FACTORS INFLUENCING SEED GERMINATION OF *CLEOME AMBLYOCARPA* BARR. & MURB. (CAPPARIDACEAE) OCCURRING IN SOUTHERN TUNISIA

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RÉSUMÉ.— *Facteurs influençant la germination des graines de* Cleome ambyocarpa *Barr. & Murb.* (Capparidaceae) *dans le Sud tunisien*.— *Cleome amblyocarpa* Barr. & Murb. est une plante herbacée annuelle de la famille des *Capparidaceae*. Elle est abondante dans les environnements sableux, les sols secs et pierreux, ainsi que les alluvions de gravier dans les zones arides tunisiennes. Des expériences conduites au laboratoire ont été effectuées pour évaluer les effets de la température (10 à 40° C) à l'obscurité. Le stress osmotique simulé par le NaCl à différentes concentrations (0 à 200 mM) ou le polyéthylène glycol 6000 (PEG-6000) à différents potentiels osmotiques (0 à -1 MPa) a été évalué à la température optimale trouvée. Les meilleurs pourcentages et vitesses de germination ont été obtenus à 25° C, mais la germination est inhibée au-dessus et en deçà de cette température. L'augmentation de l'osmolarité des solutions induites par le NaCl ou le PEG inhibe progressivement la germination des graines. À 150 mM NaCl ou un potentiel osmotique de -0,6 MPa, la germination est complètement inhibée. On assiste à une diminution de l'indice de germination au fur et à mesure que le stress osmotique est accentué. Ces résultats suggèrent que *C. amblyocarpa* semble bien tolérer la salinité et la sécheresse des écosystèmes arides durant la phase germinative.

SUMMARY.— *Cleome amblyocarpa* Barr. & Murb. is an annual herb in the family of Capparidaceae. It is abundant in sandy environments, and the gravel and stony grounds in Tunisian arid areas. Laboratory experiments were carried out to assess the effects of temperature (10 to 40° C) on seed germination in complete darkness. The osmotic stress simulated by NaCl at different concentrations (0 to 200 mM) or polyethylene glycol 6000 (PEG-6000) at different osmotic potentials (0 to -1 MPa) was evaluated at the most suitable temperature found. Greatest germination percentage and rate of germination were obtained at 25° C, but germination was inhibited by either an increase or decrease in temperature from the optimal temperature. The increase in osmolality of solutions induced by NaCl or PEG progressively inhibited seed germination. At 150 mM NaCl or osmotic potential of -0.6 MPa, germination was completely inhibited. There was a decrease in the rate of germination as osmotic stress was intensified. These findings suggest that C. *amblyocarpa* was able to tolerate well osmotically- and saline-stressful habitats of the arid ecosystems during germination stage.

Cleome amblyocarpa Barr. & Murb., an annual herb in the family of Capparidaceae, is abundant in sandy environments, and the gravel and stony grounds in arid Tunisia. It easily grows to over 50 cm in a rainy year. Its stems are rigid, erect and branched and bear alternate trifoliate leaves. The flowers are small, in leafy bunches. The fruit is dry and dehiscent, formed of long, hanging siliqua-shaped capsules that enclose hairy seeds (Chaieb & Boukhris, 1998; Molino, 2005). When dried, this herb is browsed and appreciated by animals, but the fresh plants are generally refused by animals. Its abuse seems to have certain toxicity (nerv-

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ous disorders) (Le Floc'h, 1983). This herb is also a medicinal plant used to ease pain. When mixed with *Juniperus phoenicia*, *Hammada scoparium* it treats headaches, and when mixed with *Artemisia herba-alba* it becomes a treatment for nausea, gastralgia, vomiting and colic (Molino, 2005). The anti-inflammatory activity of *C. amblyocarpa* leaf extract, observed *in vivo* as well as *in vitro*, could be due to its high flavonoid content (19%) (Bouriche *et al.*, 2003; Bouriche & Arnhold, 2010).

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 simultaneously influence germination (Baskin & Baskin, 1998) and initial establishment of plants in arid and semi-arid regions (Song *et al.*, 2005; Gorai & Neffati, 2007; Gorai *et al.*, 2009; Maraghni *et al.*, 2010). Under such conditions, seed germination and seedling emergence are often limited by temperature when moisture conditions are favourable (Evans & Etherington, 1990). Knowledge of temperature effects on germination may be useful to evaluate the germination characteristics or the establishment potential among range by species (Jordan & Haferkamp, 1989). In the field, sand temperature near the surface in the daytime can be considerably higher than air temperatures (Zhang & Maun, 1990); however, when the sand near the surface is moist, the only condition in which seeds germinate, evaporative cooling will prevent an extreme rise in sand temperature. Temperature changes may affect a number of processes controlling seed germinability, including membrane permeability and the activity of membrane-bound and cytosolic enzymes (Bewley & Black, 1994). Another crucial factor determining germination and seedling growth is soil moisture and, therefore, plays an important role in determining the distribution patterns of species (Gutterman, 1993). Although higher osmotically- and saline-stressful habitats of the arid environments decrease germination, the detrimental effect of salinity and osmotic stress are generally less severe at optimum germination temperature (Gorai & Neffati, 2007; Tlig *et al.*, 2008; Gorai *et al.*, 2009; Maraghni *et al.*, 2010).

There is little available information in literature on the ecology of seed germination of *Cleome amblyocarpa*. Therefore, the aim of the present study was to investigate the effects of temperature, and salt and water stresses on seed germination of this species.

MATERIALS AND METHODS

Seed collection site

Seeds of *C. amblyocarpa* were obtained from plants, which were collected from a location near El Fjé, Medenine (10°13' N, 33°13' E; South-East Tunisia) in June 2008. One thousand seeds weighed, on average, 2.28 ± 0.04 g. See Tlig *et al.* (2008) for details on climate.

Effects of constant temperatures on seed germination

Seeds of *C. amblyocarpa* were sterilized in Na-hypochlorite solution, subsequently washed with distilled water and air-dried before being used in the germination experiments to avoid fungus attack. 90-mm Petri dishes containing two disks of Whatman No. 1 filter papers with 5ml of test solution were prepared. Germination experiments were conducted in incubators set at 10, 15, 20, 25, 30, 35 and 40° C in complete darkness (Lumincube II, analys, Belgium; MLR-350, Sanyo, Japan). A completely randomized design was used in the germination tests. For each treatment, four replicates of 25 seeds each were used. During 20 days the number of germinated seeds were counted and removed every 2 days. A seed was considered to have germinated when the emerging radicle elongated to 2 mm.

Effects of NaCl and PEG-6000 on seed germination

The effects of NaCl or PEG solutions on seed germination were examined by incubating seeds, as described in the first experiment, at the most suitable temperature found. For salt stress, seeds were laid in distilled water (0), and in 50, 100, 150 and 200 mM NaCl solutions. However, water stress was simulated by PEG solutions of known osmotic potential (Ψ_π): 0, -0.1, -0.2, -0.4 and -0.6 MPa (Michel & Kaufmann, 1973). Distilled water equal to the mean water loss from dishes containing only water was added to each Petri dish every 2 days to maintain salt concentration near the target levels throughout the germination period.

Methods of germination expression

Two characteristics of seed germination were determined: final germination percentage and germination rate estimated using a modified Timson's index of germination velocity = $\Sigma G/t$, where G is the percentage of seed germination at 2-days interval and t is the total germination period (Khan & Ungar, 1984). The maximum value possible for our data using this index was 50 (i.e. $[20/2 \times 4 \times 25]/20$). The greater the value, the more rapid is the germination.

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 the effects of temperature, salinity and water stress on the rate and percentage of germination. Tukey test (Honestly significant differences, HSD) was used to estimate the least significant range between means.

RESULTS

Effect of temperature on seed germination

In response to the tested constant temperatures, most germination (81%) of *C. amblyocarpa* occurred at 25° C and none at 10 and 40° C (Fig. 1a). A one way ANOVA indicated that germination was significantly affected by temperature (F = 17.71; P < 0.001). Seeds of *C. amblyocarpa* were able to germinate at temperatures between 15 and 35° C and the optimal temperature corresponds to 25° C (Fig. 1a). Germination percentage was inhibited by either an increase or decrease in temperature from the optimal temperature. Polynomial regression analysis was used to determine the relationship between final germination percentages and temperature of incubation. There was a strong negative relationship with germination and temperature, with R^2 = 0.95 (data not shown). The index of germination velocity calculated by using a modified Timson's index showed that the rate of germination was significantly inhibited by either an increase or decrease in temperature from the optimal temperature (Fig. 1b; F = 27.68; P < 0.001).

Effect of NaCl-salinity on seed germination

The results of one way ANOVA revealed a significant effect of NaCl on percentage and rate of germination (F = 77.79 and 128.07, respectively; P < 0.001). The highest percentages were obtained in distilled water and were decreased with an increase in NaCl concentrations (Fig. 2a). At NaCl concentration of 150 mM, the germination was completely inhibited. There was a strong negative relationship identified between germination and salinity, with R^2 = 0.96 (data not shown). Moreover, the rate of germination was severely reduced by 63 and 85% at 50 and 100 mM NaCl, respectively, as compared to controls (Fig. 2b).

Effect of osmotic potential on seed germination

Germination percentage and rate of germination significantly declined with a decrease in Ψ_π of solutions based on the results of one-way ANOVA (F = 76.26 and 143.99, respectively; P < 0.001). Only 4% of *C. amblyocarpa* seeds germinated at -0.4 MPa, and at Ψ_π of -0.6 MPa germination was completely inhibited (Fig. 3a). Germination percentages were plotted against Ψ_π of solutions which revealed a negative relationship between them, with R^2 = 0.96 (data not shown). The rate of germination was severely reduced by 40, 58 and 99% at Ψ_π of -0.1, -0.2 and 0.4 MPa, respectively, as compared to controls (Fig. 3b).

DISCUSSION

Results from this study showed that the percentage and rate of germination of *C. amblyocarpa* seeds increase by temperature increment, with highest values occurring at 25° C then they decrease gradually. Similar results were obtained on *Panicum turgidum* (Al-Khateeb, 2006), *Phragmites communis* (Gorai *et al.*, 2006), *Reaumuria vermiculata* (Gorai & Neffati, 2007), *Diplotaxis harra* (Gorai *et al.,* 2008) and *Spartidium saharae* (Zammouri *et al.*, 2010) and *Salvia aegyptiaca* (Gorai *et al.*, 2011). Neffati (1994) showed that the variation in the thermal optimum depends on the considered species, although for the majority of southern Tunisian species germination occurred over a wide range of temperatures and that temperature of 20° C appears to enhance their germination. This variation in the thermal optimum and the germination rate between species constitutes some adaptive strategies to harsh environmental conditions (Gorai *et al.*, 2006). However, the optimum temperature for germination is not the same for all species. Neffati (1994) identified two types of plant seeds and explained this by different thermal optimum during germination stage, category 1: low optimum thermal (≤ 20° C) and category 2: high optimal thermal (> 20° C). *C. amblyocarpa* presents a high optimum temperature for germination and corresponds to category 2 of Neffati's (1994) classification. Temperatures between 10 and 30° C appeared favourable for the germination of this species. These advantages seem to place *C. amblyocarpa* among the species most able to adapt to climate change during germination stage.

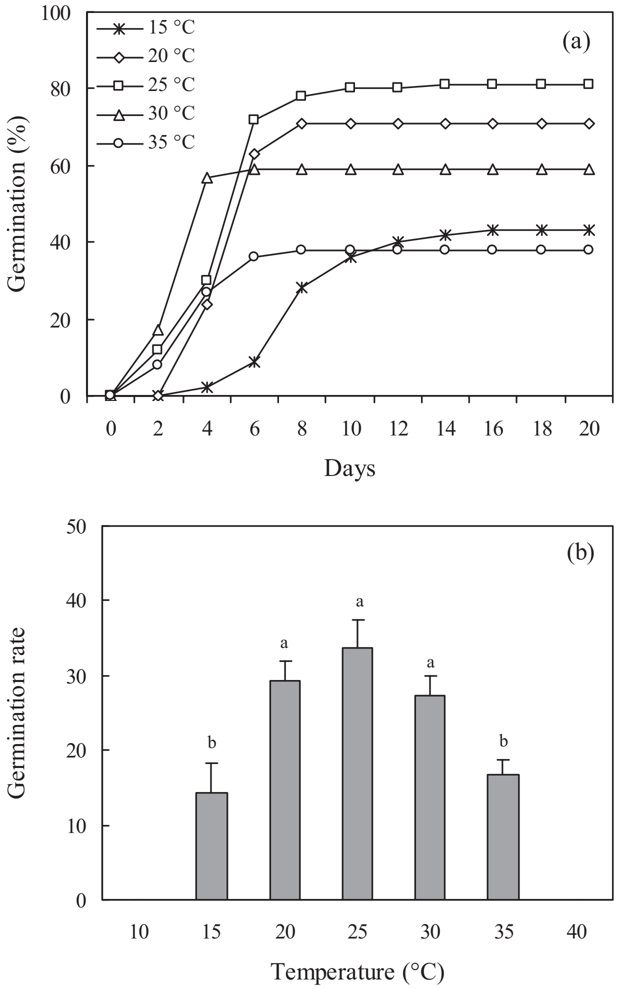

Figure 1.— Effect of temperature on seed germination of *Cleome amblyocarpa*. (a): Time course of germination during 20 days at different temperatures (10 to 40° C). At temperature 10° C and 40° C seed germination was completely inhibited (n = 4). (b): Rate of germination. Values of each parameter (mean ± SE, n = 4) having the same letter are not significantly different ($P < 0.05$) from each other (Tukey test).

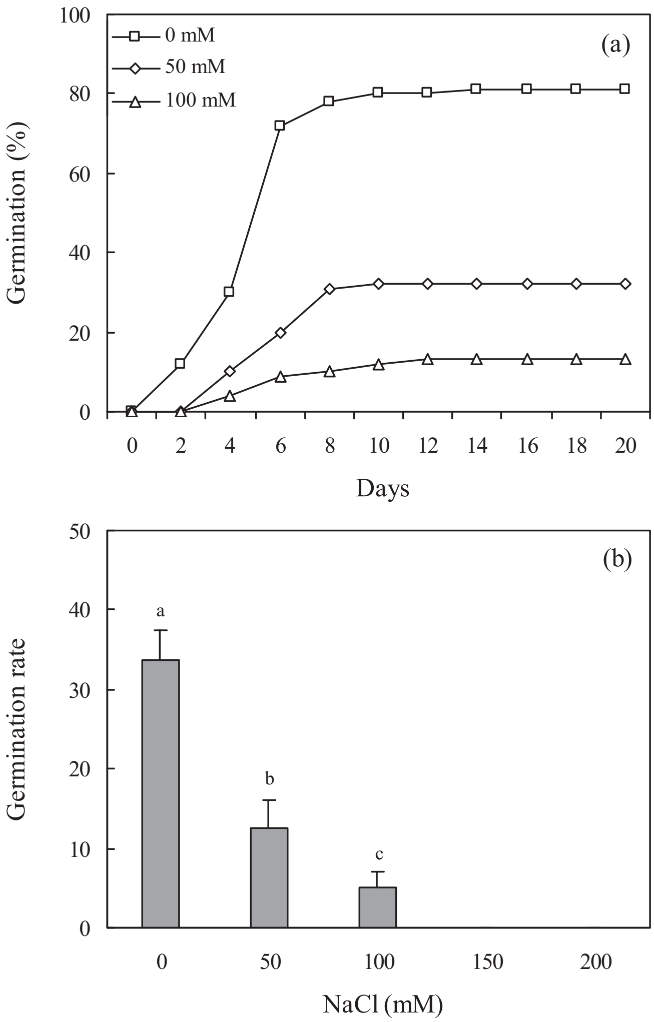

Figure 2.— Effect of salinity on seed germination of *Cleome amblyocarpa*. (a): Time course of germination during 20 days at different NaCl concentrations (0 to 200 mM). At 150 and 200 mM NaCl concentrations seed germination was completely inhibited (n = 4). (b): Rate of germination. Values of each parameter (mean ± SE, n = 4) having the same letter are not significantly different ($P < 0.05$) from each other (Tukey test).

The highest germination of *C. amblyocarpa* was obtained in distilled water. Increasing the concentration of NaCl significantly reduced the percentage of germination. These results are consistent with those obtained in other species as *Reaumuria vermiculata* (Gorai & Neffati, 2007), *Diplotaxis harra* (Tlig *et al.*, 2008), *Spartidium saharae* (Zammouri *et al.*, 2010). In Tunisia, salinity affects large areas mainly in central and south where the arid climate increases the proliferation of these territories. The effect of salinity on germination may be explained by osmotic and/ or toxic effects (Song *et al.*, 2005; Tlig *et al.*, 2008). Increasing the NaCl concentration in the medium reduces the amount of water available to the seed (osmotic effect) causes an accumulation of Na^+ in the embryo at the expense of K^+ and also leads to the synthesis of inhibitor of germination: abscisic acid under the action of Na^+. The detrimental effect of salinity on seed germination of *C. amblyocarpa* causes a decrease in both percentage and rate of germination. This suggests that this species has no physiological requirement of salt for germination.

These results are consistent with other studies showing that like halophytes glycophytes are particularly sensitive to salinity in germination phase (Gorai *et al.*, 2006; Gorai & Neffati, 2007; Zammouri *et al.*, 2010; Gorai *et al.*, 2011). Caballero *et al.* (2003) suggest that the seeds which do not germinate in the presence of salt, while keeping their viability, serve to enrich the soil seed bank. Such seeds secure the long-term existence of seed bank helping the species spreading germination over years and using opportunistically suitable germination conditions. Seeds of some species are reported to tolerate high salinity during the period when they are dormant in the soil and subsequently germinate when soil salinities are reduced (Khan & Ungar, 1997).

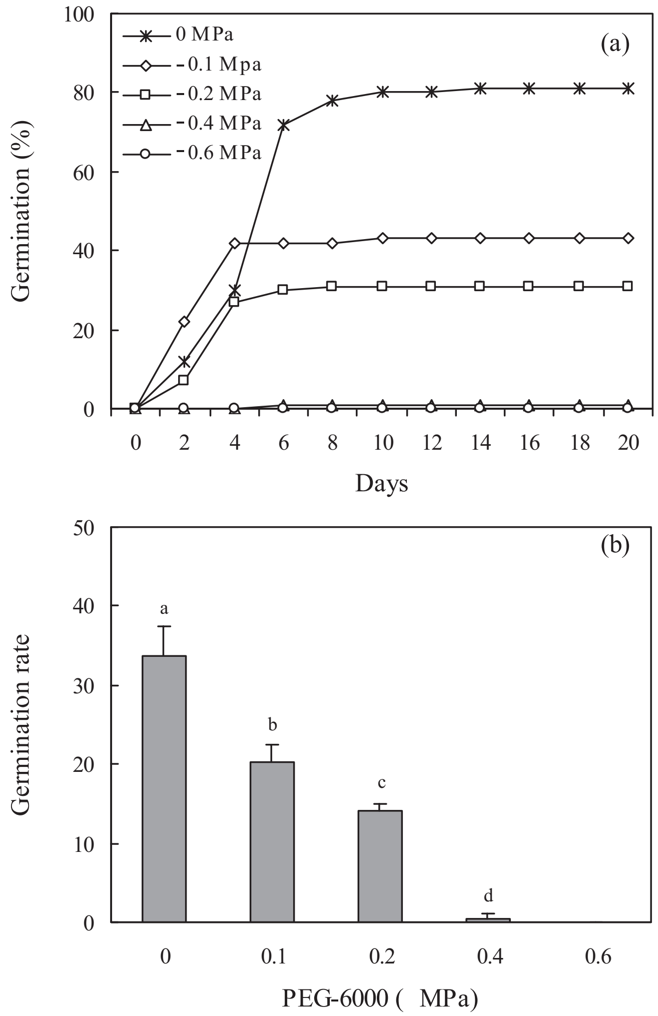

Figure 3.— Effect of osmotic potential on seed germination of *Cleome amblyocarpa*. (a): Time course of germination during 20 days at different PEG concentrations (0 to -0.6 MPa), n = 4. (b): Rate of germination. Values of each parameter (mean ± SE, n = 4) having the same letter are not significantly different ($P < 0.05$) from each other (Tukey test).

Our data show that an increase in osmolality of PEG solutions results in decreasing both the percentage and rate of germination *C. amblyocarpa*, indicating that water stress inhibits germination. At low osmotic potentials, water does not induce germination. This was in agreement with the germination behaviour of most species (Tobe *et al.*, 2006; Gorai *et al.*, 2009; Maraghni *et al.*, 2010). As rainfall is a random event in the arid zone, some plants are waiting for a certain moisture threshold is reached to germinate. This strategy, which ensures proper installation of the plant, was adopted by many sensitive species to moisture stress during germination stage. In general, annual species need to control their germination closely to minimize the risk of local extinction caused by germinating under unfavourable conditions (Fenner & Thompson, 2005).

In conclusion, this study shows that seeds of *C. amblyocarpa* were able to tolerate osmotically- and saline-stressful habitats of the arid ecosystems. Further investigations are necessary to understand the early establishment of this herb under field conditions and to determine if there are differences between seed germination stage and the early seedling growth in their responses to salinity and drought stresses.

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