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Germination Responses of *Marrubium Vulgare* L. under Various Water stress Conditions

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ABSTRACT

White Horehound (*Marrubium vulgare* L.) is a Mediterranean perennial herb belongs to the *Lamiaceae* family. 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; -0.03; -0.07; -0.2; -0.5; -1; and -1.6 MPa. The decrease of water potential progressively declined initial germination percentage (IGP) and final germination percentage (FGP). The highest germination percentages (88 ± 2 %) were obtained under control conditions without PEG, and increasing moisture stress progressively inhibited seed germination; although, germination started to regress significantly at -0.07 MPa and decline completely at -1.6 MPa. However, the Initial germination day (IGD) and mean germination time (MTG) increased with an increase in water potential. Furthermore, final germination day (FGD) was not affected significantly by water potential.
KEYWORDS: Germination; *Marrubium vulgare*; Polyethylene Glycol; water stress.

I. INTRODUCTION

Seed germination is an important and vulnerable stage in the life cycle of terrestrial Angiosperms and determines seedling establishment and plant growth, Seed germination is regulated by the interaction of environmental conditions and the state of physiological readiness [1]. Each plant species has a specific range of environmental requirements necessary for germination [2]. 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 [3,4,5]. 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 [6,7]. 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 [8], and little rainfall approximately 200 to 400 mm per year for semi-arid zones ecosystems [9]. 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 [10].

White horehound, a member of the Lamiaceae, is a perennial plant native to southern Europe, central and western Asia and North Africa [11]. As a drought tolerant plant, white horehound is found throughout areas with a minimum of 200 mm annual rainfall [12]. Furthermore, this species has very few requirements in terms of soil types, so it is often found among the first colonizers of eroded areas [13]. Because of its adaptability to different environmental conditions, white horehound can easily become a troublesome weed [14].

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 [15]. In this paper we furnish data about time, rate and synchronicity of seed germination of *M. vulgare* 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.

II. MATERIEL AND METHODS

Plant materiel

Mature seeds were collected in August 2014, from plants of *M. vulgare* growing on calcareous soil in *Tessala Mounts* (35°16’017’’N; 00°46’452’’ at 798 m north-western Algeria). This region is semi-arid with a

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typical Mediterranean climate, characterized by irregular rainfall events and a harsh dry summer period. Annual precipitation is between 290 to 420 mm and Average monthly temperatures are between 9.4 ° C and 26.6 ° C.

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 [16]. 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 n ° 1 filter paper. Each treatment consisted of 4 replicates with 25 seeds. Seeds were considered germinated when radical appeared [17], germinated seeds were counted and removed every tow day over a period of 20 days.

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 and –1.6 MPa, using the method described by Michel and Kaufmann [15]. Distilled water was used as the control (0.0 MPa). Germination tests were conducted in darkness at constant temperature of 20 °C. An irrigation with different PEG concentration equal to the mean loss from dishes was added every two days to maintain humidity.

Data analysis

Six germination variables were determined: Initial germination day (IGD), 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 [18].

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 at $p < 0.001$.

III. RESULTS AND DISCUSSION

Water potential is considered as the most important environmental signal regulating species germinations [19,20]. Analysis of variance revealed a significant effect of PEG on the parameters (IGD, FGP and MTG) while (FGD and PGI) was not really affected by water stress (Table 1).

Effect of water potential on FGP

Decrease of water potential progressively decreases germination (Fig. 1). *M. vulgare* seeds was significantly ($p < 0.001$) inhibited by PEG. The highest FGP was founded in distilled water (88 ± 2 %), followed by -0.03 MPa (85 ± 2 %). Higher PEG concentration (-1 MPa) showed substantial reduction of FGP (25 ± 4 %); germination was completely inhibited at concentration of -1.6 MPa (Fig. 1). 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 [21] in particularly under arid and semi arid environment. Our results join those of Lippai et al. [22] who reported that seeds of *M. vulgare* are negatively affected by the water potential (the FGP decreases when water potential becomes low) and seeds germinate better in the absence of stress. The decrease in germination with decrease of water potential confirms the studies of Smith [23] and Bafeel [24] who used sodium chloride solution as the osmoticum. Gharoobi *et al.* [25] suggested that concentration which causes significant reduction could be a border line of seed characteristics abatement, our results showed that at concentration of -0.07 MPa difference become significant. This can be explained by the idea that osmotic potential of this concentration is a border line for starting the reduction of germination features. The reduction of final germination percentage may be due to alteration of enzymes and hormones found in the seed [26]. 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 [27]. This osmo-regulation mechanism affects the later stages by a reduction in growth, unless if plant adapts to water deficit conditions by synthesized of carbohydrates [28], it means that when the plant is subjected to drought or salt stress, it adopts a biochemical strategy by synthesizing and accumulated carbohydrates such as soluble sugars to adjust its water potential and therefore reduce water losses [29,30,31,32,33].

Effect of water potential on IGD, MTG, FGD and IGP

Analysis of variance revealed a significant effect of PEG on the parameters (IGD and MTG). While, (FGD and IGP) was not really affected by water stress (Table 1).

IGD is also affected by stress, in the control it is found the quickest time (3.4 ± 0.4 days). This time started to prolong with the application of stress and the slowest time is registered at -1 MPa (7.4 ± 1.7 days). MTG increased with PEG concentration and we recorded (7.2 ± 0.3 days) in the absence of stress and (10.4 ± 1.1 days) at -1 MPa. The water stress thus decrease both final germination percentage 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 Australian Horehound seed [34].

Table 1. Germination characteristic variables of *M. vulgare* seeds in response to water potential (mean ± SE, n = 4).

Temperature 20 °C					
PEG concentration (Mpa)	IGD (days)	FGD (days)	IGP (%)	FGP(%)	MTG (days)
0	3.4 ± 0.4^{a}	13 ± 0.8^{a}	8 ± 6^{a}	88 ± 2^{a}	7.2 ± 0.3^{a}
-0.03	3.6 ± 0.4^{a}	12.6 ± 1^{a}	5 ± 4^{a}	85 ± 2^{a}	8.1 ± 0.5^{ab}
-0.07	4 ± 0^{ab}	14 ± 0.8^{a}	7 ± 2.3^{a}	68 ± 6^{b}	8.6 ± 0.7^{ab}
-0.2	4.5 ± 0.5^{ab}	16 ± 0.6^{a}	5 ± 4^{a}	58 ± 2^{b}	9.5 ± 0.2^{ab}
-0.5	4.7 ± 0.8^{ab}	13 ± 0.8^{a}	2 ± 2.4^{b}	40 ± 4^{c}	9.9 ± 0.5^{ab}
-1	7.4 ± 1.7^{b}	12.7 ± 1.2^{a}	2 ± 2.3^{b}	25 ± 4^{c}	10.4 ± 1.1^{b}
-1.6	-	-	-	0^{d}	-
F- value	7.024	3.93	0.963	86.50	7.04

Different lowercase letters (column) show significant differences between the averages ($p < 0.001$), according to Duncan multiple.

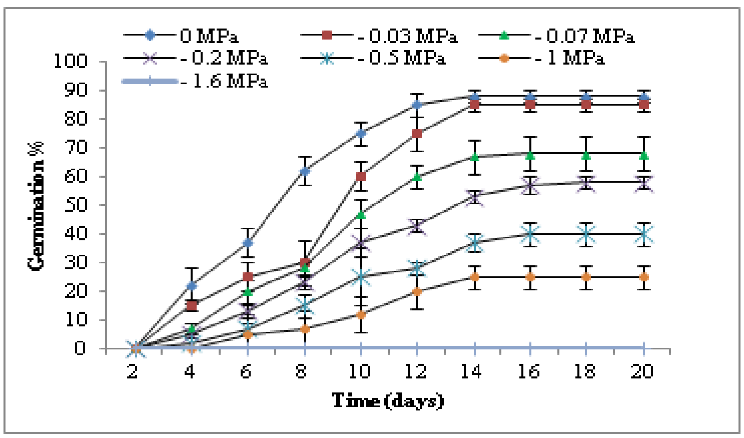

Fig. 1. Cumulative germination percentage in response to water potential. Bars represent ± S.E. (n = 4). The confidence intervals were calculated at the threshold of 5%.

Polynomial analysis

Polynomial 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 0.96 for the first (Fig. 2) and 0.97 for the second (Fig. 3). These results were similar to those of Mohammadi Nasab [35] who reported that regression coefficient of *Lens culinaris* seeds was 0.96.

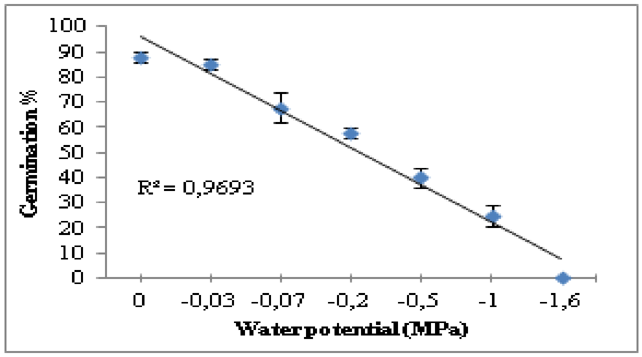

Fig. 2. Regression plot for final germination percentage in response to water potential. Bars represent ± S.E. (n = 4). The confidence intervals were calculated at the threshold of 5%.

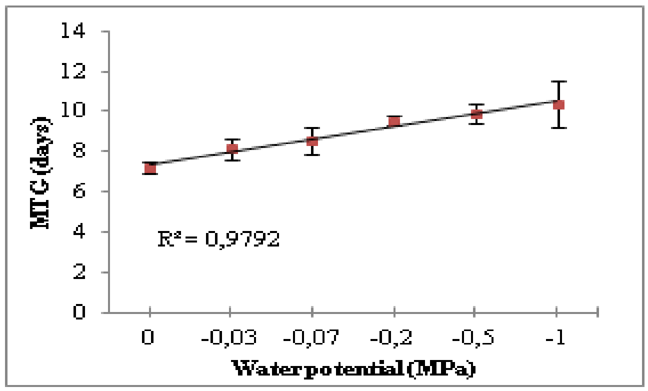

Fig. 3. Regression plot for mean time to germination in response to water potential. Bars represent ± S.E. (n = 4). The confidence intervals were calculated at the threshold of 5%.

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. 4. Actually, water stress affects significantly germination percentage (estimated by the increase of MTG). Same results has been found by Benjelloun et al. [28] when they studied the tolerance to water stress of different ecotypes of *Myrtus communis* at the germination stage.

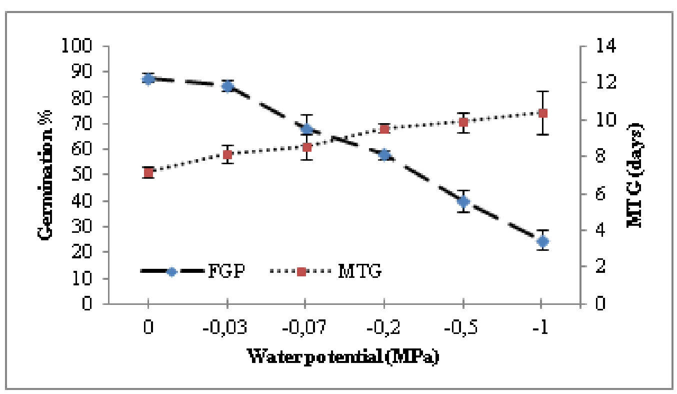

Fig. 4. Effect of various water potential concentrations FGP and MTG at 20 °C. Bars represent ± S.E. (n = 4).0 The confidence intervals were calculated at the threshold of 5%.

The seed germination of *M. vulgare* under Mediterranean climate conditions starts in autumn, when precipitation usually initiates and the temperature is adequate [14]. 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 [36]. This problem can negatively affect sexual reproduction in of many Mediterranean species [37]. Indeed, the species germination requires the availability of

resource, essentially high soil moisture content. That's why *M. vulgare* probably uses vegetative reproduction as a space colonization strategy.

CONCLUSION

These experiments, under water stress, revealed that germination of *M. vulgare* 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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