South African Journal of Botany 114 (2018) 241–249

Contents lists available at ScienceDirect

South African Journal of Botany

journal homepage: www.elsevier.com/locate/sajb

Physiological and biochemical changes in *Periploca angustifolia* plants under withholding irrigation and rewatering conditions

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A R T I C L E I N F O

Article history:
Received 24 January 2017
Received in revised form 3 November 2017
Accepted 10 November 2017
Available online xxxx

Edited by MG Kulkarni

Keywords:
Periploca angustifolia
Drought
Recovery
Water potential
Soluble sugars
Vitamins

A B S T R A C T

Periploca angustifolia (Labill.) is a multipurpose xerophytic shrub widely distributed in arid zones. It is used in rehabilitation programs; its exploitation and utilization require the understanding of its behavior under stress conditions such as drought. To evaluate drought response and at what level it becomes irreversible, a new analysis of physiological and biochemical performances of this threatened shrub was conducted to assess possible mechanisms of drought tolerance and how these relate to its ecological success. Eleven-month-old plants, grown under semi-controlled conditions, were subjected to a well-watered control treatment or progressive drought by withholding water for 7, 14 and 21 d with subsequent recovery for 7-d. After 7 days of drought, followed by rewatering period, no significant effect on all studied parameters was recorded for *P. angustifolia*. However, from 14 until 21 days of withholding irrigation, drought becomes irreversible causing plant damages, may be rewatering has no effect to reduce these damages. A water deficit of 21 days RWC (33%), chlorophyll a content ($0.083\ mg \cdot g^{-1}$ DW), transpiration 14.7%, as well as water and osmotic potential (−3.40 and −3.78 MPa) decreased significantly compared with control. While, proline content ($0.25\ \mu mol \cdot g^{-1}$ FW), soluble sugars ($80.52\ mmol \cdot g^{-1}$ DW) and vitamins C, E ($1.9\ mM \cdot g^{-1}$ FW) increased significantly that could indicate ability of osmotic adjustment in this species. Also the increases in RWC, water and osmotic potentials values after rewatering period indicate the good recovery of this plant. Based on these experiments, responses of *P. angustifolia* to water stress were typical of those of xerophytic plants.

1. Introduction

Drought is one of the major environmental factors that inhibits many metabolic processes and eventually constrains plant survival, growth, crop productivity and distribution of plants (Chaves and Oliveira, 2004; Liu, 2009). It is also considered to be the main environmental factor limiting plant growth and yield of many agronomic and horticultural crops, especially in semi-arid areas (Boyer, 1982). Plants grown under such conditions are often exposed to a long period of drought and harsh environmental conditions which affect plant growth and agriculture development (DiCastri et al., 1981). In Mediterranean ecosystems, limited water availability and current decrease of water resources are leading to the urgent need to better manage water use for irrigation in arid and semi-arid areas. The most dominant growth forms in these ecosystems are evergreen and semi-deciduous plants which adapt to their environment through physiological responses and ecological strategies by either avoiding or tolerating stress (Levitt, 1980).

Drought, like other environmental stresses, affects many physiological and biochemical processes within plants. One of the most damaging effects is associated with the photosynthetic process of the plant. Many studies have shown that drought-stress reduces stomatal conductance (gs), transpiration rate (E) and net CO_2 assimilation rate (A_{CO2}), implying that the stomata are important regulators of gas exchange (Arbona et al., 2005). The decrease of the photosynthetic activity under drought stress can be attributed to both stomatal and non-stomatal limitations (Zlatev and Yordanov, 2004). One of the earliest responses to drought is stomatal closure (Mahajan and Tuteja, 2005). Stomatal closure allows plants to limit transpiration, but it also limits CO_2 absorption, which leads to a decreased photosynthetic activity (Yang et al., 2006). Nonetheless, limitations to CO_2 absorption imposed by stomatal closure may promote an imbalance between photochemical activity of photosystem II (PSII) and the electron requirement of the Calvin–Benson cycle, leading to an excess of absorbed excitation energy and subsequent photo-inhibitory damage to PSII reaction centers (Baker and Rosenqvist, 2004).

Water-stress tolerance involves subtle changes in cellular biochemistry. It appears to be the result of the accumulation of compatible solutes and of specific proteins that can be rapidly induced by osmotic stress (Shao et al., 2007). The synthesis of osmolytes including proline

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https://doi.org/10.1016/j.sajb.2017.11.007
0254-6299/

is widely used by plants to stabilize membranes and maintain the conformation of proteins at low leaf water potentials. The synthesis and accumulation of osmolytes varies among plant species as well as among different cultivars of the same species. Proline is also known to be involved in reducing the photo damage in the thylakoid membranes by scavenging and/or reducing the production of $^1O^2$ (Reddy et al., 2004).

Periploca angustifolia Labill. (Apocynaceae) is an evergreen perennial shrub colonizing the Mediterranean basin (Spain, Morocco, Algeria, Sicily, Tunisia, Malta, Libya, Egypt, Lebanon and Syria) (Quezel and Santa, 1962). In Tunisia, this species has a wide distribution extending from Jebel Ichkeul (sub-humid bioclimate) in the North to Dhiba (upper saharian bioclimate) in the South. It commonly grows in areas where annual rainfall ranges from 100 to 400 mm (Chaieb and Boukhris, 1998). It is a range shrub with continuous vegetative production when soil humidity allows it (Ferchichi, 1996). It grows on different types of soil but prefers calcareous substrates. This indigenous shrub is the only species of this genus in Tunisia (Chaieb and Boukhris, 1998), it has a persistent and lanceolated sheets (Ozenda, 2004) and grows up to 3 m tall with short and rigid branches, which the upper ones sometimes lax and twining (Ghrabi, 2005). Although *P. angustifolia* is a species producing latex, but it is very palatable as forage by livestock in arid lands of Tunisia. Recent studies reported that this latex contains natural rubber and the type of laticifiers cells exuding was also identified (Dghim et al., 2015). In Lampedusa, this shrub was called “silk tree” and its seeds are used to make small pillows (Zito and Sajeva, 2012). In Tunisia, *Periploca angustifolia* has high pastoral, ecological and medicinal values (Le Floc'h, 1983; Ghrabi, 2005; Bouaziz et al., 2009; Dghim et al., 2013; Zito et al., 2013).

Studies focusing on germination behavior of *P. angustifolia* seeds showed that germination percentage is influenced by burial depth, temperature, salt and osmotic stress (Noumi et al., 2009; Dghim et al., 2015). A comparative study of root system growth of this shrub and *Rhus tripartitum* (Ucria), cultivated in rhizotrons, showed that in the first months following germination (in November), the root development of the shrubs was rapid in spite of the negative influence of low winter temperature on the growth of the *P. angustifolia* root system (Yobi et al., 2001).

In order to evaluate regeneration success of *P. angustifolia* Labill., a comparative study of spontaneous and transplanted (from a nursery) plants using morphological and ecophysiological traits was explored by Ennajeh et al. (2010), in the natural reserve of Oued Dekouk. This study revealed that spontaneous (pre-existing) plants of *P. angustifolia* were more vigorous and appears more adapted to aridity than transplanted (re-introduced from a nursery) ones.

Knowing ecophysiological traits of autochthon populations (especially Mediterranean shrubs, such as *P. angustifolia*) to drought is essential for protecting and conserving them in order to be used for their multipurpose (ecological, medicinal, etc.). In this study, we evaluate ecophysiological and biochemical responses of *P. angustifolia* seedling subjected to drought stress (withholding irrigation for 7, 14 and 21 days) followed by 7 days of recovery. The drought study was limited to 21-d, since a longer period leads to the appearance of chlorotic symptoms on leaves (data not shown). The relationship between water relations and biochemical behavior was also explored, to test the hypothesis that maintaining turgor potential may provide an indication of the capacity of *P. angustifolia* to access groundwater during the summer drought, which characterizes the Mediterranean-type climate of Southern Tunisia. Basic physiological and biochemical responses of this important regional species are not well understood, and such information is indispensable to improve the basis for management of this shrub in the delicate shifting ecosystems of Northern Africa.

The main purposes of this paper are to examine the main changes in ecophysiological and biochemical aspects of drought treated and recovered plants in order to consider traits that may be associated with a greater ability to resist water stress.

2. Materials and methods

2.1. Plant material and growth conditions

Seeds of *P. angustifolia* were collected from natural populations at Sidi-Toui, Southern Tunisia in 2006 (32°44′N, 11°14′E; 110 m altitude) and stored in the seed bank at the Laboratoire d'Ecologie Pastorale (IRA-Medenine, Tunisia) until their use. The pots were kept under semi-controlled conditions in a plastic greenhouse. The environmental parameters characterizing the arid climate of the experimental site during the trial are summarized in Table 1. Germination experiments were carried out in pot (18 × 20.6 cm height), which contained a mixture of 1/3 peat and 2/3 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).

2.2. Treatments and experimental set-up

Eleven months after emergence, seedlings were subjected to: full irrigation (FI), water deficit (WD) and rehydrated (R) with 10 plants per treatment with one plant in each pot. Water deficit was applied by withholding irrigation for 7, 14 and 21 days followed by 7 days of recovery period for each treatment. Water potential and photosynthesis measures were carried out before harvesting the plants. Part of the harvested plant material was stored at −80 °C; however the other part is stored as dried matter (72 h at 60 °C). Two parts were used for physiological and biochemical analyses.

2.3. Plant water relations

Relative water content (RWC) was determined for leaves of *P. angustifolia* and calculated according to the formula of Jeon et al. (2006): RWC (%) = (FW − DW) / (TW − DW) × 100. Where: fresh weight (FW) was measured at the end of the drought period, and dry weight (DW) was obtained after drying the samples at 65 °C for at least 48 h. Turgor weight (TW) was determined by subjecting leaves to rehydration for 24 h at obscurity at 4 °C.

After 7, 14 and 21 days of drought stress and a recovery of previously stressed plants, water potential (ψw) was determined using a Scholander pressure chamber (PMS Instruments, Corvallis, Oregon, USA) (Scholander et al., 1965) on the same stems after measurements of CO_2 and H_2O exchange.

After ψ_w measurement, stems were tightly enclosed in aluminium foil, frozen by immersing in liquid nitrogen and stored in a freezer at −20 °C. After thawing, a vapor pressure osmometer (Wescor 5520, Logan, UT, USA) was used to determine osmolality of the sap expressed from stems, which was converted to Ψπ by the Van't Hoff equation: $\Psi\pi = -nRT$, where n is the value reading from the instrument, R is the ideal gas constant and T is the absolute temperature (Nobel, 1991).

2.4. CO_2 and H_2O gas-exchange

Net assimilation of CO_2 (*A* or *PN*), stomatal conductance (*gs*), transpiration rate (*E*), water use efficiency (WUE = A_{CO2} / *E*) and intrinsic WUE (WUEi = *A* / *gs*) were measured periodically during the experiment using the longest stem in an LCpro + portable photosynthesis system equipped with a broad leaf chamber (ADC BioScientific Ltd., UK). The stems were irradiated with a PFD of 1500 mol m^{-2} s^{-1} of internal light source and the CO_2 concentration in the chamber was set at 360 mol mol^{-1}. Readings were logged every 30 s until we obtained stable values.

2.5. Chlorophyll and carotenoid content

Photosynthetic pigments content (chlorophyll and carotenoid) was determined using fresh shoots. One-hundred milligram was ground