability and effect of physico-chemical pretreatments on breaking their dormancy

The first germination trials showed that untreated *T. santae* seeds are dormant, however, the colorimetric test with 2,3,5-Triphenyltetrazolium chloride revealed a viability rate of 75%. Only the pretreatments below could break seed dormancy:

- Pre-soaking in GA3 1,500 ppm for 72h (FGP = 14%);
- Chemical scarification with sulfuric acid for 5 minutes associated with soaking in GA3 1,500 ppm for 72 hours (FGP = 75%).

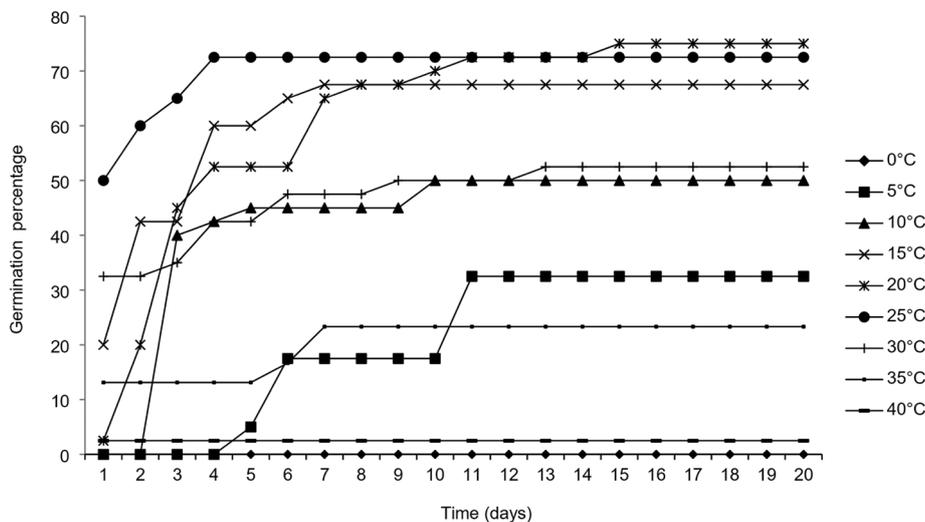


Fig. 2. Germination kinetic curves of *Teucrium santae* seeds at different temperatures.

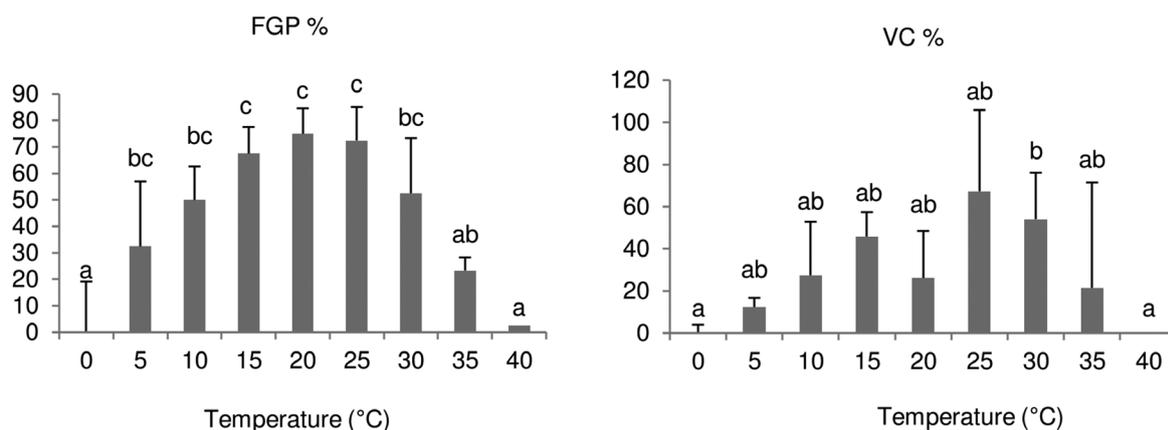


Fig. 3. Variation of FGP and VC of *Teucrium santae* seeds as a function of temperatures. The different letters indicate a significant difference between the averages by the Tukey test ($p > 0.05$).

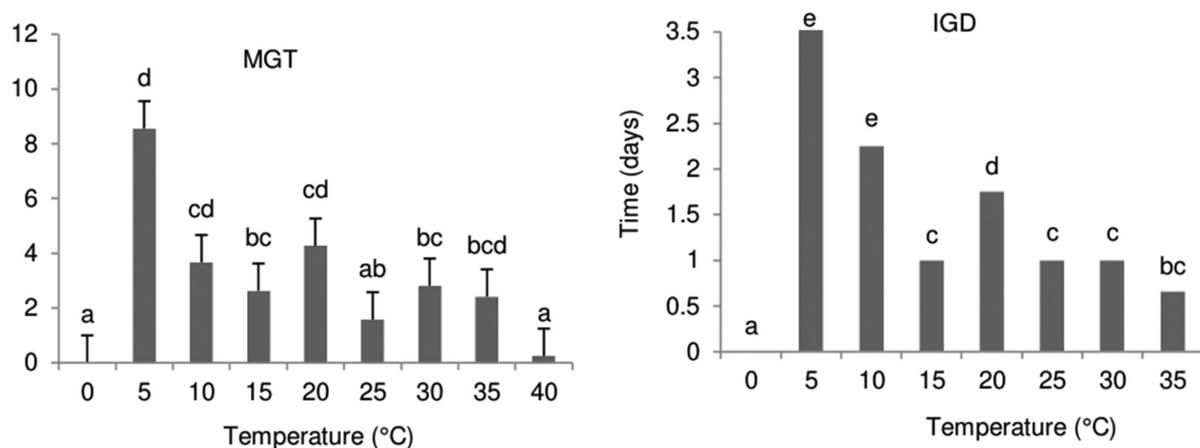


Fig. 4. Variation of MGT and IGD of *Teucrium santae* seeds as a function of temperatures. MGT, mean germination time; IGD, initial germination day. Different lowercase letters show significant differences between the averages.

The photoperiod's impact on germination

The germination attitude of *T. santae* seeds appears to be similar in both light and continuous darkness (Table 1). All germination parameters are insignificantly different ($p > 0.05$).

Effects of temperature on germination parameters

The kinetics curves for the various temperatures investigated are displayed in Fig. 2, each curve has three phases: a latency phase (the time interval preceding the germination commencement), an exponential phase (the beginning and acceleration of germination), and a stationary phase (the germination stabilization).

T. santae seed's germination ability (Figs 2–3) is expressed in temperatures between 5 °C and 35 °C; the highest FGPs were obtained at temperatures ranging from 15 °C to 25 °C with 20 °C being the thermal optimum (FGP = 75%). The lowest FGP (2.5%) is recorded at 40 °C. Extreme temperatures of 0 °C and 45 °C are detrimental to germination. Analysis of variance demonstrated the effect of temperature to be significant on the FGP ($p < 0.01$) and

non significant on the VC ($p > 0.05$).

As shown in Fig. 4, at 5 °C, both MGT and IGD were observed to be the longest, whereas the shortest ones were obtained at 40 °C (MGT = 0.25 days; IGD = 3 days). The analysis of variance revealed that both MGT and IGD ($p < 0.05$) are significantly influenced by the temperature factor.

Effects of water stress on germination

The germination kinetic curve for each examined water potential includes the three phases described previously (Fig. 5).

Compared to the control test, where the highest FGP (75%) is recorded, this latter gradually decreases simultaneously with the water potential drop.

Indeed, FGP begins to regress from -0.2 MPa to reach a minimum value of 12.5% at -1 MPa. Germination is completely inhibited at -1.2 MPa (Fig 5). The depressive effect of the water potential's diminution on FGP was confirmed by the analysis of variance and Tukey's test ($p < 0.01$) (Table 2).

The mean germination time varies along with the

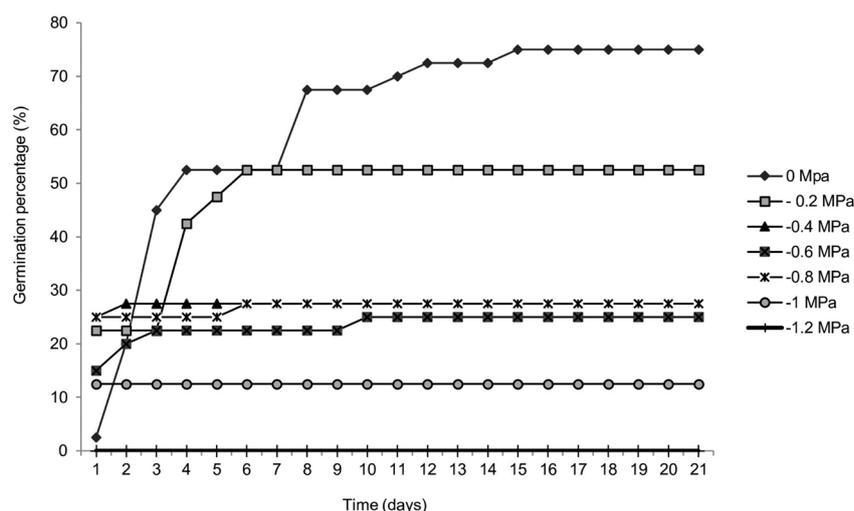


Fig. 5. Germination kinetic curves of *Teucrium santae* seeds at different water potentials.

Table 2. Variation of FGP, VC, IGD and MGT of *Teucrium santae* seeds as a function of different water potentials

Water potential(MPa)	FGP (%)	VC (%)	IGD (days)	MGT (days)
0	75 ± 10 c	26.2 ± 11.08 abc	3 ± 0.57 b	4.28 ± 0 c
-0.2	52.5 ± 34.04 bc	36.77 ± 8.60abc	1.75 ± 1.5 b	3.05 ± 1 bc
-0.4	32 ± 12.58 ab	22.5 ± 3.4 ab	1 ± 0 ab	4.53 ± 0.75 c
-0.6	25 ± 12.9 ab	67.5 ± 30.4 bc	1 ± 0 ab	1.88 ± 1.23 ab
-0.8	27.5 ± 12.9 ab	84.57 ± 31.25 c	1 ± 0 ab	1.40 ± 0.8 ab
-1	12.5 ± 9.57 a	75 ± 50 bc	1 ± 0.5 ab	0.75 ± 0.5 a
-1.2	0 a	0 a	0 a	0 a
F value	9.527***	37.290ns	3.900***	13.418***

Mean value ± Standard deviation. FGP, final germination percentage; VC, velocity coefficient; IGD, initial germination day; MGT, mean germination time; F, Snedecor Fisher variable. Different lowercase letters (column) show significant differences between the averages. ***, significant differences at $p < 0.01$; ns, non significant at $p > 0.01$.

variations in the water potential. The maximum value of 4.53 days is registered at -0.4 MPa while the lowest one (0.75 days) corresponds to -1 MPa.

The high obtained values of the regression's coefficient for FGP ($R^2 = 0.914$), IGD ($R^2 = 0.768$) and MGT ($R^2 = 0.744$) elucidate the water deficit's deleterious effect on these parameters. Conversely, water stress does not seem to affect the VC ($R^2 = 0.021$) (Table 2, Fig. 6).

Discussion

Preliminary germination tests conducted at an average temperature of 20 °C on untreated seeds revealed that they are dormant, but viable. The tetrazolium colorimetric test confirmed this viability.

FERRADOUS et al. (2017) explain the mechanism of this test. The tetrazolium test examines the activity of certain enzyme systems. The loss of activity in these systems tends to parallel the loss of seed vitality. Dehydrogenase enzymes participate in the respiratory activity of biological systems. During the process of respiration, intermediate molecules are produced, on which enzymes act. The hydrogen ions are transferred (in several phases) to the tetrazolium, which, by combining with the hydrogen, is reduced to an insoluble and coloured (red) formazan.

Since the absence of respiration prevents the production of formazan, the dead seeds remain uncoloured.

The temperature of 20 °C chosen to carry out the first germination tests corresponds approximately to the sampling site's average monthly temperature during the autumn season, when the majority of Lamiaceae family species germinate (DADACH and MEHDADI, 2016; DADACH et al., 2018). In fact, the temperatures ranging from 15 °C to 25 °C are considered as the thermal optimum for numerous Mediterranean species (RAO et al., 2006), which further explains the selection of this temperature.

MARTYN (1946) and BASKIN and BASKIN (2004) studied the dormancy of a large number of Lamiaceae species and concluded that seeds belonging to this family are mostly either physiologically dormant (PD) or 'non-dormant' (ND). This agrees with our results, in which the non-pretreated *T. santae* seeds did not germinate. The highest germination percentage could be obtained only after the seeds were chemically scarified with sulfuric acid and soaked in GA₃ 1,500 ppm. The germination could not be stimulated by the use of chemical scarification alone, while soaking in 1,500 ppm GA₃ relatively improved it. This pretreatment is known to be used to break embryonic dormancy (FAO, 1987). According to FINCH-SAVAGE and LEUBNER-METZGER (2006), and in contrast to deep physiological dormancy, which it is ineffective against, GA₃

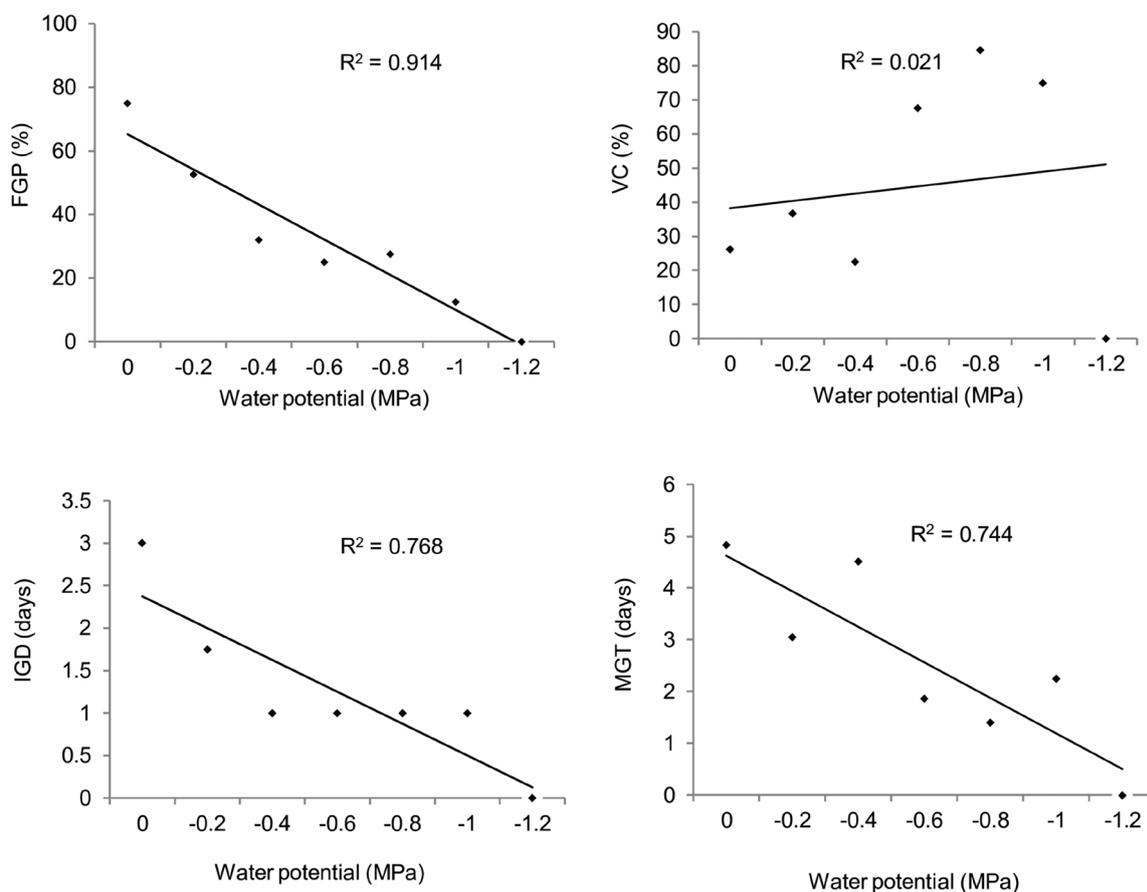


Fig. 6. Linear regression lines between water potential and FGP, VC, IGD, and MGT. FGP, final germination percentage; VC, velocity coefficient; IGD, initial germination day; MGT, mean germination time; R^2 , Regression coefficient.

breaks the non-deep physiological dormancy of seeds.

The work of BASKIN and BASKIN (2004) states that physical dormancy is the result of a superposition of impermeable layers in the seed coat and that mechanical or chemical scarification can break this dormancy. In our study, *T. santae* seeds dormancy could not be broken by chemical scarification alone; however, when chemical scarification was combined with GA_3 pretreatment, the germination percentage was promoted and dormancy could be released. This indicates that the hard seed coat can be considered an inhibitory trait and likewise, the embryo certainly contains a physiological component causing its dormancy. Therefore, *T. santae* seeds are probably affected by a double, non-deep physiological and physical dormancy. Physical dormancy may have a link with the seed size. In this regards, SMITH et al. (1996) and RODRIGUES-JUNIO et al. (2018) hypothesize a relationship between seed size and the physical dormancy affecting them. Smaller seeds have a tendency to be more dormant than larger ones. Furthermore, JAYASURIYA et al. (2008) assumed that small seeds low water content influences their ability to open water gaps in the seed coat. These water gaps are expected to be released when the internal pressure potential caused by vapors rising inside the seed increases (JAYASURIYA et al., 2008).

Physiological dormancy could be due to the GA_3 /Abscisic acid (ABA) ratio. The induction and breaking of seed dormancy is mainly under the control of ABA and

GA_3 . ABA promotes seed dormancy and germination inhibition while GA_3 prompts timely seed germination (GAO and AYELE, 2014; SHU et al., 2016; RODRIGUES et al., 2015). The balance between these two phytohormones is regulated by environmental conditions, a significant supply of gibberellin is necessary for the neutralization of ABA activity in seeds (SKUBACZ and DASZKOWSKA-GOLEC, 2017). Although it is reported that GA_3 stimulates germination, due to a cycle of overlapping interactions between environmental factors and seeds biochemical and enzymatic activities, the precise mechanism leading to germination improvement has been confirmed yet (GUPTA and CHAKRABARTY, 2013).

In our study, the seeds responded positively to all GA_3 applications, which could refer that their dormancy can be explained by an imbalance of the ABA/ GA_3 ratio associated with a seed coat inhibitory effect. This agrees with the research of FARAHANI et al. (2014) who also revealed a combined dormancy affecting *Teucrium chamaedrys* seeds. *T. santae* seed dormancy is one of the factors explaining the difficulties of natural regeneration by sowing and, as a result, the species vulnerability.

Concerning the photoblastic response, the findings demonstrated that *T. santae* seeds are of indifferent photosensitivity as the FGP, VC, MGT, and IGD in both light and continuous darkness are similar. Most plants requiring light to germinate contain in their seeds an inactive amount of

phytochrome (photoreceptor) which is activated when light rays are absorbed. Conversely, seeds that do not need light to germinate contain a sufficient amount of active phytochrome (QUAIL, 2010), this apparently applies to our seeds.

The germination of seeds of any plant takes place within a specific range of temperatures called cardinal temperatures (BEWLEY, 1997). In our study, *T. santae* minimum and maximum cardinal temperatures are 5 °C and 40 °C respectively, with the thermal optimum being 20 °C.

Obtaining high germination percentages in this temperature interval can be explained by the fact that these temperatures favor the absorption of water by the seeds, the improvement of their enzymatic process and their respiration as well as the solubilization of stored nutrients, therefore, facilitating their utilization (WEI et al., 2021). This thermal optimum corresponds to the average temperatures of the autumn season (≈ 20 °C) that characterize this species' native environment where germination takes place. Moreover, SOIL SURVEY STAFF (2014) categorizes the Mediterranean region's soils as "xeric" in reference to the winter's significant precipitation, followed by the summer's dry period, inducing an average annual soil temperature between 15 °C and 22 °C. This thermal germination optimum also typifies the seeds of several Lamiaceae species such as *Teucrium marrum* (BENVENUTI et al., 2006), *Marrubium vulgare*, *Sideritis incana* and *Stachys ocymastrum* (DADACH et al., 2018) and *Lavandula stoechas* (GHADERI-FAR et al., 2021). Likewise, *Teucrium massiliense* and *Teucrium flavum* studied by PORCEDDUO et al. (2021) were found to have an optimal germination in temperatures oscillating between 10 and 25 °C.

In contrast, *T. santae* germination process is severely hindered and even inhibited outside cardinal temperatures (5 °C–40 °C), as a result of excessive oxidative stress and damage to membranes caused by these extreme temperatures (BITA and GERATS 2013; HASANUZZAMAN et al., 2013).

In addition, our results indicate that the velocity coefficient or germination speed does not appear to be correlated with temperature variation, which is consistent with DOVE'S (2010) experimentation that demonstrated the influence of temperature on the velocity coefficient to be either positive or neutral; but not neutralizing. Furthermore, LUNA et al. (2019) illustrated that the VC of some Cistaceae seeds were less affected by temperature than their FGP. The degree of influence that temperature can exert on the germination speed may be linked to the characteristics that each species has acquired during its domestication process (RAMIREZ SANTIAGO et al., 2020).

Overall, the IGD and the MGT shorten when the temperature exceeds the germination thermal optimum of *T. santae* to reach a minimum value of around 1 day at 40 °C. This joins DUNCAN et al. (2019) findings which consider that the rapid germination of seeds in arid natural ecosystems constitutes a strategy that plants use to get adapted to unfavorable conditions.

In our experiment, the seed's tolerance to drought was expressed at all examined water potential levels excluding the level -1.2 MPa, where the germination was

completely inhibited; however, the water deficit influence on FGP was highly significant, as this parameter decreased drastically from 75% at the control test to 12.5% at -1 MPa. It is the case for most seeds of arid-region plants, which fail to germinate when the water potential of the soil is below -1.2 MPa (BASKIN and BASKIN, 2014).

Our findings revealed that *T. santae* seeds can germinate in water potentials levels as low as -1 MPa. This reflects the ability of this species to resist water stress during the germination phase. This tolerance to water deficit may be linked to the calcicole nature of this species (QUEZEL et SANTA, 1963). In calcareous soils, the accumulation of Ca^{2+} generates calcium carbonate precipitates around the roots (JAILLARD, 1985), restricting the surface of the pores between the soil particles and, as a result, the soil's capacity to retain water, thus, inhibiting plant growth (LUJIE et al., 2020). We hypothesize that *T. santae* seeds are adapted to fluctuating or reduced water availability. These results agree with those of FERRIOL et al. (2006) on *Teucrium carolipau* (calcicole species) which showed a remarkable tolerance to water stress.

The decline in FGP caused by the water potential level drop may be explained by the changes in enzyme activity during germination (WEI et al., 2021). The availability of water is a crucial condition for seed germination since it determines the imbibition and subsequent activation of metabolic processes such as rehydration, repair mechanisms (membranes, proteins, and DNA), cell elongation, and radicle growth (OBROUCHEVA, 1991). In the presence of sufficient water resources, the vacuole expands and fuses with the other vacuoles, thus producing an osmotic compartment, which precedes and becomes a mandatory step for the initiation of cell elongation (OBROUCHEVA et al., 2017). The absence of water impedes these processes, decreasing germination success each time the water deficit becomes more severe.

The MGT and the IGD values decrease progressively when the water potential drops. This can be explained by the fact that seeds in arid conditions use a rapid germination strategy to ensure the persistence of the species to which they belong (DUNCAN et al., 2019). The same observations were highlighted by DADACH et al. (2015), as a simultaneous diminution in IGD and MGT and a decrease in water availability were observed on *Thymus serpyllum* seeds that withstand low water potentials at the germination stage.

Conclusion

At the end of this work, it appears that the seeds of *T. santae* have a high rate of viability, but are unable to germinate since they are affected by a double dormancy, a physiological and physical dormancy. This dormancy can be broken by chemical scarification using sulfuric acid followed by soaking in gibberellic acid 1,500 ppm for 72 hours.

Germination tests carried out on seeds subjected to this pretreatment have shown that they are of indifferent photosensitivity since their germination behavior in light

and in continuous darkness is comparable. Their germination thermal optimum is between 15 °C and 25 °C. Water stress exerts a depressive effect on the various germination parameters. The threshold of tolerance to this stress is located at –1 MPa beyond which germination is inhibited.

Exploiting the collected results of this study, *Teucrium santae* seeds can withstand exposure to extreme cold and heat conditions and a certain level of water deficiency. The wide range of tolerance of *T. santae* seeds expresses the possibility of planning reintroduction plans in their natural habitat. In this regard, our results can be useful to facilitate the production of new seedlings. Seed double dormancy has apparently been one of the factors constraining its natural regeneration by sowing; nevertheless, this can be overcome by subjecting seeds to the pre-treatment mentioned previously in this study.

In order to further understand the factors that govern *T. santae* seed germination physiology, it would be important to inspect the effect of other environmental stresses such as salt stress or to study the impact of osmopriming on mitigating the negative effects of water and salt stress on the germination of *T. santae* seeds. In view of accomplishing this study, it would also be crucial to study the physiology of seedling growth. The control of all these parameters will certainly be useful within the framework of the *ex-situ* conservation biology of this taxon, in particular for the production of plants intended to reinforce the natural populations.

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