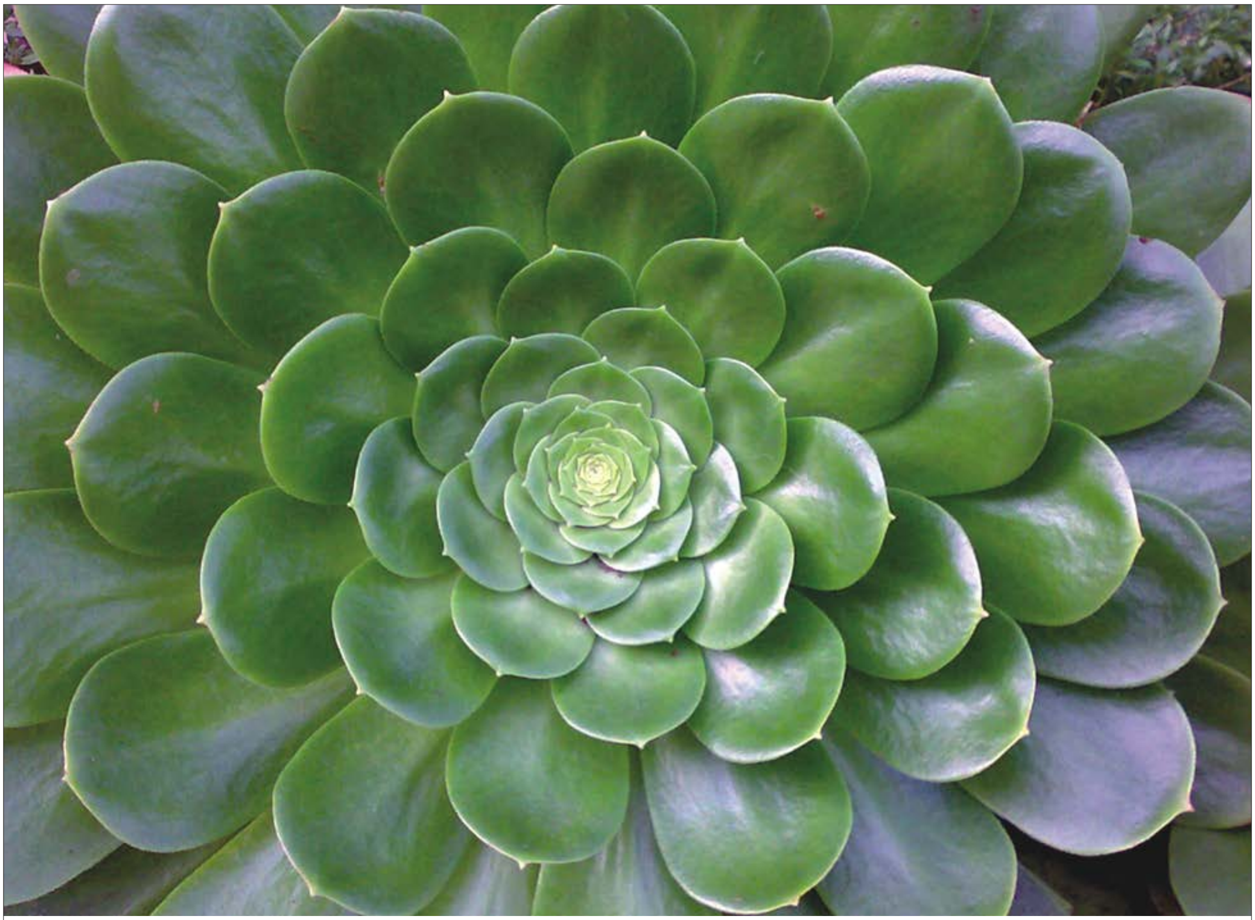

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Effect of Water Stress on Seed Germination of *Thymus serpyllum* L. from *Tessala mount*

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ABSTRACT

Thymus serpyllum L. is a xerophytic perennial herb belongs to the *Lamiaceae* family commonly used for medicinal purposes. The aim of this study is to determine the effects of water potential on germination. Seeds were germinated under stress of aqueous polyethylene glycol (PEG) solutions mixed to create water stress of -0.03, -0.07, -0.2, -0.5, -1, -1.4 and -1.6 MPa. Distilled water was used as the control (0.0 MPa). A preliminary experiment showed 20°C as the optimum germination temperature for seeds of this species. The decrease of water potential progressively declined Initial Germination Percentage (IGP) and Final Germination Percentage (FGP). The highest germination percentages (90%) were obtained under control conditions (without PEG) and increasing moisture stress progressively inhibited seed germination. Although, germination started to regress significantly at -0.5 MPa and decline completely at -1.6 MPa. However, the Initial Germination Day (IGD) and Mean Time of Germination (MTG) increased with decrease in water potential. We conclude that *T. serpyllum* is able to act as pioneer plant due to the ability of seeds to germinate under lowest water potential.

Key words: Germination, polyethylene glycol, temperature, *Thymus serpyllum* L., water stress

INTRODUCTION

Seed germination is an important and vulnerable stage in the life cycle of terrestrial Angiosperms, determines seedling establishment and plant growth. Seed germination is regulated by the interaction of environmental conditions and the state of physiological readiness (Steckel *et al*., 2004). Each plant species has a specific range of environmental requirements necessary for germination (Baskin and Baskin, 1998). 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 (Rojas-Arechiga *et al*., 1998; Flores and Briones, 2001; Ramirez-Padilla and Valverde, 2005). Indeed, the environmental conditions of the area of species occurrence are essential to determine the seed characteristics and its germination responses. Mainly, the temperature, water availability, soil or substrate type and the rate of gas exchange can promote or inhibit the germination and then influence the seed germination process (Fenner and Thompson, 2005; Cota-Sanchez and Abreu, 2007). 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.

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 200-400 mm per year for semi-arid zones ecosystems. 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).

A considerable number of medicinal and aromatic plants are locally adapted and considered as native to arid zones of the world (Bannayan *et al*., 2006). The potential of water tolerance and the economical value of medicinal and aromatic plants make them suitable alternative crops in dry land agro-ecosystems (Koocheki and Nadjafi, 2003).

Thymus is considered a genus, characteristic of the Mediterranean, as representatives of all its sections except *Thymus* sect. *Serpyllum* (Mill.) Benth. and sect. *Hyphodromi* (A. Kern.) Halacsy (subsect. *Serpyllastrum villar*) founded in the other Regions (Morales, 2002). *Thymus serpyllum* (Lamiaceae family) is a well-known as wild thyme (Nikolic *et al*., 2014) and has been extensively used in folk medicine for many years (Aziz and Habib-Ur-Rehman, 2008). The information sources indicate that this plant likes soil of medium acidity (Ellenberg, 1991). Loziene (2002) have noted that this species can live in low alkaline sole and it is found in habitats where there is good lightning.

One methodological approach commonly used to detect water stress as a limiting factor for seed germination is the application of PEG as an osmotic medium (Michel and Kaufmann, 1973). In this study, furnished data about time, rate and synchronicity of seed germination of *T. serpyllum* under water stress in order to better understand the drought tolerant. Information from this study, provides basic knowledge about germination requirements that can be used to determine tolerance of drought stress and recruitment of this species.

MATERIALS AND METHODS

Plant materiel: Seeds of *T. serpyllum* were collected in late summer, 2014 from Tessala mount in Algeria (province: Sidi Bel Abbes). This mount is semi-arid with a typical Mediterranean climate (average annual rainfall between 290 and 420 mm, Average monthly temperatures are between 9.4-26.6°C, the minimum average temperature of the coldest month varies between 2.5-3.3°C and the mean maxima of the hottest month rarely exceed 30°C). Seeds for the germination experiments were collected after maturation (harvest station is located in the following geographical coordinates: 35°16'19.19" N; 00°46'02.14" W at 802 m).

Germination experiments: After collection, immature and insect-damaged seeds were removed. Surface seeds were sterilized by soaking in sodium hypochlorite (NaOCl 1%) for 5 min and then thoroughly rinsed with deionised water (Nadjafi *et al*., 2009). Germination experiments were conducted in darkness in incubators (Memmert type). The seeds were placed in Petri dishes of 9 cm diameter fitted with two layers of Whatman No. 1 filter paper. Each treatment consisted of 4 replicates with 25 seeds. Seeds were germinated, when radical appeared (Zeng *et al*., 2014), germinated seeds were counted and removed every tow day over a period of 20 days.

Optimization of germination temperature: Since, no information is available on seed germination of *T. serpyllum*, a preliminary experiment was carried out in order to determine its

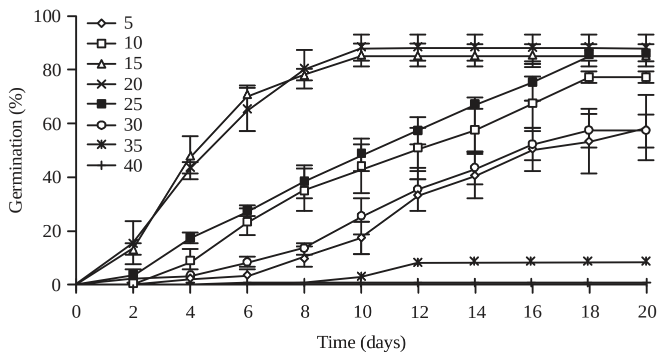

Fig. 1: Cumulative germination percentage at different incubation temperatures (°C). Bars represent ±SE (n = 4). The confidence intervals were calculated at the threshold of 5%

optimal temperature. Germination experiments were conducted in incubators set at 5, 10, 15, 20, 25, 30 and 40°C. Distilled water equal to the mean water loss from dishes was added every two days to maintain humidity. We found that the seeds germinated rapidly in distilled water in the range 10-30°C. The optimal temperature for germination was 20°C and the maximum percentage (88%) was achieved after 10 days (Fig. 1). Hence, 20°C was chosen for the study of seed germination responses to water stress in this species.

Effect of water potential: Polyethylene glycol (PEG600) was dissolved in distilled water to make solutions that had the osmotic potentials of -0.03, -0.07, -0.2, -0.5, -1, -1.4 and -1.6 MPa, using the method described by Michel and Kaufmann (1973). Distilled water was used as the control (0.0 MPa). Irrigation with different PEG concentration equal to the mean loss from dishes was added every two days to maintain humidity.

Data analysis: Five germination variables were determined; Final Germination Day (FGD), Initial Germination Percentage (IGP), Final Germination Percentage (FGP), germination kinetics and Mean Time of Germination (MTG).

MTG was calculated as follows:

$$MTG = \Sigma_i (n_i x\ d_i)/N$$

where, n_i is the number of seeds germinated at day i, d_i is the incubation period in days and N is the total number of germinated seeds (Redondo-Gomez *et al*., 2007).

Statistical analysis: Data were analysed using SPSS for windows, version 20. Analysis of variance (ANOVA) was carried out to test effects of the main factors on the final germination percentage and mean time of germination. Duncan test was used to estimate least significant range between means.

RESULTS

Effect of water potential on FGP: The decrease of water potential progressively decreases germination (Fig. 2). *Thymus serpyllum* seeds was significantly ($p<0.05$) inhibited by PEG concentration higher than -0.2 MPa (Table 1). The highest FGP was founded in distilled water

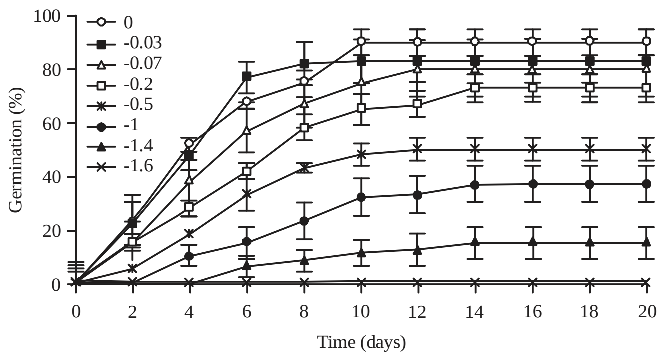

Fig. 2: Cumulative germination percentage in response to water potential (MPa). Bars represent ±SE (n = 4). The confidence intervals were calculated at the threshold of 5%

Table 1: Germination characteristic variables of *Thymus serpyllum* seeds in response to water potential (Mean±SE, n = 4)

	Temperature (20°C)			
PEG concentration (MPa)	FGD (days)	IGP (%)	FGP (%)	MTG (days)
0	9.7 ± 0.5^{ab}	23.0 ± 6.2^{a}	90 ± 5^{a}	4.6 ± 0.2^{a}
-0.03	8.4 ± 0.4^{a}	22.0 ± 7^{ab}	83 ± 10^{a}	4.4 ± 0.2^{a}
-0.07	11.4 ± 0.8^{bc}	15.0 ± 4.3^{ab}	80 ± 9^{a}	5.6 ± 0.6^{ab}
-0.2	11.6 ± 1^{bc}	12.0 ± 2.4^{ab}	73 ± 10^{a}	6.1 ± 0.5^{bc}
-0.5	11.9 ± 0.8^{abc}	8.0 ± 2^{ab}	50 ± 4^{b}	6.7 ± 0.4^{c}
-1	12.0 ± 1.2^{bc}	8.0 ± 2.3^{ab}	37 ± 7^{bc}	7.4 ± 0.6^{cd}
-1.4	13.4 ± 1.1^{c}	5.0 ± 0^{b}	15 ± 6^{cd}	8.4 ± 0.4^{d}
-1.6	-	-	0^{d}	-
F- value	8.96	4.18	57.44	26.78

Different lowercase letters (column) show significant differences between the averages ($p<0.05$), according to Duncan multiple

(90%), followed by -0.03 MPa (83%). Higher PEG concentration (-1.4 MPa) showed substantial reduction of FGP (15%), germination was completely inhibited at a concentration of -1.6 MPa (Fig. 1).

Effect of water potential on FGD, IGP and MTG: Analysis of variance revealed a significant effect of PEG on the parameters FGD, IGP and MTG (Table 1). At -0.03 MPa it is found the quickest time of FGD (8.4 days). This time started to prolong with the application of stress and the slowest time is registered at -1.4 MPa (13 days). The IGP regressed with increasing water stress with the lowest percentage at concentration of -1.4 MPa (5%). However, MTG increased with PEG concentration and we recorded (4.6 days) in the absence of stress and (8.4 days) at -1.4 MPa.

Polynomial analysis: Linear regression analysis was used to determine the relationships between the two parameters (FGP/MTG) and water stress at temperature of 20°C. There is a strong relationship between (FGP/MTG) and PEG concentration with a regression coefficient of 0.94 for the first (Fig. 3) and 0.96 for the second (Fig. 4).

Relationship between FGP and MTG at various water potential concentrations: Nevertheless, a clear distinction between the effect of PEG concentration on the FGP and MTG is evident, when examining, Fig. 5. Actually, water stress affects significantly germination percentage (estimated by the increase of MTG).

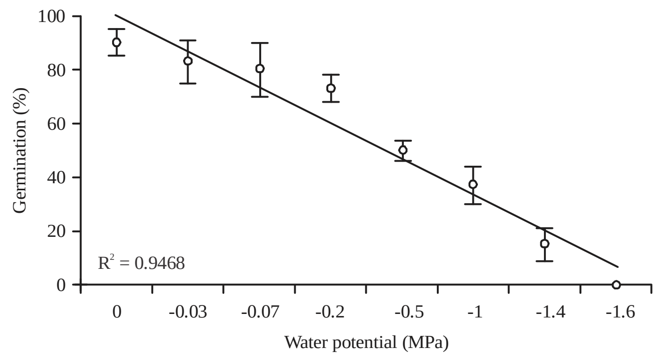

Fig. 3: Regression plot for Final Germination Percentage (FGP) in response to water potential. Bars represent ±SE (n = 4). The confidence intervals were calculated at the threshold of 5%

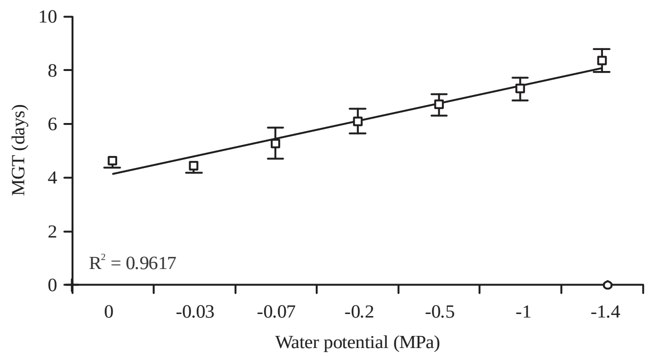

Fig. 4: Regression plot for Mean Time to Germination (MTG) in response to water potential. Bars represent ±SE (n = 4). The confidence intervals were calculated at the threshold of 5%

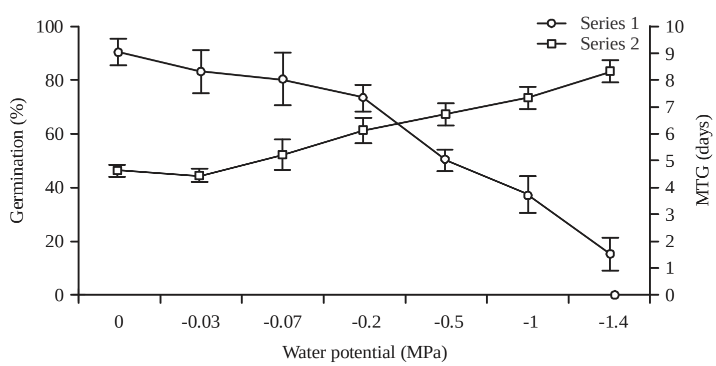

Fig. 5: Effect of various water potential concentrations FGP and MTG at 20°C. Bars represent ±SE (n = 4). The confidence intervals were calculated at the threshold of 5%

DISCUSSION

The negative effect of water stress on germination was reported by many authors for severals plants species (Amara *et al*., 2013; Rios-Rojas *et al*., 2014; Zhou *et al*., 2015). For this species, water soil has more complicated effects on germination than temperature because water is the initial factor for seed imbibitions and germination; it is directly and indirectly involved in subsequent germination metabolic stages (Cavalcante and de Perez, 1995) in particularly under arid and semi arid environment. Our results join those of Abbad *et al*. (2011), who reported that seeds of *Thymus maroccanus* and *Thymus broussonetii* are positively affected by the water potential and seeds germinate better in the absence of stress.

The decrease in germination with decrease of water potential confirms the studies of Smith (1991) and Bafeel (2014) that used sodium chloride solution as the osmoticum. At a concentration of -1 MPa it is found that the FGP drops below 50%, this could be explained by the idea that osmotic potential of 0.5 MPa is a border line for starting the reduction of germination features. The reduction of FGP may be due to alteration of enzymes and hormones found in the seed (Botia *et al*., 1998). It could also be a deficit of hydration of seeds due to high osmotic potential causing inhibition of the mechanisms leading to the output of the radicle out of the integuments and therefore delay seed germination (Gill *et al*., 2003). This osmo-regulation mechanism affects the later stages of a growth with slowdown due to the lack and/or unavailability of carbohydrates (Benjelloun *et al*., 2013).

The water stress thus decrease both FGP and speed of germination, which may be due to the seeds being unable to imbibe sufficient water from their surroundings, when as water availability becomes increasingly restricted. Such delays had been observed for *Thymus koteschanus* and *Thymus daenensis* seeds (Bagheri *et al*., 2011).

The seed germination of *T. serpyllum* under Mediterranean climate conditions starts in autumn, when precipitation usually initiates and the temperature is adequate (Benvenuti *et al*., 2001). 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). This problem can negatively affect sexual reproduction in of many Mediterranean species (Ramirez *et al*., 2008). Indeed, the species germination requires the availability of resource, essentially high soil moisture content. That's why *T. serpyllum* probably uses vegetative reproduction as a space colonization strategy.

CONCLUSION

These experiments, under water stress, revealed that germination of *T. serpyllum* is affected by water availability with a limit of about -1.6 MPa under which germination is inhibited. Therefore, germination may occur when suitable moisture conditions are present. Undoubtedly, future projections of climate change will impact in the population dynamics of this species by influencing seed germination and consequently their potential for natural regeneration.

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