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SHORT COMMUNICATION

Germination responses of *Ballota hirsuta* seeds under conditions of temperature, salinity and water stress

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Summary Temperature, salinity and water deficit can be major environmental constraints which reduce distribution of indigenous plants in the Mediterranean region. Laboratory experiments were carried out to assess the effect of temperature, sodium chloride (NaCl) and polyethylene glycol 6000 (PEG) on germination of *Ballota hirsuta* seeds. The germination responses of seeds were determined over a wide range of temperature (5 to 35°C), salinity (0 to 136 mM NaCl) and water stress (0 to -1 MPa PEG). Germination percentage was optimum at 20°C (78%), and showed a decline at lower (5°C, 25%) or higher (30°C, 18%) temperature values and total inhibition of germination at 35°C. Under salinity and water stress conditions, there was a significant deterioration in most germination parameters such as lower final germination percentage, increased mean germination times and lower germination rates.

Additional keywords: *Ballota hirsuta*, NaCl, PEG, salt stress, seeds, water deficit

Introduction

Ballota hirsuta L. (Lamiaceae) is a Mediterranean wild shrub that is mainly used in traditional medicine (Kechar *et al.,* 2016). Aqueous plant extract of *B. hirsuta* is known to inhibit growth in larvae of *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) (Pascual-Villalobos and Robledo, 1999). *Ballota hirsuta* is currently under biotic and abiotic pressures such as overexploitation, global warming, soil salinity and drought (Underwood *et al*., 2009). Knowledge on the effects of the environmental conditions on seed germination would assist to carry out reintroduction and conservation programs of existing populations in Tessala mount, Algeria.

Several environmental factors such as temperature, light, pH, and soil moisture are known to affect seed germination (El-Keblawy and Al-Rawai, 2006). Amongst those, temperature is the most prominent factor regulating germination and establishment of plants (Koger *et al*., 2004). Use of cardinal temperatures makes it possible to estimate geographical limitations for seed germination and select the most suitable time for plant establishment (Ramin, 1997).

Soil salinity is a major problem limiting plant distribution and productivity. Therefore, tolerance to salinity during germination is critical for the establishment of plants growing in arid and semi-arid regions (Khan and Gulzar, 2003). In such salinity conditions, seed germination would be successful only after high rainfall precipitation as soil salinity is usually reduced due to leaching (Redondo-Gomez *et al*., 2007).

Water deficit is an important constraint disrupting plant production worldwide (Kaya *et al*., 2006). Under drought stress, seed germination is inhibited due to low water potential that results in a decline in water uptake (Farooq *et al*., 2009). A common methodology to measure the water stress effects on seed germination is the application of polyethylene glycol (PEG) as an osmotic medium (Michael and Kaufman, 1973).

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The objective of this study was to determine the effect of temperature, osmotic stress, and salt stress on seed germination patterns of *B. hirsuta*.

Materials and methods

Seed collection site and germination experiments

Mature seeds were collected from plants of *B. hirsuta* growing in Tessala mounts located in North-Western Algeria. The climate in this region is semi-arid with typical Mediterranean characteristics (average annual rainfall is between 290 and 420 mm and average monthly temperatures are between 9.4-26.6°C).

Intact seeds were surface sterilized by sodium hypochlorite solution (0.58%) for 5 minutes, clean washed with distilled water and left to dry out. Germination studies were conducted on seeds placed on two disks of filter papers Whatman No. 1 in Petri dishes. The experiments were conducted in darkness. For the test of temperature effect, seeds were incubated at cardinal temperatures of 5, 10, 15, 20, 25, 30 and 35°C, moistened with distilled water. Tests on salinity and water deficit stress were conducted under optimum seed germination temperature conditions (20°C). In particular, seed germination was tested at saline concentrations of 0, 2, 4, 6 and 8 g/l NaCl, which were transformed to molarity (M), and at PEG solutions of 0, -0.03, -0.07, -0.2, -0.5 and -1 MPa. Three replicates of 20 seeds per treatment were used. Germinated seeds were counted every two days up until the final germination.

Methods of germination expression and statistical analysis

The following seed germination parameters were determined: Final germination percentage (FGP), initial germination day (IGD), final germination day (FGD), mean time of germination (MTG) and germination rate (GR).

MTG was calculated as follows:

$MTG = \Sigma i\ (n_i \times d_i)/N$ (Redondo-Gomez *et al.*, 2007).

Where:

n_i : number of seeds germinated at day *i*,

d_i : incubation period in days,

N : total number of germinated seeds.

GR is defined as days needed to reach 50% of final germination percentage (Farooq *et al.*, 2005) and it was calculated as follows:

$$GR = \frac{[(N/2)-N_1) \times (T_2-T_1)]}{N_2-N_1} + T_1$$

Where:

N : total number of germinated seeds,

N_1 : number of germinated seeds slightly less than $N/2$,

N_2 : number of germinated seeds slightly higher than $N/2$,

T_1 : incubation period corresponding to N_1,

T_2 : incubation period corresponding to N_2.

Analysis of variance (ANOVA) was carried out to test effects of the main factors. Duncan test was used to estimate least significant difference between means. Data were analyzed using SPSS for windows, version 20.

Results

Regarding the effect of temperature on seed germination, seeds of *B. hirsuta* can germinate at temperatures between 5°C and 30°C, however germination percentages are low (25% and 18%, respectively). The optimal temperature was 20°C (78% germination) (Table 1). At the optimum temperature for germination, the shortest mean time of germination (MTG = 10.8 days) and initial germination day (IGD = 5.4 days) were recorded, although not statistically significantly different to those of higher temperatures. At high temperatures (30°C), the shortest final germination day (FGD = 13 days) was measured. The rate of germination, decreased at temperatures over 20°C.

Salt stress significantly inhibited germination of *B. hirsuta* seeds (Table 2). The highest germination percentage (FGP = 72%)

was measured in distilled water. The germination percentage decreased significantly as salinity increased (at 102 mM NaCl, 7% germination) with total inhibition of germination at 136 mM NaCl. The initial germination day and final germination day were significantly delayed at 102 mM NaCl (0 mM, 6 days vs 102 mM, 10.4 days for IGD, and 0 mM, 17.6 days vs. 102 mM, 11.5 days for FGD). Germination rate declined significantly when salinity increased to 68 mM NaCl (0 mM, 11 days vs. 68 mM, 8.84 days).

Water stress significantly inhibited germination of *B. hirsuta* seeds (Table 3). Control showed the maximum germination percentage (FGP = 72 %) (although statistically significantly higher only compared to that at -0.5 MPa PEG), the shortest mean time of germination (MTG = 10.8 days) and the quickest initial and final germination day (IGD = 5.6 days, FGD = 16.6 days) (although not statistically significantly different to the MTG and IGD values of the -0.03 MPa PEG). The germination percentage decreased significantly when water stress increased at -0.5 MPa PEG (FGP = 23% germination) with total inhibition of germination at -1.0 MPa PEG. Initial germination day was delayed when PEG was higher than -0.07 MPa and germination rate increased at PEG values higher than -0.07 MPa, compared to the control (0 MPa, 9.64 days vs -0.2 MPa, 14.44 days).

Table 1. Germination parameters of *Ballota hirsuta* seeds in response to temperature (mean ± SE, n = 3): initial germination day IGD (days); final germination day FGD (days); final germination percentage FGP (%); mean time of germination MTG (days); germination rate GR (days).

Temperatures (°C)	IGD (days)	FGD (days)	FGP (%)	MTG (days)	GR (days)
5	12.6 ± 1.2^{a}	18.7 ± 1.2^{a}	25 ± 4^{a}	17.6 ± 1.8^{a}	15.14 ± 0.9^{a}
10	11.4 ± 0.4^{a}	18.4 ± 0.4^{a}	53 ± 8^{b}	17.2 ± 3.2^{ab}	14.38 ± 0.6^{a}
15	7.6 ± 0.8^{b}	18.2 ± 1.2^{a}	68 ± 5^{bc}	14.8 ± 1.4^{abc}	14.75 ± 1.1^{a}
20	5.4 ± 0.4^{c}	16.0 ± 0.8^{b}	78 ± 4^{c}	10.8 ± 1.2^{c}	10.30 ± 1.4^{bc}
25	5.8 ± 0.5^{bc}	18.6 ± 0.6^{a}	57 ± 7^{b}	12.2 ± 1.2^{bc}	11.22 ± 1.6^{b}
30	6.4 ± 0.6^{bc}	13.0 ± 0.8^{b}	18 ± 4^{a}	12.5 ± 0.8^{bc}	8.50 ± 0.0^{c}
35	-	-	-	-	-
F-value	58.31	6.82	71.70	6.70	19.63

[1] Different lower case letters (column) show significant differences between the means (P ≤ 0.05) according to Duncan's Multiple Range test.

Table 2. Germination parameters of *Ballota hirsuta* seeds, incubated at 20°C, in response to salinity (mean ± SE, n = 3): initial germination day IGD (days); final germination day FGD (days); final germination percentage FGP (%); mean time of germination MTG (days); germination rate GR (days).

NaCl (mM)	Parameters				
	IGD (days)	FGD (days)	FGP (%)	MTG (days)	GR (days)
0	6.0 ± 0.8^{a}	17.6 ± 1.8^{ab}	72 ± 5^{a}	11.6 ± 0.6^{a}	11.0 ± 0.9^{ab}
34	7.4 ± 0.4^{a}	19.0 ± 2.6^{a}	40 ± 8^{b}	13.2 ± 1.5^{a}	12.5 ± 2.8^{a}
68	7.6 ± 0.8^{a}	14.6 ± 1.4^{bc}	25 ± 7^{bc}	10.4 ± 0.8^{a}	8.84 ± 1.1^{b}
102	10.4 ± 1.4^{b}	11.5 ± 1.6^{c}	7 ± 3^{cd}	9.6 ± 1.2^{a}	9.67 ± 1.0^{ab}
136	-	-	-	-	-
F- value	19.77	15.66	38.19	4.11	2.83

[1] Different lower case letters (column) show significant differences between the means (P ≤ 0.05) according to Duncan's Multiple Range test.

Table 3. Germination parameters of *Ballota hirsuta* seeds, incubated at 20 °C, in response to water stress (mean ± SE, n = 3): initial germination day IGD (days); final germination day FGD (days); final germination percentage FGP (%); mean time of germination MTG (days); germination rate GR (days).

PEG (MPa)	IGD (days)	FGD (days)	FGP (%)	MTG (days)	GR (days)
0	5.6 ± 0.5[a]	16.6 ± 2.2[a]	72 ± 9[a]	10.8 ± 0.8[a]	9.64 ± 0.3[a]
-0.03	6.6 ± 0.8[ab]	20.6 ± 1.4[bc]	68 ± 10[a]	12.6 ± 1.2[ab]	11.92 ± 1.3[ab]
-0.07	7.8 ± 1.2[bc]	22.8 ± 0.8[c]	62 ± 8[a]	13.4 ± 1.5[bc]	12.92 ± 2.3[bc]
-0.2	8.6 ± 0.8[c]	24.2 ± 1.2[c]	55 ± 4[a]	15.8 ± 2.2[cd]	14.44 ± 1.2[c]
-0.5	9.0 ± 1.5[c]	17.8 ± 0.6[ab]	23 ± 5[b]	16.2 ± 1.8[d]	11.37 ± 0.3[ab]
-1	-	-	-	-	-
F- value	12.35	17.21	26.50	15.50	5.65

[1] Different lower case letters (column) show significant differences between the means (P ≤ 0.05) according to Duncan's Multiple Range test.

Discussion

Germination patterns of *B. hirsuta* are similar to that of some other medicinal plants that could maintain a relatively high germination percentage over a wide range of temperatures (Bannayan *et al*., 2006). In our study, it was shown that the best germination patterns (maximum final germination percentage (FPG), the shortest mean time of germination (MTG) and initial germination day (IGD)) were measured at temperature range between 10 to 25°C with the optimum temperature at 20°C. This specific thermal optimum also characterizes other Mediterranean species of arid and semi-arid regions belonging to the Lamiaceae family such as *Lavandula dentata*, *Teucrium gnaphalodes*, *Thymbra capitata* and *Thymus hyemalis* (Estrelles *et al*., 1999; Kadis and Georghiou, 2010). Additionally, Corme (1993) reported that seeds of *Salvia officinalis* and *Salvia sclarea* (Lamiaceae) could germinate satisfactorily in a wide range of temperatures 10-25°C and 10-30°C, respectively.

Salt stress significantly inhibited germination of *B. hirsuta* seeds and showed a deterioration of values regarding the final germination percentage and the onset of germination as compared to distilled water. This is in agreement with studies demonstrating that distilled water is the most suitable medium for germination of seeds (Huang *et al*., 2003; Deng *et al*., 2014). Our results showed that *B. hirsuta* seed germination was more affected by salinity as compared with other species in the literature e.g. *Campis radicans*, 20% germination at 160 mM NaCl (Chachalis and Reddy, 2000). The negative response of salinity on seed germination might be due to a potential reduction in cellular water or a decrease in hydration of proteins and the enzymatic activity involved in the germination process (Noguchi and Macias, 2005).

Water stress also significantly inhibited germination of *B. hirsuta* seeds by affecting negatively the initial and final day of germination and increasing the mean time of germination. The percentage of seed germination was dramatically decreased at -0.5 MPa PEG. Our results were similar with previous reports on the germination of other species belonging to Lamiaceae family including those of Abbad *et al*. (2011) who reported a decrease in germination capacity of *Thymus maroccanus* and *Thymus broussonetii* seeds at -0.53 MPa. Krichen *et al*. (2014) reported that the germination of *Stipa tinaciccima*, a plant distributed in semi-arid climate areas (similar to *B. hirsuta*), was also very sensitive to a range of water potential from 0 to -0.8 MPa. Plants adapted to arid conditions such as *B. hirsuta*, might have the ability to absorb sufficient water amounts during seed germination from their surroundings even when there is a water restriction.

Ballota hirsuta germinates over a broad range of temperatures and environmental conditions corresponding to the spring season in the Mediterranean region. Seed germination of *B. hirsuta* was negatively affected by salinity and water stress indicating that these conditions would restrict its distribution in the natural habitats and its potential utilization as a medicinal plant. This information should also be taken into account in programs of reintroduction of the plant in Algeria that should be implemented in non-saline soil and in zones with high rainfall precipitation.

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ΣΥΝΤΟΜΗ ΑΝΑΚΟΙΝΩΣΗ

Βλαστικότητα σπόρων του *Ballota hirsuta* L. υπό οριακές συνθήκες θερμοκρασίας, αλατότητας και νερού

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Περίληψη Η θερμοκρασία, η αλατότητα και η έλλειψη νερού μπορεί να είναι σημαντικοί περιβαλλοντικοί περιορισμοί στην εξάπλωση των αυτοφυών φυτών στην περιοχή της Μεσογείου. Στην παρούσα μελέτη διεξήχθησαν εργαστηριακά πειράματα για την εκτίμηση των επιδράσεων της θερμοκρασίας, του χλωριούχου νατρίου (NaCl) και της πολυαιθυλενογλυκόλης 6000 (PEG) στη βλαστικότητα των σπόρων του ιθαγενούς Μεσογειακού θάμνου *Ballota hirsuta*. Η βλαστικότητα των σπόρων προσδιορίστηκε σε ένα ευρύ φάσμα θερμοκρασιών (5 έως 35°C), αλατότητας (0 έως 136 mM NaCl) και υδατικής στέρησης (0 έως -1 MPa PEG). Η βλάστηση των σπόρων ήταν βέλτιστη στους 20°C (78%), μειώθηκε σε χαμηλότερες (5°C, 25%) ή υψηλότερες θερμοκρασίες (30°C, 18%) ενώ στους 35°C σημειώθηκε αναστολή της βλάστησης. Κάτω από συνθήκες υψηλής αλατότητας και υδατικού στρες, παρατηρήθηκε σημαντική μείωση στις περισσότερες παραμέτρους βλάστησης δηλαδή χαμηλότερο τελικό ποσοστό βλάστησης, αυξημένος μέσος χρόνος βλάστησης και μικρότερος ρυθμός βλάστησης.

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