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Full Length Research Paper

Influence of temperature and water potential on laboratory germination of two Moroccan endemic thymes: *Thymus maroccanus* Ball. and *Thymus broussonetii* Boiss.

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***Thymus broussonetii* and *Thymus maroccanus* are under intensive utilization because of their wide ranging medicinal potential. In an effort to improve and promote the cultivation of this over-exploited medicinal herb, the effects of temperature and water potential on the germination of their seeds were investigated. Six water potentials (0, -0.07, -0.14, -0.22, -0.32 and -0.53 MPa) and three incubated temperature (15, 20 and 25°C) were tested. The result showed that seeds of *T. maroccanus* and *T. broussonetii* germinated rapidly and reached highest germination percentage in distilled water under no stressful condition of germination at all temperature tested. Decrease in osmotic potential progressively inhibited seed germination of the two thyme species. Germination was severely decreased at -0.53 MPa indicating that the two thyme species resistance limits to the water stress is between -0.32 and -0.53 MPa. The cumulative effect of water potential and temperature on the final germination depends on the species. For *T. broussonetii*, no significant effect of the interaction between water potential and temperature was observed. The temperature between 15 and 20°C seem to be favorable for the germination of the two thyme species. Regarding to the water stress, *T. broussonetii* appears to be more tolerant than *T. maroccanus*.**

Key words: *Thymus maroccanus, Thymus broussonetii,* water potential, temperature, seed germination, Morocco.

INTRODUCTION

Thyme species (Lamiaceae) are amongst medicinal plants that are largely used in the Mediterranean basin (Ismaili et al., 2004). Many of them are used as medicinal remedies against a variety of diseases, aromatic, culinary as well as food preservative (Dababneh, 2007). As a medicinal property, it has been reported that many thyme species possesses numerous biological activities such as antispasmodic (Meister et al., 1999), antibacterial (Marino et al., 1999; Essawi and Srour, 2000), antioxidant (Miura et al., 2002), antifungal activities (Soliman and Badeaa, 2002; Dob et al., 2006) and anti-inflammatory effect (Ismaili et al., 2002; Elhabazi et al., 2006). Morocco is rich by about twenty one thyme species of which twelve are endemic (Benabid, 2000). Most of these species are used as medicinal remedies, but some of them are more appreciated than others. In Southwestern Morocco, particularly in Essaouirra and Marrakech regions, *Thymus broussonetii* called locally as “Za'atar Essaouiri” and *Thymus maroccanus* known locally as “Za'ater” or “Azukeni” present a great demand by the local population (Bellakhdar, 1996) and also a high economic importance for many collectors of the regions. In these areas, the inflorescences, leaves and stems barks of this two species were used as powder, decoction or infusion form to treat digestive disorder, diarrhea, fever, coughs, cold and numerous infected areas (Bellakhdar, 1996; Sijelmassi, 1993). Previous observation showed that the population of these two species has become low and

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scattered in the wild, which may be attributed essentially to its intensive harvesting (up-rooting) and also to the land conversion (Belaqziz et al., 2009). In addition, these species are collected essentially during the flowering period, before seed set, making low regeneration and causing their gradual degradation. Many herbalists and collectors from the region recognized the fact and reported that this situation affected greatly their financial income and subsequently their livelihood.

In order to ensure the sustainable utilization and to meet the growing demand of these wild species, it has become necessary, therefore, to develop rapid methods of their commercial cultivation (*in domo* conservation). This solution is largely adopted in Europe, China and India for many medicinal plants (Zschocke et al., 2000; Hamilton, 2004). It was reported that seeds culture is an alternative and easy method of commercial propagation and is being used widely for the commercial propagation of a large number of plant species, including many medicinal plants (George and Sherrington, 1984). It is evident that before this goal could be achieved, basic information on the seed germination of these wild medicinal species in response to several environmental constraints was essential. In arid natural environments that characterize many regions in Morocco, numerous uncontrolled factors may influence germination, particularly the scarcity of water. It was recognized that the water stress is the most limiting factor to plant germination and their productivity in arid regions (Rodrigues et al., 2009). It was also reported that temperatures and water stress interacted in their effect on cumulative germination and the germination rate of several species, revealing significant variations in germination (Battaglia, 1993; Lopez, 1999).

The aim of this report was to study the germination response and to verify the influence of the temperature and water potential in the seeds germination of two over exploited Moroccan endemic thymes (*T. broussonetii* and *T. maroccanus*).

MATERIALS AND METHODS

Seed source

In July 2006 (period of mature fruits), inflorescences of *T. broussonetii* and *T. maroccanus* were collected, respectively from Essaouirra and Marrakech regions, Southwest Morocco. Seeds were separated from the inflorescence, cleaned and dry stored at room temperature after surface sterilization with 3% (w/v) sodium hypochlorite (NaOCl) for 20 s and rinsed three times with deionized water.

Germination study

Seeds were placed to germinate in 9 cm petri dishes on filter paper moistened with 5 ml of test solution. Four replicates of 30 randomly selected seeds each were used for each treatment. A seed was considered to have germinated at the emergence of the radicle (radicle > 1 mm) (Bewley and Black, 1994). Water potential solutions (0.0 control, -0.07, -0.14, -0.22, -0.32 and -0.53 MPa) were prepared by calculating the amount of polyethylene glycol (PEG 6000) using the equation of Michel and Kaufmann (1973). These water potential solutions (Ψ) were used based on a preliminary test for water stress tolerance limits of the species. PEG 6000 solutions were renewed every 48 h under sterile conditions to ensure relatively constant water potential in the treatment. In our test, we used the PEG (6000) because it has been more effective as it does not penetrate in the cells, is not degraded and does not cause toxicity, due to its high molecular weight (Hasegawa et al., 1984). Assays were performed in the dark at 15, 20 and 25°C in incubator with automatic temperature control (± 0.1°C). Germination was noted on alternate days for 14 days. The germination parameters evaluated are germination rate expressed as the percentage of seeds germinated after 14 days.

Statistical analysis

The data were subject to the statistical analyses. Analysis of variance was used (ANOVA) (SPSS, 1999). $P<0.05$ was used to define statistical significance. If a significant difference was determined among means, a Student-Neuman-Keuls (SNK) test was used to determine significant difference between pairwise comparisons among individual treatments. Germination data were arcsine transformed before statistical analysis to ensure homogeneity of variance.

RESULTS AND DISCUSSION

Seeds of *T. maroccanus* and *T. broussonetii* germinated rapidly in distilled water under no stressful condition of germination at all temperature tested, and reached final germination percentage in less than 6 days (Figures 1 and 2). This data indicate that seeds of the two thymes species were not dormant. Change in temperature had no significant effect on the final germination of *T. broussonetii* seeds in distilled water (Figure 1) but significantly affected the final germination of *T. maroccanus* seeds (Figure 2). At lower (15°C) and moderately (20°C) temperature, seeds at 0.0 MPa (control) had about 90% germination as compared to less than 50% germination at the higher temperature (25°C). These observations confirm the results previously reported by Belaqziz et al. (2008, 2009) on the two thyme species. Decrease in osmotic potential progressively inhibited the germination of the two thyme species. Germination was drastically reduced at -0.53MPa indicating that the two thyme species resistance limits to the water stress is between -0.32 and -0.53 MPa (Figures 1 and 2). It was reported that the decrease in germination rate particularly under osmotic stress may be due to the fact that seeds seemingly develop an osmotically enforced dormancy under water stress condition.

This situation was interpreted as an adaptative strategy to prevent germination under stressful environment thus ensuring proper establishment of the seedlings (Singh et al., 1996). The negative effect of water stress on germination was reported by many authors for severals plants species (Stefanello et al., 2006; Mensah et al., 2006; Salvatore et al., 2004). The cumulative effect of

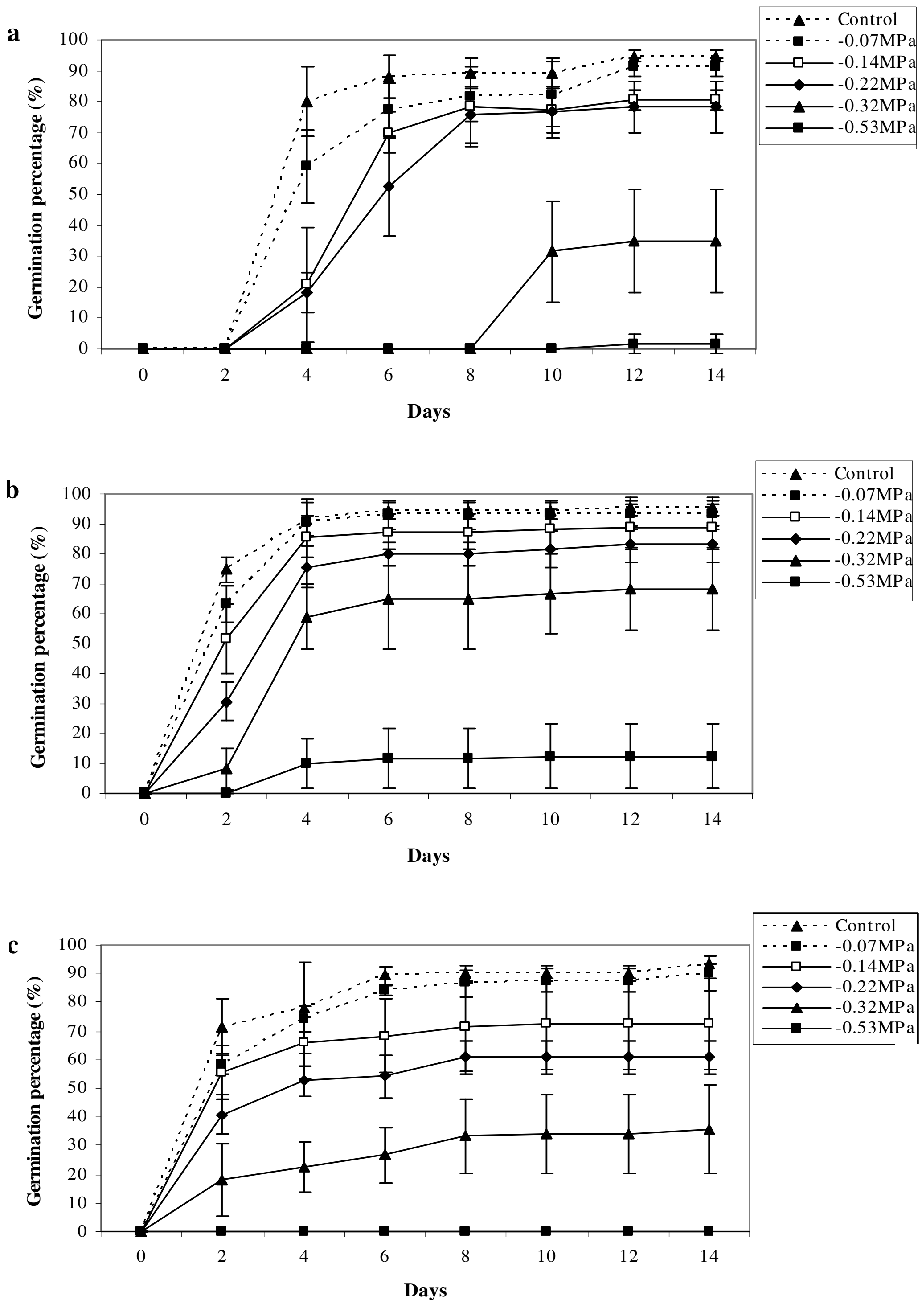

Figure 1. Germination rate of *Thymus broussonetii* seeds at different water potential at (a) 15°C, (b) 20°C and (c) 25°C.

water potential and temperature on the final germination depends on the species. A two-way ANOVA indicated that germination of *T. broussonetii* seeds was significantly affected by temperature and water potential. However, no significant effect of the interaction between water potential and temperature was observed (Table 1). In fact, for each water potential, no significant differences were observed between final seed germination for each incubated temperature. Regarding the evolution of seeds germination, it appears that the temperature at 20°C

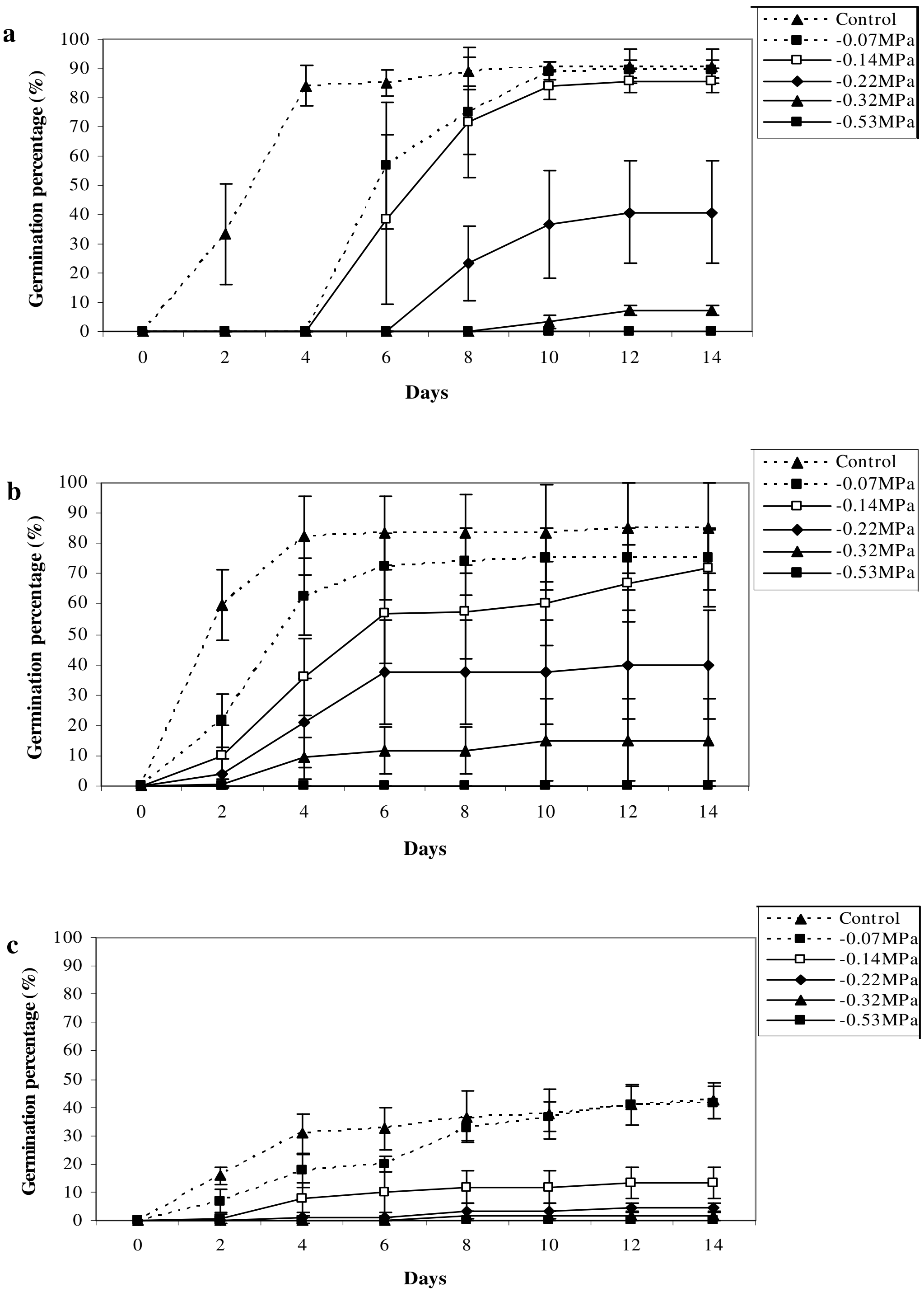

Figure 2. Germination rate of *Thymus maroccanus* seeds at different water potential at (a) 15°C, (b) 20°C and (c) 25°C.

yielded maximum germination rate (Figures 1 and 3). Osmotic potentials higher than -0.22 MPA had little effect on germination capacity of *T. broussonetii* (Figures 1 and 3). Germination of *T. maroccanus* seed was affected significantly by temperature, water potential and by the interaction of the two factors (temperature × water potential) (Table 1). In fact, the maximum final seed germination rate occurred at lower (15°C) and medium temperature (20°C) (Figure 3). The lowest seed germination was observed at higher temperature (25°C) (Figure 3). This result indicated that, in general, the temperature requirement to achieve maximum germination of the two

Table 1. Effects of water potential and temperature on the final germination percentage of *Thymus broussonetii* Boiss and *Thymus maroccanus* Ball.

Species	Source of variance	df	F value	P level
Thymus broussonetii	Temperature (T)	2	9.931	0.000*
	Water potential (Ψ)	5	140.461	0.000*
	T × Ψ	10	1.444	0.187ns
	Error	54		
Thymus maroccanus	Temperature (T)	2	128.51	0.000*
	Water potential (Ψ)	5	223.62	0.000*
	T × Ψ	10	0.221	0.000*
	Error	54		

(*) Significant at P<0.05; ns is not significant.

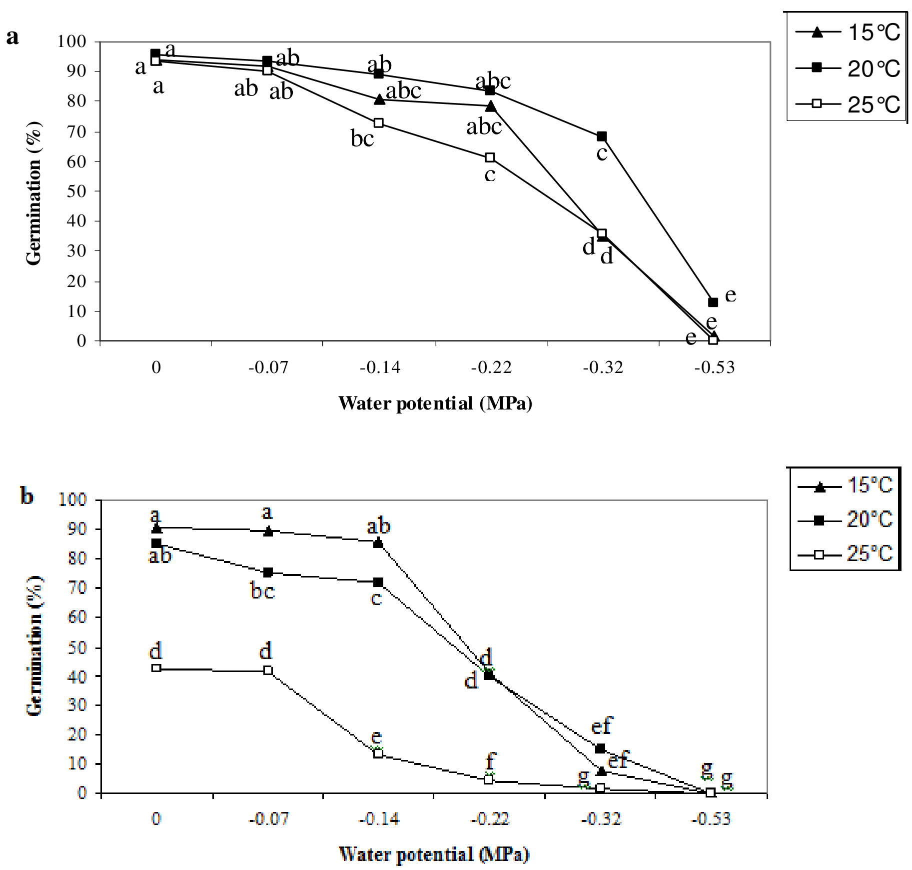

Figure 3. Final germination percentage of (a) *Thymus broussonetii*; and (b) *Thymus maroccanus* seeds at different water potentials at three temperatures (15, 20 and 25°C). Different letters indicate significant difference at $p < 0.05$.

two thyme species ranged from 15 to 20°C. This range of temperature was reported by Thanos et al. (1995) as the optimal temperature for germination of several thyme species.

The presence of an optimum temperature above and below the rate of germination declines has been noted in several reviews (Bewley and Black, 1994; Bradford, 1995). The decline in rate of germination with increasing ambient temperature might partly results from the decline in the inhibition rate. In fact, it was reported that the rate of water penetration into seeds is critical to the success of germination (Bewley and Black, 1994).

Conclusion

From this study, it can be concluded that low water potential and high temperature inhibit germination of the two thyme species. The temperature ranging from 15 to 20°C is more suitable for germination of the two thyme species because of better conditions to the germination process. The reduction of the availability of water reduces the germination and the limits for seeds germination of the two thyme species occur between 0 and -0.22 MPa. Comparing the two species, it appears that *T. broussonetii* is more tolerant to the water stress than *T. maroccanus*. This situation reflects the specific ecological distribution of each species. In fact, *T. broussonetii* is mainly located in arid and semi arid bioclimatic on the Atlantic coast between 20 to 400 m above sea level, whereas *T. maroccanus*, present essentially in the mountains between 100 to 2750 m above sea level, is largely distributed between arid and sub-humid bioclimatic (Morales, 1994; Tahiri, 1996).

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