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# South African Journal of Botany

journal homepage: [www.elsevier.com/locate/sajb](http://www.elsevier.com/locate/sajb)

## Ecophysiological requirements on seed germination of a Mediterranean perennial grass (*Stipa tenacissima* L.) under controlled temperatures and water stress

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### ARTICLE INFO

#### Article history:

Received 1 February 2014

Received in revised form 7 July 2014

Accepted 7 July 2014

Available online 26 July 2014

Edited by H El-Maarouf-Bouteau

#### Keywords:

Seed germination

Steppes

*Stipa tenacissima* L.

Temperature

Water potential

### ABSTRACT

*Stipa tenacissima* L. (alpha grass) steppes are one of the most representative ecosystems in arid Mediterranean ecosystems. On the one hand these steppes, which are perpetually exposed to climate and strong anthropogenic pressure, have undergone severe degradation. On the other hand, the ability of *S. tenacissima* to regenerate naturally is significantly reduced. In this study the germination response and seedling emergence of *S. tenacissima* are examined in relation to the main environmental factors (water stress and temperature) under laboratory-controlled conditions. The main aim of this paper was to investigate the influence of temperature over a temperature range (10 °C–30 °C) and water stress induced by the solutions of polyethylene glycol (PEG)-6000 (0 to –1.6 MPa) for a period of 30 days, on the germination behavior of *S. tenacissima* seeds. The results showed that temperatures between 10° and 20 °C seem to be favorable for the germination of this species, with optimum temperatures among accessions found in 20 °C. When seeds were water-stressed, germination severely decreased at –0.8 MPa, indicating that the accession resistance limits to the water stress, and was completely inhibited at –1.6 MPa. Consequently, the final germination percentage (FGP) decreased and the mean time germination (MTG) increased. Based on the empirical data of the germination rate, we estimated that the parameters of the thermal time and hydrotim models showed different values in all accessions which proves the difference between accession adaptive capacities.

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### 1. Introduction

Germination is a critical stage in the life cycle of plants particularly in arid and semi-arid land, which is subjected to various adverse conditions, and this has serious impact on the subsequent stand of vegetation. Success of natural propagation depends mainly on the response of the seeds towards the interference of various external factors. Therefore, seed germination success may reflect upon population size, distribution and abundance (Flores and Briones, 2001; Ramírez-padilla and Valverde, 2005; Rojas-Aréchiga et al., 1998). Indeed, the environmental conditions of the area of species occurrence are essential to determine the seed characteristics and its germination responses. Mainly, the temperature can promote or inhibit the germination and then influence the seed germination process (Cota-Sánchez and Abreu, 2007; Flores et al., 2006; Ramírez-padilla and Valverde, 2005; Simão et al., 2007; Valverde et al., 2004), water availability, soil or substrate type and the rate of gas exchange (Bewley and Black, 1994; Fenner and Thompson, 2005). All of those factors influence the germination alone or associated

among them, so each portion or population of seeds shows different responses to the environment variations. Understanding germination is therefore of important ecological relevance.

North Africa has a Mediterranean climate, which is characterized by its seasonality in temperature, and precipitation, which leads to a hot drought period in summer and a cool wet period in winter (Joffre et al., 1999), and little rainfall approximately 200 to 400 mm per year for semiarid zones ecosystems (CIA, 2009). This peculiarity of the Mediterranean climate has important implications on plant germination physiology, since dry summer conditions limit water availability and thus germination, while cool winter temperatures also limit germination during the season with high water availability (Rundel, 1996). Spatial and temporal distribution of precipitation in these ecosystems is considered to be highly episodic and unpredictable (Terradas, 1991). The germination season, and for extension the temperature requirements for germination, might be crucial for plant survival. For instance, it is obvious that a species with high, massive germination process relatively independent of temperature may not be favored, because any short precipitation would cause the germination of their seeds, but seedlings will not be capable to overcome the following summer stresses (Lloret et al., 1999). Moreover, the presence of dormancy that delays germination is often advantageous in a competitive or seasonal environment (Harper, 1977; Vleeshouwers et al., 1995).

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One of the most important factors controlling the plants behaviors is genes, which significantly affected seed germination. Several studies have shown that phylogenetic relatedness of plant species within a community could account for a significant proportion of interspecific variations in reproductive traits such as seed germination and seed mass (Bu et al., 2007, 2008; Smith-Ramírez et al., 1998; Verdú, 2006). Seed germination can be regulated not only through genotypic characteristics (Guterman, 1993), but also by environmental conditions, being soil temperature the most important environmental factor controlling seed germination (Beardsell and Richards, 1987). Furthermore, the fluctuations or changes in climatic conditions can affect plant distributions and abundance (Scifres and Brock, 1971). Hence, precipitation and temperature are the most important factors that have a great influence on *Stipa tenacissima* seedling recruitment. In this case, the use of local seed provenance is advisable because it is better adapted to local habitat conditions. The importance of using local seed provenance is generally recognized as the best practice in biodiversity conservation and restoration (Carr, 2005; Krauss et al., 2005). As growing *S. tenacissima* in its native habitat would preserve it, the critical challenge facing restoration practitioners is the identification of the suitable environmental factors and appropriate seed provenance. These factors may be taken into account in the establishment of *S. tenacissima* in steppes. On the other hand, because the lack of *S. tenacissima* seedlings in steppes proves that the species suffers germination disability, it is critical to discover the causes of lack of germination and limited seedling recruitment.

*S. tenacissima* steppes are one of the most representative ecosystems in arid Mediterranean ecosystems. *S. tenacissima* develops several adaptation characteristics to climatic and edaphic aridity (Balaguer et al., 2002; Pugnaire et al., 1996; Ramirez et al., 2007; Valladares and Pugnaire, 1999) like its resistance to long drought periods (Pugnaire and Haase, 1996; Pugnaire et al., 1996), resprouting ability (Martínez-Sánchez et al., 1997), and ecological amplitude (Le Houérou, 1969). Besides its ecological value, *S. tenacissima* shows a high economic importance. In fact, this species is used as a raw material for manufacturing paper, thread, baskets, jute (Belkhir et al., 2012; Ramírez et al., 2006), rope, mats (Akchiche and Messaoud Bouregghda, 2007; Belkhir et al., 2012), and livestock (Genin et al., 2007).

The main objective of this study is to determine the most adequate ranges of temperature and water availability for the germination of *S. tenacissima* under controlled conditions. In fact, germination studies provide valuable information about the ability of the species to germinate and the influence of seed provenance in this factor, which is important for its preservation and restoration of steppes. In addition, limitations of some populations to regenerate under the current climatic constraints can explain the vulnerability of this species to future global change projections.

## 2. Material and methods

### 2.1. Plant material and seed collection site

Seeds of *S. tenacissima* were collected from five provenances originating in the western region of North Africa, with different geographical

localizations. The names of the studied accessions, their geographical localization and the climatic characteristics of the collect site were summarized in Table 1. After collection, seeds were allowed to air-dry and stored under ambient temperature.

Previous to the germination test, a sample of seeds was tested for viability, applying the tetrazolium test (AOSA, 2000). Four replications of 25 seeds were used to test viability using triphenyl tetrazolium chloride (TTC; Hampton and Tecrony, 1995). For this purpose, seeds were longitudinally cross-sectioned through the embryo and immersed in a 0.1% TTC solution for 12 h in the dark. Subsequently, the cut seeds were examined for pink staining.

### 2.2. Effect of temperature

A total of 4000 seeds per treatment were used. From each accession, sixteen replications of 50 randomly selected seeds were placed on filter paper in 9 cm Petri dishes moistened with different quantities of distilled water selected according to different temperatures. Then, the Petri dishes were covered with polyethylene sheets to prevent evaporation. The dishes were placed in a dark incubator (LMS Cooled Incubator), maintained at different temperatures: 10 °C, 15 °C, 20 °C, 25 °C and 30 °C (Table 2). The choice of this temperature range was made according to the observed temperatures in the Tunisian arid ecosystems, as well as the optimum temperature growth of species, where photosynthesis typically exhibits an optimum temperature range of 10 to 35 °C (Yamori et al., 2013). It is well-known that a seed is proven to germinate at the emergence of the radicle (radicle length > 1 mm) (Bewley and Black, 1994). The counting of germination was performed daily in each Petri dish for 30 days.

### 2.3. Effect of water potential

Germination response to water stress was tested for different water potentials (0, -0.2, -0.5, -0.8, and -1.6 MPa) (Table 2), whose values simulate the soil water potential generally observed in the Tunisian arid ecosystems during the rainfall season. The water potential effects on germination were examined by incubating seeds at the optimum germination temperature (20 °C, see the Results section). Different water potentials were obtained with solutions of polyethylene glycol (PEG) 6000. The concentrations of PEG 6000 were determined according to the Michel and Kaufman (1973) equations. Petri dishes were covered with polyethylene sheets to prevent evaporation and then kept in a dark incubator (LMS Cooled Incubator). Four replicates of 50 seeds for each provenance were made.

### 2.4. Thermal time and hydrotime calculations

To determinate the temperature and water relation parameters of seed germination in different accessions, germination rate data were analyzed based on the thermal time and hydrotime models by means of repeated probit analysis. This approach is described in detail by Bradford (1990) and Cheng and Bradford (1999).

**Table 1**  
Geographical origin and environmental conditions of the five Tunisian accessions of *Stipa tenacissima*.

Accession name	Code	Latitude	Longitude	Seed collection date	Mediterranean bioclimatic stage	Winters	Annual mean rainfall isohyets (mm)	Texture of soil
Hassi El Frid	P1	35°2'5.21"N	8°54'26.58"E	June 2012	Superior arid	Cool	200 et 400 mm/an	Sandy loam
Majel Bel Abbes	P2	34°50'26.64"N	8°31'34.36"E	June 2012	Superior arid	Cool	200 et 400 mm/an	Sandy loam
Sbeitla	P3	35°14'3.82"N	8°57'47.12"E	June 2012	Superior arid	Cool	200 et 400 mm/an	Sandy loam
Zelfane	P4	35°22'13.70"N	8°48'55.99"E	June 2012	Superior arid	Cool	200 et 400 mm/an	Sandy clay loam
El Gonna	P5	34°43'18.76"N	10°34'20.14"E	May 2012	Lower arid	Mild	100 et 200 mm/an	Sandy loam

**Table 2**  
Tested treatments and measurement in *Stipa tenacissima* seeds. Initial germination date (IGD), final germination date (FGD), final germination percent (FGP), initial germination percent (IGP), mean time germination (MTG) and seed weight (SW) were used to evaluate germination performance of seeds. Mean time germination (MTG) was calculated according to the following equation:  $MTG = \sum Dn / \sum n$  (Moradi Dezfouli et al., 2008), where n is the number of seeds germinated on day D, and D is the number of days counted from the beginning of germination.

Treatments	Used seed number per treatment	Experiment duration	Observed parameters	Solution types	Solution quantities added (ml)
Temperatures (°C)	10	50	30 days	Distilled water	3.5
	15				
	20				
	25				
	30				
Water potential (–MPa)	0	50	30 days	Distilled water + PEG (6000)	4.5
	0.2				
	0.5				
	0.8				
	1.6				

### 2.5. Statistical analysis

The statistical analysis was carried out by means of ANOVA tests and differences among provenances by post-hoc Tukey test at  $P < 0.05$ . All data were analyzed with the R statistical package (R Development Core Team 2012, version 3.0.1).

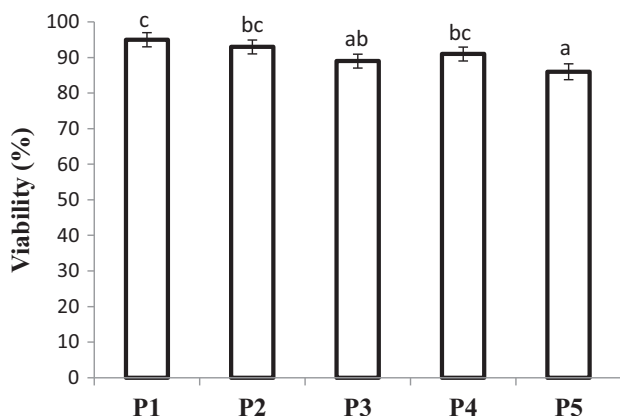
## 3. Results

### 3.1. Viability test

Viability, as indicated by TTC, varied between accessions (Fig. 1). In general, seeds of *S. tenacissima* used in the experiment had a high viability percentage. The response of seed viability differed significantly among provenances. Among provenances, the P1 had the highest viability percentage  $95 \pm 2\%$ , and then P2 with  $93 \pm 2\%$ . The accessions P3, P4, and P5 showed a viability percentage respectively of  $89 \pm 2\%$ ,  $91 \pm 2\%$ , and  $86 \pm 2.3\%$ .

### 3.2. Effect of temperature on seed germination

The main results have shown that seeds of *S. tenacissima* were able to germinate at temperatures below  $25^\circ\text{C}$  (Fig. 2). In fact, the species manifested better germination rates at  $20^\circ\text{C}$ . At  $25^\circ\text{C}$  and  $30^\circ\text{C}$ , the germination considerably decreased in all accessions. Therefore, germination percentages of *S. tenacissima* seeds were affected significantly by



**Fig. 1.** The seed viability (%) of five provenances of *S. tenacissima*. Different letters over the bars indicate significant differences among accessions (Tukey test,  $P < 0.001^{***}$ ).

temperature. On the other hand, for all accessions and temperatures, the analysis of variance (ANOVA) shows a high significant effect of the provenance on the germination percentage ( $P < 0.001$ ).

The germination percentage under optimal temperature of germination ( $20^\circ\text{C}$ ) (Table 3) showed significant differences among the five studied *S. tenacissima* accessions. According to post-hoc analysis, P1 and P2 were gathered in an independent group as the highest FGP accessions. Concerning P3 and P4, they were in another group, and P4 was also included in a different group with P5; P4 and P5 as the lowest FGP accessions. Therefore, in the optimal conditions of germination, P1 and P2 germination percentages were better than the others.

The MTG also varied significantly among the five accessions. Indeed, the most important germination speed accession (P2) did not necessarily show the highest germination percentage (P1), while the highest MTG was in correspondence with the lowest germination percentage. There were two groups of accessions, in the first of which P1, P2, and P3 were assembled as the fastest speed of germination accessions and the second is made up of the other accessions with the highest MTG accessions. The temperature of  $20^\circ\text{C}$  recorded faster IGD as well as low MTG, indicating rapid germination and high final germination percentage (FGP).

There was a significant variation in seed weight among accessions. However, the difference in seed weight did not affect germination percentages because important germination percentages did not necessarily show a high seed weight.

Seed germination dynamics of different accessions of *S. tenacissima* in response to temperature were well described by the thermal-time model at both sub-optimal and supra-optimal temperatures (Table 4). Median germination rates (GR (50)) for different accessions were plotted against germination temperature to show the trend of change in germination rates with temperature (Fig. 3(A)). These rates showed continuous increase at sub-optimal temperatures (from  $10^\circ\text{C}$  to  $20^\circ\text{C}$ ). However, the rates' curve decreased at supra-optimal temperatures (from  $20^\circ\text{C}$  to  $30^\circ\text{C}$ ). Furthermore, germination rates of different accessions correlated strongly at sub-optimal and supra-optimal temperature ranges ( $R^2 > 0.70$ ).

At sub-optimal temperatures,  $T_b$  values varied among accessions. The greater value of the  $T_b$  was represented by P2, whereas the lower one is the value of P4. At supra-temperatures, the ceiling temperature  $T_c(50)$  values were very similar for all accessions. However, there was a great difference in median thermal time  $\theta_T(50)$  value among accessions at suboptimal temperature. Then as well, at supra-optimal temperature, there was a great difference in thermal time constant ( $\theta_T$ ) value between accessions. Hence, the thermal time showed a big difference among accessions. Further, the accession with a minimum  $T_b$  has a higher  $\theta_T(50)$ . Hence, the model

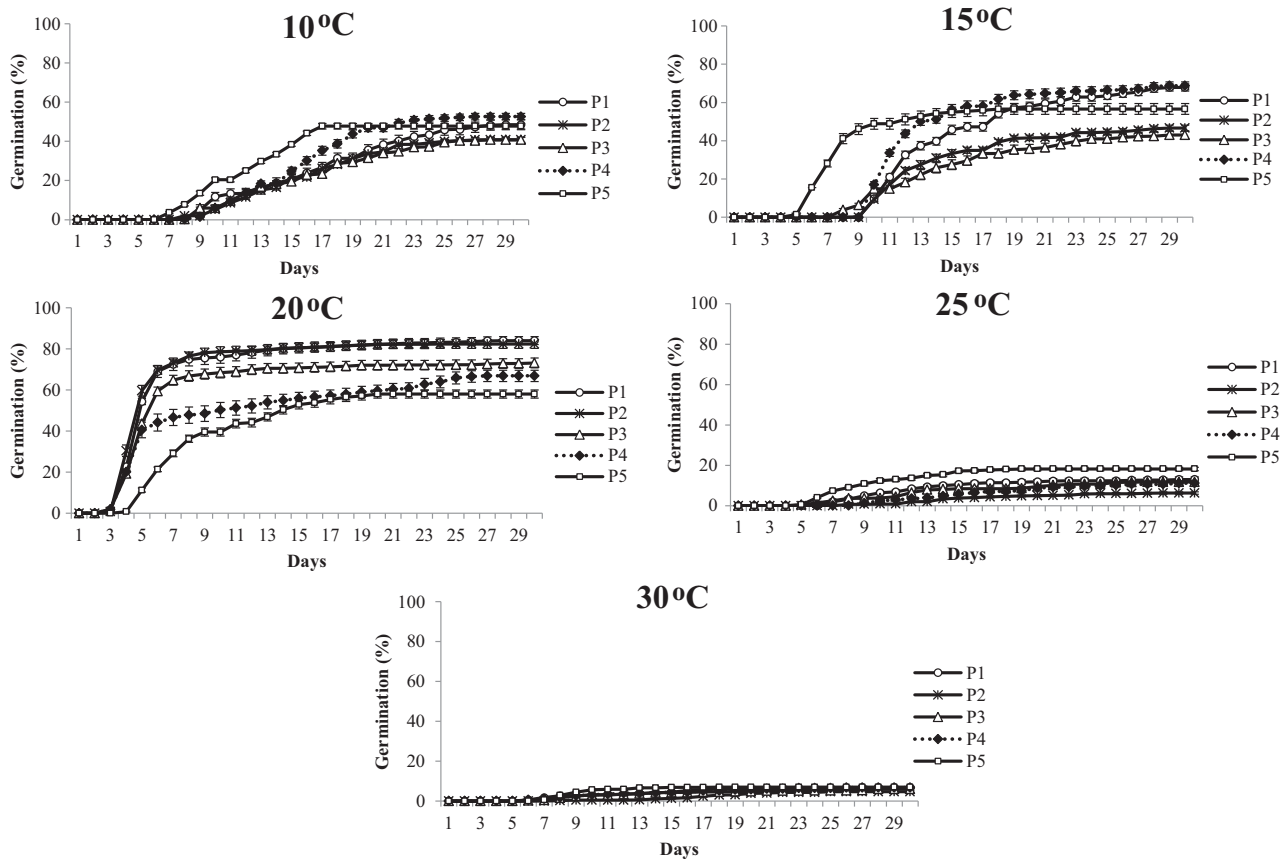


Fig. 2. Effect of temperature on germination percentage of five provenances of *S. tenacissima* for 30 days.

showed a correspondence between a lower  $T_b$  and a higher thermal time requirement for seed germination.

### 3.3. Effect of water stress on seeds germination

The germination responses of *S. tenacissima* seeds to water stress condition simulated by PEG addition at the optimum germination temperature (20 °C) are shown in Fig. 4. There was no significant difference between 0, –0.2 and –0.5 MPa stress levels according to the germination percentages of different accessions. The germination percentage was significantly reduced at –0.8 MPa for all accessions. But when the water potential was lower than –0.8 MPa, germination percentages were completely inhibited (0% at –1.6 MPa). The decrease in water potential of the solution decreased germination. On the other hand, the germination speed was affected by water stress (Table 5). In fact, the water potential of –0.8 MPa caused a significant increase in the MTG, compared to the value observed at control (0 MPa) in all accessions.

Moreover, in low water potential, MTG did not show a significant difference between accessions, which had almost the same IGD and FGD with decreased water potential. Besides, the *S. tenacissima* accessions had almost the same values of germination percentages and MTG under water stress conditions.

Fig. 3(B) showed the relationship between germination rate (GR (g)) and water potentials. For all accessions, the germination rate of *S. tenacissima* decreased with rising water stress.

The hydrotime models described the germination dynamics of different accessions in response to water potential (Table 6). In fact, they showed a large deviation in hydrotime constant among accessions (5.14°Cd for P1 vs 26.97°Cd for P5). Further, the  $\Psi_b(50)$  values varied among the seed accessions. The accession P1 presented the lowest  $\Psi_b(50)$  (–1.02 MPa), whereas the accession P2 exhibited the highest value (–1.22 MPa). The accessions P4 and P5 had a higher thermal time and hydrotime requirement for seed germination.

Table 3

Germination characteristic variables of different provenances of *S. tenacissima* under optimal temperature of germination (20 °C). Different letters denote statistically significant differences by a Tukey's multiple comparison test ( $P < 0.05$ ).

Temperature 20 °C						
Provenances	IGD (days)	FGD (days)	IGP (%)	FGP(%)	MTG (days)	SW (g)
P1	3.38 ± 0.5 <sup>a</sup>	19.31 ± 6.04 <sup>b</sup>	9.63 ± 10.18 <sup>a</sup>	84 ± 7.83 <sup>c</sup>	6.25 ± 0.87 <sup>a</sup>	0.32 ± 0.01 <sup>d</sup>
P2	3.69 ± 0.48 <sup>a</sup>	14.38 ± 4.7 <sup>a</sup>	20.875 ± 15.34 <sup>b</sup>	82.38 ± 8.95 <sup>c</sup>	5.54 ± 0.6 <sup>a</sup>	0.25 ± 0.01 <sup>a</sup>
P3	3.56 ± 0.51 <sup>a</sup>	19.63 ± 7.23 <sup>b</sup>	11.88 ± 8.81 <sup>ab</sup>	73.13 ± 9.69 <sup>b</sup>	6.03 ± 0.72 <sup>a</sup>	0.31 ± 0.01 <sup>c</sup>
P4	3.5 ± 0.63 <sup>a</sup>	24.56 ± 2.58 <sup>c</sup>	9.63 ± 9.16 <sup>a</sup>	66.88 ± 11.24 <sup>ab</sup>	8.53 ± 2.21 <sup>b</sup>	0.26 ± 0.01 <sup>b</sup>
P5	4.75 ± 0.58 <sup>b</sup>	18.875 ± 1.09 <sup>ab</sup>	8.75 ± 5.16 <sup>a</sup>	58 ± 8.099 <sup>a</sup>	9.025 ± 0.68 <sup>b</sup>	0.27 ± 0.013 <sup>b</sup>
P-value	<0.001***	<0.001***	0.007**	<0.001***	<0.001***	<0.001***

Values are mean ± S.E. ( $n = 16$ ), \*\* ( $P < 0.01$ ), \*\*\* ( $P < 0.001$ ).



**Table 4**

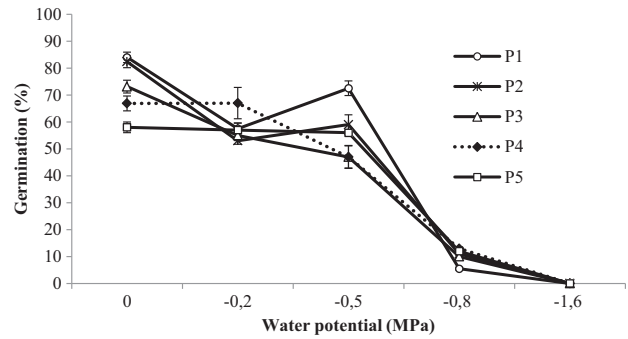
Germination parameters of different accessions of *S. tenacissima* seeds at constant temperatures based on thermal time model analysis.  $\theta_T(50)$  thermal time for 50% of seeds to germinate,  $\sigma_{\theta_T}$  standard deviation,  $T_b$  constant base temperature at supra-optimal temperature range,  $\theta_{T_c}$  constant thermal time at supra-optimal temperature and  $T_c(50)$  ceiling temperature for 50% of seeds, and  $\sigma_{T_c}$  standard deviation at supra-optimal temperature.

Temperature range	Parameter	P1	P2	P3	P4	P5
Sub-optimal temperature	$T_b$ (°C)	7.89	10.61	8.28	3.8	2
	$\theta_T(50)$ (°Cd)	49.50	32.05	111.11	200	250
	$\sigma_{\theta_T}$	0.30	0.29	0.38	0.76	1.13
	$R^2$	0.80	0.76	0.77	0.928	0.945
	$T_c(50)$ (°C)	28.64	28.47	28.84	28.88	31.28
Supra-optimal temperature	$\theta_{T_c}$ (°Cd)	43.07	28.77	81.80	111.11	142.85
	$\sigma_{T_c}$	0.06	0.06	0.07	0.08	0.10
	$R^2$	0.7669	0.7525	0.7792	0.7963	0.884

## 4. Discussion

### 4.1. Temperature and water stress

Temperature and water potential are considered as the most important environmental signals regulating species germinations (Chauhan and Johson, 2008; Norsworthy and Oliveira, 2006). Since temperature plays an important role in seed germination, the present paper undertakes the study of demonstrating that the temperature range reaches a maximum of germination percentage of different *S. tenacissima*



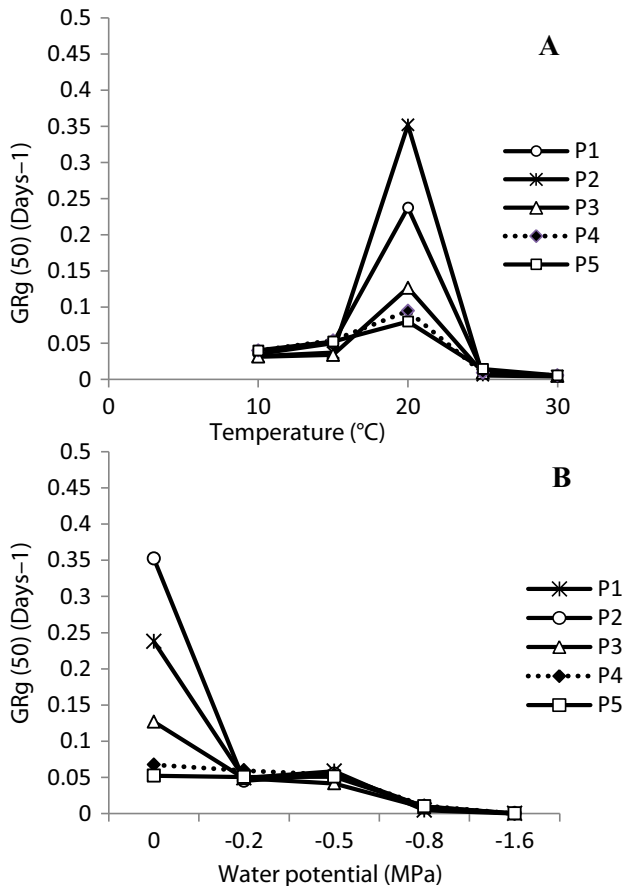
**Fig. 4.** Effects of water potential on the cumulative germination percentage of *S. tenacissima* seeds from the five studied provenances at 20 °C.

accessions between 10 °C and 20 °C. This result is similar to that reported in germination temperature ranges of other *Stipa* species or populations (Hamasha and Hensen, 2009; Hu et al., 2013; Ronnenberg et al., 2008). Furthermore, it is a typical range of germination for many Mediterranean plants (Barragán et al., 1999; Baskin and Baskin, 1998; Thanos, 2000). Likewise, the results' analysis showed a high significant effect of the provenance on the germination percentage for all temperatures. This result is in agreement with Besnier (1989) who reported that temperature ranges for germination depend largely on geographic origin of the species or populations. Subsequently, the differences in germination between accessions may be considered as a mechanism of adaptation to the environmental conditions in the natural habitats. According to many authors (Bischoff et al., 2006; Lopez et al., 2003; Pico et al., 2003) differences among provenances, particularly in germination, can be inflated by heterogeneous environmental condition sites at which seeds were collected. Other physiological parameters interfere in the germination regulation for example the low germination percentage, at 25 °C and 30 °C, may be due to significant degrees of secondary dormancy (Baskin and Baskin, 1998). The dormancy can be a strategy developed by the species to avoid germination during the period of the year in which the survival chances of the seedlings are very low (Gasque and García-Fayos, 2003). Moreover, the high temperature during the summer season may affect a number of ecophysiological processes determining the ability of seeds to germinate including membrane permeability, activity of membrane-bound proteins and cytosol enzymes (Bewley and Black, 1994). According to Murdoch and Ellis (1992), the loss of dormancy in Poaceae depends not only on high temperature in the storage environment but also on the time spent on it. Therefore, the seeds of *S. tenacissima* germinate better after summer. In this regard, Gasque and García-Fayos (2003) have found that the germination of *S. tenacissima* is completely absent before autumn.

In the present study, the germination of *S. tenacissima* began at 3–4 days with an initial germination percentage between 9% from 20% and developed a high speed of germination that did not exceed nine days. Hence, the species might adapt a strategy to germinate quickly when germination conditions are favorable.

The different responses of germination to optimum temperature among the five studied accessions can be explained by genetic and environmental factors which have a great influence on seed germination (Hamasha and Hensen, 2009). In this context, Gasque and García-Fayos (2003) have proven that there is a significant variation in seed germination among individuals, populations and years in *S. tenacissima*, which also seems to be subjected to both genetic and maternal influence.

Seed viability of *S. tenacissima* was found to be in the range of 86–95%, a result similar to the finding of Ronnenberg et al. (2008), which has already been described in other species of the genus *Stipa*. Seed viability is influenced by events that occur during seed development. *S. tenacissima* grows in different habitats over a wide geographical



**Fig. 3.** Median germination rates (GR50) (1/t50) of different accessions of *S. tenacissima* seeds incubated at a range of temperature from 10 °C to 30 °C (A) and water potentials of 0 to –1.6 MPa (B).

**Table 5**

Final germination percentage and mean time germination of different provenances of *S. tenacissima* under different water stress potentials. Different letters denote statistically significant differences by a Tukey's multiple comparison test ( $P < 0.05$ ).

Provenances	Water potential (–MPa)									
	Control		0.2		0.5		0.8		1.6	
	FGP(%)	MTG (days)	FGP(%)	MTG (days)	FGP(%)	MTG (days)	FGP(%)	MTG (days)	FGP(%)	MTG (days)
P1	84 ± 7.83 <sup>c</sup>	6.25 ± 0.87 <sup>a</sup>	57.5 ± 4.12 <sup>a</sup>	11.06 ± 0.39 <sup>b</sup>	72.5 ± 5.51 <sup>b</sup>	9.58 ± 0.1 <sup>b</sup>	5.5 ± 1 <sup>a</sup>	12.29 ± 1.02 <sup>b</sup>	0 ± 0	0 ± 0
P2	82.38 ± 8.95 <sup>c</sup>	5.54 ± 0.6 <sup>a</sup>	53 ± 2.58 <sup>a</sup>	11.08 ± 0.19 <sup>b</sup>	59 ± 7.39 <sup>ab</sup>	8.58 ± 0.43 <sup>a</sup>	11 ± 2.58 <sup>b</sup>	11.72 ± 0.4a <sup>b</sup>	0 ± 0	0 ± 0
P3	73.13 ± 9.69 <sup>b</sup>	6.03 ± 0.72 <sup>a</sup>	55 ± 3.83 <sup>a</sup>	9.74 ± 0.21 <sup>a</sup>	47 ± 8.41 <sup>a</sup>	9.16 ± 0.68 <sup>ab</sup>	10 ± 1.63 <sup>b</sup>	10.46 ± 0.33 <sup>a</sup>	0 ± 0	0 ± 0
P4	66.88 ± 11.24 <sup>ab</sup>	8.53 ± 2.21 <sup>b</sup>	67 ± 11.94 <sup>a</sup>	10.12 ± 0.49 <sup>a</sup>	58 ± 8.64 <sup>ab</sup>	8.77 ± 0.29 <sup>ab</sup>	13 ± 1.15 <sup>b</sup>	11.18 ± 0.86 <sup>ab</sup>	0 ± 0	0 ± 0
P5	58 ± 8.099 <sup>a</sup>	9.025 ± 0.68 <sup>b</sup>	57 ± 5.29 <sup>a</sup>	9.78 ± 0.09 <sup>a</sup>	56 ± 1.63 <sup>a</sup>	8.83 ± 0.26 <sup>ab</sup>	12 ± 2.83 <sup>b</sup>	11.02 ± 0.9 <sup>ab</sup>	0 ± 0	0 ± 0
P-value	<0.001***	<0.001***	<0.065 NS	<0.001***	<0.002**	<0.024*	<0.001***	<0.036*	–	–

Values are mean ± S.E. ( $n = 4$ ), \* ( $P < 0.05$ ), \*\* ( $P < 0.01$ ), \*\*\* ( $P < 0.001$ ), NS = non significant.

range and is adapted to different environments, thereby showing natural variation. Differences in viable seed percentage marked the importance of accession factor in seed viability. In this context, Ronnenberg et al. (2006) reported that the variability in seed viability could also be attributed to annual precipitation fluctuations. In addition, the results revealed that there is a relation between viability and accession germination, the higher viability the higher germination percentage in the majority of tested temperatures. The accessions P1 and P2 have higher viability percentage. Contrariwise, the accession P5 has the lowest seed viability.

In addition to the temperature, water stress affects the germination of different accessions of *S. tenacissima*. However, for this species water soil has more complicated effects on germination than temperature because water is the initial factor for seed imbibition and germination and is directly and indirectly involved in subsequent germination metabolic stages (Cavalcante and Perez, 1995) in particularly under arid and semi-arid bioclimates. In this study, germination percentages were considerably reduced at –0.8 MPa, while they were completely inhibited at –1.6 MPa. Therefore, the accessions of *S. tenacissima* were found to be moderately tolerant to water stress. This result confirms previous reports on the germination of other *Stipa* species including those of Bonvissuto and Busso (2007), who reported that a decrease in germination capacity in *Stipa neaei* seed occurred at –0.59 MPa, and those of Chaieb et al. (1992) and Neffati and Akrimi (1997) who found that *Stipa lagascae* tolerated water potentials that reached –0.7 MPa, then was inhibited at –1.6 MPa. Additionally, Hu et al. (2013) pointed that the germination ability of *Stipa bungeana* is affected by water stress, with a water potential value that does not exceed –0.2 MPa. Moreover, the mean time germination (MTG) increased with the decrease in water potential especially at –0.8 MPa. The greater the water stress conditions, the lower the total germination and speed of germination (Brown, 1995) will be. Consequently, drought, high temperature and soil water stress can be considered as the major obstacle to the regeneration of *S. tenacissima* in Tunisian and North African steppes.

The seed germination of different accessions of *S. tenacissima* under Mediterranean climate conditions starts in autumn, when precipitation usually initiates and the temperature is adequate (Galmés et al., 2006). Subsequently, some of the seeds will be stored in the soil seed bank instead of germinating immediately after dispersal. The species forms transient soil seed banks with germination taking place from autumn

to early spring (Gasque and García-Fayos, 2003). However, the global change effects on the Mediterranean climate is likely to provide more frequent and longer drought periods, increases of the temperature and changes in precipitation patterns in arid regions (Osborne et al., 2000; Rey et al., 2011). This problem can negatively affect sexual reproduction in this species (Ramírez et al., 2008). Indeed, the species germination requires the availability of resource, optimum range of temperature and high soil moisture content. That's why, *S. tenacissima* probably uses vegetative reproduction as a space colonization strategy (Haase et al., 1995; White, 1983). The use of vegetative reproduction is better as *S. tenacissima* plant is well adapted to acclimatize with aridity (Balaguer et al., 2002; Pugnaire et al., 1996; Ramirez et al., 2007; Valladares and Pugnaire, 1999). Therefore, the germination requirements of *S. tenacissima* are different from those required by the adult plant. This indicates that *S. tenacissima* may be the worst affected by climate change scenario in the Mediterranean region (Ramírez et al., 2008) because the decrease of seed germination can lead to the species extinction risk.

#### 4.2. Thermal time and hydrotime model

The thermal time and hydrotime models provide insight into how physiological and environmental factors interact to regulate the germination behavior of seed populations (Bradford, 1995, 2002). It has been clearly demonstrated that seed dormancy is a reflection of high (more positive) values of the  $\Psi_b(g)$  threshold, and that conditions that break dormancy (after-ripening, hormones, etc.) shift the  $\Psi_b(g)$  distribution to lower (more negative) values (Allen and Meyer, 1998; Bradford, 1996; Christensen et al., 1996; Meyer et al., 2000; Ni and Bradford, 1992, 1993). Subsequently, *S. tenacissima* had a low  $\Psi_b$  that may be a condition that break dormancy. Further, Bradford and Somasco (1994) reported that the low  $T_c$  values are often associated with seed dormancy, as in relative dormancy or thermo-inhibition exhibited by seeds whose germination is prevented at warm temperatures. Hence, *S. tenacissima* presented a low  $T_c$  values in different accessions, it is clear that the species may be in dormancy at warm temperatures.

Thermal time model coefficients describe a different thermal-response scenario at two test range temperatures. In fact, P1 and P2 accessions appear to have the most rapid germination (lowest  $\theta$ ). This discrepancy can be explained by differences in  $T_b$  among accessions, as P1 and P2 have a higher  $T_b$ . In this context, Trudgill et al. (2000) suggest that species adapted to cooler temperatures have a lower base temperature and a higher thermal time requirement for seed germination. This relationship correlated with the result found in the present study. Therefore, the accessions P4 and P5 are more adapted to germinate in the winter than the other accessions. Furthermore, the *S. tenacissima*  $T_c$  values did not exceed 31 °C, so the species cannot germinate in summer temperatures. On the other hand, Hu et al. (2013) discover that the thermal time and hydrotime model depend with other environmental factors. They found that *S. bungeana* seeds had significantly higher  $T_b$

**Table 6**

Germination parameters of different accessions of *S. tenacissima* seeds at 20 °C based on hydrotime model analysis.  $\theta_H$  constant hydrotime,  $\Psi_b(50)$  the base water potential for 50% of seeds, and  $\sigma_{\Psi_b}$  standard deviation.

Parameter	P1	P2	P3	P4	P5
$\Psi_b(50)$ (MPa)	–2.10	–1.22	–1.31	–1.46	–1.51
$\theta_H$ (MPa days)	5.14	6.48	15.27	22.00	26.97
$\sigma_{\Psi_b}$	0.40	0.45	0.42	0.37	0.43
$R^2$	0.06	0.43	0.66	0.86	0.82

and  $\theta_T(50)$ , and  $T_c(50)$  and  $\theta_T$  in light than in darkness and that light has a great effect on  $\Psi_b(50)$ . So, the thermal time and hydrotime model coefficients may be influenced by various environmental factors.

In conclusion, these experiments, under controlled abiotic factors (temperature and water stress), revealed that *S. tenacissima* has an optimal range of germination in the range between 10 and 20 °C, with an optimum temperature at 20 °C. While, high temperatures (i.e. 30 °C) limit the germination. In addition, germination is also affected by water availability with a limit of about  $-0.8$  MPa under which germination is inhibited. Therefore, germination may occur when suitable moisture and temperature conditions are present. Undoubtedly, future projections of climate warming will impact in the population dynamics of this species by influencing seed germination, and consequently their potential for natural regeneration. Therefore, *S. tenacissima* steppes would undergo a regression response to the predicted severe future conditions according to climate change scenarios (IPCC, 1996, 2001).

### Acknowledgment

The authors would like to express their thanks to Frank Monkman, a teacher in Marysville Getchell High School, for having proofread this manuscript.

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