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Rapid recovery of photosynthesis and water relations following soil drying and re-watering is related to the adaptation of desert shrub *Ephedra alata* subsp. *alenda* (Ephedraceae) to arid environments

Mustapha Gorai[a,*], Wafa Laajili[b], Louis S. Santiago[c], Mohamed Neffati[b]

[a] Département des Sciences de l'Environnement, Institut Supérieur de Biologie Appliquée de Médenine, Université de Gabès, Médenine 4119, Tunisia
[b] Laboratoire d'Ecologie Pastorale, Institut des Régions Arides, Université de Gabès, Médenine 4119, Tunisia
[c] Department of Botany and Plant Sciences, University of California, 2150 Batchelor Hall, Riverside, CA 92521, USA

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A B S T R A C T

Ephedra alata subsp. *alenda* is the most important pioneer plant of the moving and semi-stable sand dunes in the deserts and steppes of south Tunisia and occurs naturally in the Grand Erg Oriental, one of the most extreme habitats for plant growth on the planet. A new analysis of physiological performance of this medically important and internationally threatened xerophytic shrub was conducted to assess possible mechanisms of drought tolerance and how these relate to its ecological success. Five-month old plants, grown under controlled climatic conditions, were subjected to a well-watered control treatment or progressive drought by withholding water for 14 d with subsequent recovery for 7 d. Soil water depletion significantly reduced stem relative water content (RWC) water potential (Ψ_w) and osmotic potential (Ψ_π). *Ephedra* displayed more negative Ψ_w and Ψ_π values of ca. −3.5 and −4.1 MPa, respectively, at the end of the drought treatment, and were associated with turgor loss. Low stem Ψ_w reduced stomatal conductance (g_s), photosynthetic CO_2 assimilation rates (A_{CO_2}), transpiration (E) and internal CO_2 concentration (C_i). However, instantaneous (WUE; $A_{CO_2}\,E^{-1}$) and intrinsic (WUE$_i$; $A_{CO_2}\,g_s^{-1}$) water use efficiency (WUE) increased gradually as water deficit was intensified. Stomatal closure therefore only exerted limited control against dehydration and could not compensate for decreases in soil water status, typical of anisohydric behavior. Drought-stressed stems accumulated high levels of proline up to 480% of control values, highlighting a pivotal role in osmotic adjustment during intense water deficit. In contrast, the osmotic adaptation to soluble sugars was limited. Drought-stressed plants increased A_{CO_2}, E, g_s and C_i and decreased WUE and WUE$_i$ during the first 48 h after re-watering, such that they reached similar values to those of control plants by the end of the experiment. Stem proline levels of drought-stressed plants returned to near control values with re-watering. Overall, rapid recovery of photosynthesis following drought-breaking moisture appears to be a critical mechanism allowing *E. alata* to withstand and survive dry environments.

1. Introduction

Plants growing in Mediterranean-type ecosystems are subjected to prolonged periods of high temperatures and water deficit during annual summer drought, interspersed by short cool winters with variable precipitation (Cowling et al., 1996). Currently, the faster-than-predicted change in global climate (IPCC, 2007) and a variety of climate change scenarios suggest an increase in aridity for the semi-arid regions of the globe and the Mediterranean region in the near future. Under these conditions, the mechanisms by which different species respond to the summer drought largely determine their success and regeneration (Hernàndez et al., 2010).

Drought stress is known to shape many physiological and biochemical attributes in plants, especially in desert habitats. The most dominant growth forms in the Mediterranean ecosystem are evergreen and semi-deciduous plants. They adapt to their environment through physiological responses, in addition to ecological strategies, coping with water shortages by either (i) stress avoidance (so-called "drought avoiding species") or (ii) stress tolerance ("drought tolerant species") (Levitt, 1980). This classification roughly corresponds to the actually widespread categorization of plants across the continuum of stomatal regulation of water

* Corresponding author. Tel.: +216 75633919; fax: +216 75633918.
E-mail address: gorai.mustapha@yahoo.fr (M. Gorai).

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status in isohydric vs anisohydric species (Tardieu and Simonneau, 1998). Isohydric species maintain plant midday water potential at relatively constant values regardless of drought conditions via stomatal closure potentially resulting in mortality by carbon starvation during extreme drought, whereas anisohydric species display large midday water potential fluctuations by maintaining relatively high gas exchange rates even during arid season potentially leading to hydraulic failure during extreme drought (McDowell et al., 2008; Nardini et al., 2014). Recent progress in plant hydraulics has generated a coupled hypothesis of carbon starvation and hydraulic failure as mechanisms that explain how plants succumb to drought-induced mortality (McDowell, 2011). However, the outcome of such responses depends on several interacting variables, including the frequency and severity of the stress, plant genotype and plant developmental stage (Alpert, 2000; Otte, 2001; Chaves et al., 2003). Desert plants regulate their water status and adapt to their heterogeneously stressful environment through a multiplicity of ecophysiological features by following two major strategies in relation to drought, based upon variation in phenology (deciduousness) and ability to tolerate water deficit (persistence) (Ackerly, 2004). Soil depth and texture are considered to be the most important edaphic properties that influence plant moisture regimes in arid environments with episodic rainfall (Grigg et al., 2008; Dhief et al., 2009).

Ephedra alata Decne. subsp. *alenda* (Stapf) Trab., a dioecious species, is the most important pioneer plant of the moving and semi-stable sand dunes in the deserts and steppes of south Tunisia and occurs naturally in the Grand Erg Oriental (Nabli, 1989) and grows up to 5 m tall near Rjim Mâatoug, Tunisa (Chaieb and Boukhris, 1998). Other than its sand stabilization value, *E. alata* has gained popularity because of its secondary product chemistry—it is an important source of pharmaceutical compounds (Smith, 1977; Nawwar et al., 1984, 1985; Abourashed et al., 2003; Hayashi et al., 2010). Local nomadic populations also use it for pasture and for fuel, but overuse in this respect has contributed to its decrease (Nabli, 1989; Chaieb and Boukhris, 1998). Recently, Al-Qarawi et al. (2011) reported that seed borne fungi play an important role in deterioration of seed quality, and therefore declines in *E. alata* populations. In natural habitats, *E. alata* flowers during late winter and spring, and seeds start to mature from mid-May to early August (Derbel and Chaieb, 2013), after which they are dispersed by wind. In arid areas, the ability to overcome multiple and simultaneous stresses is of great importance for successful establishment of plants. Seeds of *E. alata* germinate best in spring season at a fluctuating regime of 28 °C day and 13 °C night temperatures, whereas seed germination decreases with increasing osmolality of growth media (Al-Taisan et al., 2010). Detailed knowledge of how *E. alata* responds to drought is vital for protection of its natural populations, and equally important for conserving its ecological restoration value as a stabilizer of shifting sand substrates and its cultural value as a medicinal remedy. In particular, we address the ecophysiological traits that determine the ability of a species to cope with drought and attempt to explain its distribution and ecological fitness in the Mediterranean region (Lambers et al., 1998).

Mediterranean shrubs represent excellent models for studying plant responses to drought because they are generally tolerant and well adapted to decreased soil water availability during the seasonal drought. Several native species are potentially interesting under aspects of dune stabilization and extension of plant cover, including *E. alata*. The coordinated changes in water (osmotic) relations and net CO_2 assimilation rate are key issues for understanding the tolerance of *E. alata* to drought stress. We present detailed physiological mechanisms for coping with extreme seasonal water deficit on this evergreen Mediterranean shrub species for the first time. In this context, we evaluated stem gas exchange properties, stem water relations and the role of organic solute accumulation in osmotic adjustment of *E. alata* seedlings exposed to low soil water availability, and during a period of subsequent recovery from drought stress. The relationship between stem water relations and stomatal behavior was also explored, to test the hypothesis that maintaining turgor potential may provide an indication of the capacity of a species to access groundwater during the summer drought, which characterizes the Mediterranean-type climate of southern Tunisia. Basic physiological responses of this important regional species are not well understood, and such information is indispensable to improve the basis for management of this plant species in the delicate shifting sands ecosystems of Northern Africa.

2. Materials and methods

2.1. Study species

Ephedra is the only genus in the family Ephedraceae within the Gnetales order, the closest living relatives of the Angiosperms (Friedman, 1996, 1998). It comprises ca. 50 species native to arid and semi-arid regions of North Africa, Asia, Europe and North- and Central America (Price, 1996; Caveney et al., 2001). Most of these species are perennial evergreen shrubs that can exceed 1 m in height, with slender and joined photosynthetic stems. Of these, only *E. alata* subsp. *alenda* (Stapf) Trab., *Ephedra altissima* Desf., *Ephedra fragilis* Desf. and *Ephedra nebrodensis* Guss. are found in Tunisia (Cuénod et al., 1954; Le Floc'h et al., 2010). The genus is characterized by scale-like or needle-like leaves, which are either decussate or in whorls of three and are fused at the base to form a nodal sheath (Price, 1996).

2.2. Seed collection and site description

Seeds of *E. alata* were collected from natural populations at Tiert, southern Tunisia in July 2008 (30°52′N, 10°09′E). They were dried in open air, cleaned and then stored in the seed bank at the Laboratoire d'Ecologie Pastorale (IRA-Médenine, Tunisia) until their use. The site is characterized by an arid-type climate with dry and hot summers and cold winters. The annual rainfall is low, rarely exceeding 100 mm. The rains are infrequent and irregular, sometimes with no rain during long periods of several years. Mean annual temperature is 20 °C. The soil texture is sandy with increasing particle diameters with depth. The dunes reach a height of approx. 8–12 m.

The site of Tiert is dominated by *E. alata*, *Calligonum azel*, *Calligonum comosum*, *Cornulaca monacantha*, *Genista saharae*, *Helianthemum confertum*, *Limoniastrum monopetalum*, *Retama raetam*, *Stipagrostis pungens*, *Traganum nidatum* and *Zygophyllum album*. These species play a significant role in the vegetation physiognomy in these sites where the vegetation cover is approximately 4–11%. The vegetation is composed of three strata: a phanerophyte stratum with a height exceeding two meters, a nanophanerophyte stratum of less than two meters to which *E. alata* belongs; and a chamaephyte stratum of less than 0.5 m height.

2.3. Treatments and experimental set-up

Seeds were surface sterilized in 5% sodium hypochlorite for 5 min, subsequently washed with deionized water and air-dried before being used in experiments to avoid fungal infection. Germination experiments were conducted in an incubator set at 15 °C. Three germinated seeds were transplanted into each pot (18 × 20.6 cm height), which contained a 1:2 mixture of peat and sand with a drainage layer at the bottom of the pot. Each pot was irrigated every two days with rain water to field capacity (FC). Thirty days after emergence, seedlings were thinned to one per pot and irrigated with a half-strength Hewitt's solution (Hewitt, 1966).