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REGULAR ARTICLE

INFLUENCE OF WATER STRESS ON SEED GERMINATION CHARACTERISTICS IN INVASIVE *DIPLOTAXIS HARRA* (FORSSK.) BOISS (BRASSICACEAE) IN ARID ZONE OF TUNISIA

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SUMMARY

Diplotaxis harra (Forssk.) Boiss (Brassicaceae) has a wide ecological and geographical amplitudes in Tunisia and grows under a variety of environmental conditions. A laboratory experiment was performed to evaluate the effect of water potential on seed germination and recovery responses after transfer to distilled water. The germination responses of seeds at 15°C in complete darkness were determined over a wide range of PEG-6000 solutions of different osmotic potentials: 0 to –1 MPa. Greatest germination was obtained in distilled water (71%), and increases in osmolality of solutions progressively inhibited seed germination, less than 10% of the seeds germinated at –0.8 MPa. No germination was observed for the treatment of –1 MPa. The rate of germination decreased as osmotic potential decreased. When seeds were transferred to distilled water after 20 days of water stress, the recovery of germination increased with an increase in pretransfer PEG-6000 treatments. Seeds subjected to high osmotic potential had higher recovery percentages.

Keywords: *Diplotaxis harra*, Germination, Recovery, Temperature, Water stress

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1. Introduction

In arid ecosystems, the establishment of species may differ in their life cycle (annual/perennial), life form (shrubs/ herbaceous), response to drought (tolerance/ avoidance), time of flowering, reproductive effort, seed dispersal, and germination behaviour. Plants in these ecosystems have developed complementary adaptations and survival strategies throughout the stages of their life cycles. Among these stages, the seed has the highest resistance to extreme environmental factors, whereas the seedling has the lowest (Gutterman, 2002). In extreme desert conditions, it is the pattern of germination response to environmental factors that determines whether a particular species can appear or not in a specific year. Successful establishment of plants largely depends on successful germination. Germination is a crucial stage in the life cycle of plants and tends to be highly unpredictable over space and time. Several environmental factors such as temperature, salinity, light, and soil moisture (Evans and Etherington, 1990; El-Keblawy and Al-Rawai, 2005; Zheng, et al 2005; Gorai and Neffati, 2007) interact in the soil interface, which regulate seed germination. Water availability is one of the environmental factors that influences most the germinative process (Bewley and Black, 1994; Larson and Kiemnec, 1997). A threshold amount of rainfall is necessary for desert annuals to germinate in their growing season, though the seeds of some species germinate in low soil water potential. Neffati (1994) reported that the seeds of desert plants germinate faster than those from other habitats. In these regions, germination occurs during rainy seasons when soil salinity levels are usually reduced (Gorai and Neffati, 2007).

Diplotaxis harra (Forssk.) Boiss of the family of Brassicaceae, is an annual herb that grows in many regions in arid Tunisia (Tlig et al 2008). It is a ramified stem herb which occurs in both sandy and gypseous soils reaching a height of 50 to 60 cm (Pottier-Alapetite, 1979; Chaieb and Boukhris, 1998) and produces many small seeds that tend to persist for longer in the soil, which improved the existence of persistent soil seed banks further (Hegazy, 2001; Tlig et al 2008). *D. harra* has ephemeral, modular and coppiced life-cycles and relatively wet years cause the ephemeral plants to live longer and become perennials, with ability to shift from a r- to a K-selected strategy. The intra-population variations and trade-off between r- and K-life-cycles ensures plant survival under unpredictable desert environments (Hegazy, 2001).

In the field, establishment of *D. harra* varies according to the season (cool/warm), the geographical and ecological distribution as well as the amount of rainfall. Seasonal fluctuations of temperature and rain in deserts of Tunisia explain the variable establishment pattern of this plant during the course of a year, governed by seasonally different of temperatures and the rainfall events (Tlig et al 2008). There is little available information in literature on the ecology of seed germination of *D. harra*. Thus, the objectives of this study were (1) to measure the responses of *D. harra* to a wide range of osmotic potential levels obtained with polyethyleneglycol, simulating drought conditions, in order to evaluate the resistance of seeds to water stress; and (2) to investigate the response of recovery of germination under such conditions.

2. Materials and Methods

Seed collection site

Seeds of *Diplotaxis harra* were obtained from plants which were collected from a location near El Fjé, Médenine (10° 39'N, 33° 30'E; Southeast Tunisia) in June 2006. One thousand seeds weighted, on average, 400 mg. See Tlig et al. (2008) for details on climate.

Germination experiments

To determine the germination response of this species to a gradient of water potentials we used different concentrations of polyethylene glycol (PEG). PEG is a nonpenetrating, inert osmoticum and forms a colloidal solution, the effect of which is similar to the matric properties of soil particles. The method gives a good estimate of germination behaviour in relation to soil moisture under field conditions (Hadas, 1977). To simulate water stress, PEG-6000 solutions equivalent to the following osmotic potentials, prepared according to Michel and Kaufmann (1973), were used: 0, –0.1, –0.2, –0.4, –0.6, –0.8, and –1 MPa. Seeds were surface sterilized in 0.58 % sodium hypochlorite for one minute, subsequently washed with distilled water and air-dried before being used in the germination experiments to avoid fungus attack. Ninety-millimetre Petri dishes containing two disks of Whatman No. 1 filter papers with 5 ml of test solution were prepared. Germination experiments were conducted in incubator (Lumincube II, analys, Belgium; MLR-350, Sanyo, Japan) set at 15°C in complete darkness (Tlig et al 2008). A completely randomised design was used in the germination tests. For each treatment, four replicates of 25 seeds each were used. During 20 days the germinated seeds were counted and removed every second day. A seed was considered to have germinated when the emerging radicle elongated to 2 mm. Solutions were renewed every 2 days under sterile conditions to ensure relatively constant $\Psi\pi$ in the treatments.

Methods of germination expression

Three characteristics of germination were determined: final germination percentage, rate of germination, and recovery of germination. The rate of germination was estimated using a modified Timson's index of germination velocity = $\Sigma G/t$, where G is the percentage of seed germination at 2-day intervals and t is the total germination period (Khan and Ungar, 1984). The maximum value possible for our data using this index was 50 (i.e. 1000/ 20). The greater the value, the more rapid is the germination. All seeds from the previous germination tests which did not germinate after 20 days at different PEG-6000 treatments, were placed in new Petri dishes with filter paper moistened with distilled water, and incubated under the same conditions for additional 20 days to study the recovery of germination. The recovery percentage was determined by the following formula: $(a-b)/(c-b)*100$, where a is the total number of seeds germinated after being transferred to distilled water, b is the total number of seeds germinated at different osmotic potentials, and c is the total number of seeds.

Statistical analysis

Germination data were arcsine transformed before statistical analysis to ensure homogeneity of variance. Data were analysed using SPSS for Windows, version 11.5 (SPSS, 2002). A one-way analysis of variance (ANOVA) was carried out to test effects of water stress on the rate and final percentage of germination. Tukey test (Honestly significant differences, HSD) was used to estimate least significant range between means.

3. Results and discussion

Water stress significantly ($P < 0.001$) affected the final germination percentage of *D. harra* (Table 1).

Table 1. A one-way ANOVA of the effect of PEG-6000 on germination of *Diplotaxis harra*

Dependent variable	df	Mean-square	F-ratio
Percentage of germination	6	0.385	35.618
Rate of germination	6	0.064	37.464
Germination recovery	5	0.040	19.133

Note: Data represent F-values significant at $P < 0.001$.

Maximum seed germination was obtained in distilled water and an increase in PEG-6000 solutions of different osmotic potentials resulted in a gradual decrease in percent germination, and only 5% of seeds germinated at –0.8 MPa (Fig. 1a,b). Seeds germinated rapidly in distilled water during the initial 2 days, while the delay of germination increased with increasing osmolality and was more obvious at –1 MPa (Fig. 1a). *D. harra* germinate better at intermediate temperatures (10, 15 and 20°C), with a thermal optimum at 15°C (Tlig et al 2008). This behaviour is a typical strategy of Mediterranean plants with optimal temperatures ranging between 15 and 20°C (Thanos et al 1995; Baskin and Baskin, 1998). The temperature requirements of germination can be interpreted as an adaptation of this plant species to seasonal fluctuations of temperature and rain in deserts of Tunisia, because seed germination starts following rains in early winter, when temperatures were decreasing (Tlig et al 2008).

Figure 1. Changes in germination percentage of *Diplotaxis harra* as influenced by PEG-6000 solutions of different osmotic potentials: (●) 0 MPa, (○) –0.1 MPa, (▲) –0.2 MPa, (Δ) –0.4 MPa, (■) –0.6 MPa, (□) –0.8 MPa, and (♦) –1 MPa. (a): germination kinetics. The symbols are the observed numbers of germinated seeds, expressed as % of sown seeds. (b): Germination percentage after 20 days on the indicated PEG-6000 solutions (open bars). Black bars: recovery percentage of germination after being transferred to distilled water for additional 20 days. Values of the germination percentages (mean ± 95% confidence limits, n = 4), having different letter are significantly different at P < 0.05 (Tukey test).

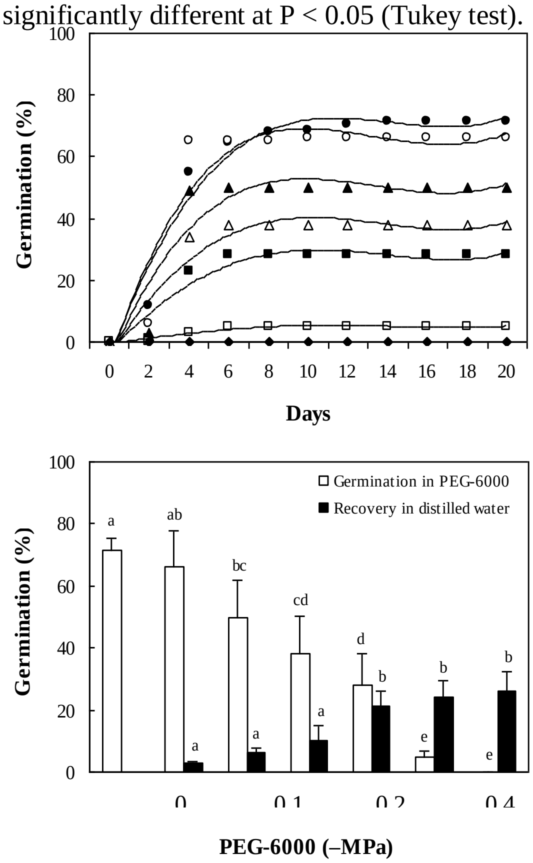
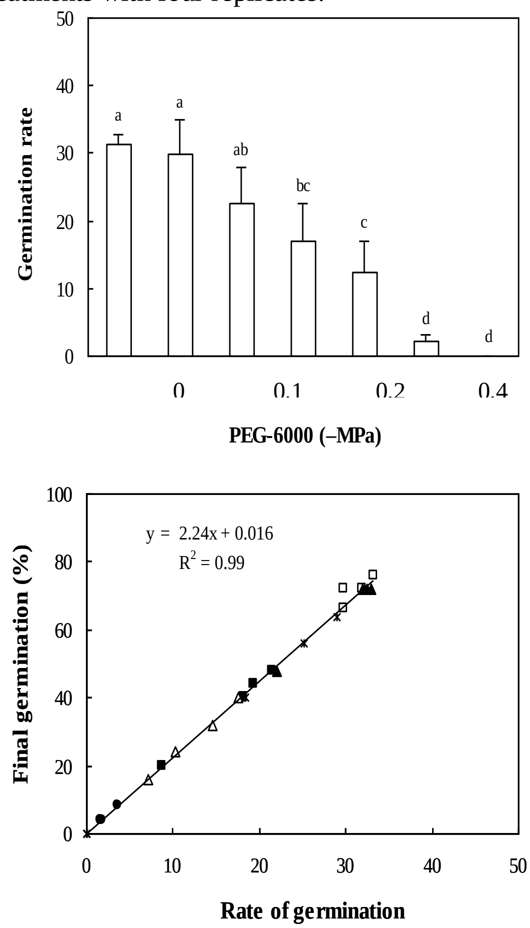


Figure 2. Mean rate of germination of *Diplotaxis harra* seeds (a) and relationship between final germination percentage and rate of germination (b) as influenced by PEG-6000 solutions of different osmotic potentials: (●) 0 MPa, (○) –0.1 MPa, (▲) –0.2 MPa, (Δ) –0.4 MPa, (■) –0.6 MPa, (□) –0.8 MPa, and (♦) –1 MPa. Values of rate of germination (mean ± 95% confidence limits, n = 4) at each osmotic potential having different letter are significantly different at P < 0.05 (Tukey test). Lines describing the relation between these parameters were obtained by linear regression. Values (n = 28) are from the six PEG-6000 treatments with four replicates.



In saline and dry soils, water potential is not very different to that of desiccated seeds. Therefore, at high osmotic potentials water does not enter the seeds and induces germination. Our results show that the highest germination was obtained in distilled water (71%), and an increase in osmolality of PEG solutions results in decreasing both the rate and percentage of germination. This was in agreement with the germination behavior of most species (Evans and Etherington, 1990; Murillo-Amador et al 2002; Zheng et al 2005; Tobe et al 2006). Imbibition is an essential prerequisite of germination (Almansouri et al 2001), and its rate and extent is governed by the surrounding soil water potential and the resistance to movement of water in the soil-seed system (Evans and Etherington, 1990).

When seeds of *D. harra* were transferred to distilled water after 20 days of water stress treatment, the recovery of germination percentages increased significantly with an increase in pretransfer PEG-6000 treatments (Table 1, Fig. 1b). Seeds subjected to high osmotic potential had higher recovery percentages. Tlig et al. (2008) reported that *D. harra* was moderatly salt tolerant at the germination stage. Germination percentage decreases with increasing salinity and comes to the limit at 200 mM. Salt stress decreases both rate and percentage of germination, but recovery is possible as long as salinity is low (Tlig et al 2008). A fraction of moistened seeds that did not germinate with decreasing osmotic potential builds up a seed bank in the soil. Such seeds secure the long term existence of seed bank helping the species spreading germination over years and using opportunistically suitable germination conditions (Caballero et al 2003). However, the lack of summer germination in the field may be as well the result of dormancy that reduces the risk of seedling mortality, when moisture is limited and salinity is increased (Baskin and Baskin, 1998).

The rate of germination, calculated by using a modified Timson's index, decreased as osmotic potential was intensified (Fig. 2a). A one-way ANOVA of the rate of germination indicated a significant effect ($P < 0.001$) of drought stress (Table 1). Linear regression analysis was used to determine the relationships between final germination percentage and rate of germination at different osmotic potential levels. There was a strong positive relationship identified between these parameters, with a coefficient of determination $R_2 = 0.99$ (Fig. 2b). Seed imbibition rate, germination percentage and germination rate generally decrease as soil water potential decreases (Murillo-Amador et al 2002; Song et al 2005; Sosa et al 2005), either by drought or by higher salinity.

Seed-soil contact has been assumed to be the most important factor for rapid transfer of water from soil to seed. Moreover, in some species 85 % or more of the water absorbed by seeds can be directly attributed to vapor (Wuest, 2007). It is well known that wetting by solutions of increased osmolality – as a rule high NaCl concentrations or PEG solutions – may have a preconditioning effect. Preconditioning, or priming, increases the uniformity and rate of germination in comparison with non-primed seeds.

In general, annual species need to control their germination closely to minimize the risk of local extinction caused by germinating under unfavorable conditions, but perennial species are free of such a control since the lack of a seedling cohort is far less threatening for population survival (Baskin and Baskin, 1998; Fenner and Thompson, 2005). In accordance with this generality, *D. harra*, a common annual species in the desert of Tunisia, has the ability to shift from r- to K-selected life-cycle and to take advantage of years with sufficient rainfall or years of less environmental stress (Hegazy, 2001). Germination of *D. harra* occurs during late winter and early spring, at a time when seedlings can survive, while no germination occurred in summer, when temperatures were at their yearly maximum. In addition, an inhibited germination under the harsh environmental conditions would preserve the seeds enabling sufficient germination of *D. harra* at right time and proper sites, thus improving the probability of successful seedling establishment (Tlig et al 2008).

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