Plant Physiology and Biochemistry 118 (2017) 589–599

Contents lists available at ScienceDirect

Plant Physiology and Biochemistry

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

Research article

Photosynthetic limitation and mechanisms of photoprotection under drought and recovery of *Calotropis procera*, an evergreen C_3 from arid regions

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ARTICLE INFO

Article history:
Received 26 May 2017
Received in revised form
23 July 2017
Accepted 30 July 2017
Available online 31 July 2017

Keywords:
A/C_i analysis
Gas exchange
Oxidative stress
Photosynthetic metabolism
Sugars metabolism
Water deficit

ABSTRACT

Calotropis procera is a C_3 plant native from arid environmental zones. It is an evergreen, shrubby, non-woody plant with intense photosynthetic metabolism during the dry season. We measured photosynthetic parameters and leaf biochemical traits, such as gas exchange, photochemical parameters, A/C_i analysis, organic solutes, and antioxidant enzymes under controlled conditions in potted plants during drought stress, and following recovery conditions to obtain a better insight in the drought stress responses of *C. procera*. Indeed, different processes contribute to the drought stress resilience of *C. procera* and to the fast recovery after rehydration. The parameters analyzed showed that *C. procera* has a high efficiency for energy dissipation. The photosynthetic machinery is protected by a robust antioxidant system and photoprotective mechanisms such as alternative pathways for electrons (photorespiration and day respiration). Under severe drought stress, increased stomatal limitation and decreased biochemical limitation permitted *C. procera* to maintain maximum rate of Rubisco carboxylation ($V_{c,max}$) and photosynthetic rate (A_{max}). On the other hand, limitation of stomatal or mesophyll CO_2 diffusion did not impair fast recovery, maintaining $V_{c,max}$, chloroplast CO_2 concentration (C_c) and mesophyll conductance (g_m) unchanged while electron flow used for RuBP carboxylation (J_c) and A_{max} increased. The ability to tolerate drought stress and the fast recovery of this evergreen C_3 species was also due to leaf antioxidative stress enzyme activity, and photosynthetic pigments. Thus, these different drought tolerance mechanisms allowed high performance of photosynthetic metabolism by drought stressed plants during the re-watering period.

1. Introduction

Drought is main abiotic stressor regulating plant biomass. All predictive models for future climate depend on drought maintenance, especially in semiarid areas such as the Brazilian Northeast (Santos et al., 2014). Low water availability closes plant stomata through a signal sent from roots to shoots leading to decrease in gas exchange. Therefore, increased stomatal limitation causes increased CO_2 resistance. Additionally, leaf internal resistance may contribute to reduced gas exchange (Lawlor and Cornic, 2002; Chaves et al., 2009). On the other hand, photosynthetic limitation by stomatal conductance under progressive water deficit gradually decreases with non-stomatal limitation increase (Hu et al., 2010).

Several species suffer damage in the photosynthetic metabolism under abiotic stress, leading to biomass losses. The main cause of damage is accumulation of reactive oxygen species (ROS) (Chaves et al., 2009). ROS may play a double role, causing damage but also as stress signalling molecules (Chaves et al., 2009; Foyer et al.,

Abbreviation: A, net photosynthetic rate; A_{max}, maximum photosynthetic rate; C_c, chloroplast CO_2 concentration; ETR, electron transport rate; EXC, the relative energy excess at the PSII level; F_v/F_m, maximum quantum efficiency of PSII; F_v'/F_m', PSII maximum efficiency; g_s, stomatal conductance; g_m, mesophyll conductance; J_c, electron flows used for RuBP carboxylation; J_o, electron flows used for RuBP oxygenation; l_s, stomatal relative limitation; l_{mc}, mesophyll conductance relative limitation; l_b, biochemical relative limitation; NPQ, non-photochemical quenching; P_r, photorespiration rate; qP, photochemical quenching; R_d, day respiration; RWC, relative water content; SM, soil moisture; $V_{c,max}$, maximum rate of Rubisco carboxylation; WUE, water use efficiency.

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http://dx.doi.org/10.1016/j.plaphy.2017.07.026
0981-9428/

2009). The antioxidant enzyme system has been extensively studied (Liu et al., 2011; Silva et al., 2015). However, little attention has been given to other mitigating effects of oxidative stress, such as metabolism of soluble sugars and some free amino acids, which may play double roles, acting both as ROS producers and scavenging molecules (Sperdouli and Moustakas, 2012; Keunen et al., 2013; Van den Ende and El-Esawe, 2013).

Low CO_2 availability can limit photosynthetic metabolism of C_3 plants. In general, plants are less tolerant to high temperatures and show high photorespiration rates under a combination of high light intensity, temperature, and under drought stress (Foyer et al., 2009; Lawson et al., 2014). However, previous studies on *Calotropis procera* showed this species has inverse characteristic behaviour for this type of photosynthetic metabolism (Tezara et al., 2011; Frosi et al., 2013). These plants show low stomatal conductance with high rates of CO_2 assimilation under severe water restriction and high temperature, besides fast recovery after re-watering (Tezara et al., 2011; Frosi et al., 2013).

The combination of high drought tolerance, fast and full recovery after re-watering is critical to *C. procera* survival in arid and semiarid areas, avoiding biomass losses. Despite several studies having discussed photosynthetic aspects in C_3 plants under drought stress, our knowledge of their capacity to recover after re-watering is still incomplete (Chaves et al., 2009; Hu et al., 2010; Tezara et al., 2011; Frosi et al., 2013; Lawson et al., 2014). In fact, maintaining photosynthesis during severe drought is critical for full recovery after re-watering (Hu et al., 2010) and to keep high CO_2 assimilation during the dry season in tropical environments (Frosi et al., 2013). Photosynthetic limitation analyses will contribute to the knowledge of photosynthetic machinery involvement in fast response to re-watering and survival of *C. procera* under drought.

Therefore, *Calotropis procera* is a good model to identify physiological attributes enabling high performance of photosynthetic metabolism under severe water restriction and subsequent recovery after re-watering. Our hypotheses include: (i) the high efficiency of energy dissipation enables this species to perform photosynthetic metabolism under water restriction; (ii) stomatal limitation of *C. procera* decreases and biochemical limitation increases under severe drought; (iii) fast recovery of *C. procera* is permitted by no diffusional limitation CO_2 (stomatal or mesophyll). Furthermore, we believe this species has efficient array of photoprotective mechanisms supporting photosynthesis under water deficit and re-watering. Therefore, this study aimed at (i) assessing photosynthetic performance, and (ii) evaluating photochemical and biochemical mechanisms of water deficit tolerance in young plants of *C. procera* under drought and subsequent recovery.

2. Materials and methods

2.1. Plant material and growth conditions

Calotropis procera (Aiton) W. T. Aiton (Apocynaceae) is an evergreen, shrubby, native species from desert areas (from the northwest of Africa to the southwest of Asia) with C_3 photosynthetic metabolism, flowering and fruiting peak in dry season in the Brazilian semiarid zone. It has become invasive species in deforested areas (Frosi et al., 2013).

Calotropis procera seeds were collected in Pernambuco, Brazil (7°57′8.37″ S, 38°17′54.07″ W). The climate is BSh, according to Köppen's classification, with average annual rainfall of approximately 750 mm concentrated between January and May (Santos et al., 2014). Seeds were sterilized with 0.5% sodium hypochlorite solution (v/v) for 5 min, germinated in Petri dishes, and maintained in germination chamber at 25 °C for 12 h photoperiod. After germination, seedlings were maintained in growth chamber at 25 °C, 70% relative humidity, and 12 h photoperiod for 15 days being watered daily. Then, young plants were transferred to pots containing 8 kg of red yellow Podzolic soil and kept in a greenhouse. The soil chemical composition was phosphorus (P) (40 mg dm^{-3}), potassium (K) (0.45 cmolc. dm^{-3}), aluminum (Al) (0.0 cmolc. dm^{-3}), calcium (Ca) (5.05 cmolc. dm^{-3}), magnesium (Mg) (0.95 cmolc. dm^{-3}) and pH 6.6.

After three months of germination, young plants were divided into two treatments: a control (irrigated with 100% the pot capacity, 400 mL) and a drought (interruption of water irrigation). Each treatment had seven plants. The maximum stress was determined when stomatal conductance was close to zero (10th day after irrigation interruption). Then, pots were rehydrated for five days from the end of the afternoon of the 10th day.

2.2. Leaf relative water content (RWC), soil water status and leaf osmotic potential (Ψ_s)

The leaf relative water content (RWC) was measured according to Barrs and Weatherley (1962). Soil moisture (SM) was measured using Falker HFM2030 m (HidroFarm, Porto Alegre, RS, Brazil), and data were transformed from volumetric to gravimetric by $U\ (\%) = \theta \times 100/D_s$, where U (%) is gravimetric water content, θ (%) is volumetric water content, and D_s is soil density (g cm^{-3}). The osmotic potential (Ψ_s) was measured using frozen leaf tissue (500 mg), which was thawed and centrifuged at 10,000 g for 10 min at 4 °C to extract the cell sap. The osmolarity (c) of the sap was measured using vapor pressure osmometer (Silva et al., 2010). The Ψ_s was calculated by $\Psi_s = -c \times 2.58 \times 10^{-3}$, according to the Van't Hoff equation.

2.3. Gas exchange and chlorophyll fluorescence

Gas exchange and chlorophyll fluorescence were measured using IRGA (Li- 6400, Li-Cor, Lincoln NE, USA) attached to a modulated fluorometer (6400–40), with gas flow of 400 μmol s^{-1}. Measurements were performed from 0800 h to 0900 h at constant photosynthetic photon flux density (PPFD) of 900 μmol m^{-2} s^{-1} on mature, but not senescent, fully expanded leaf. Net photosynthetic rate (A) and stomatal conductance (g_s) were measured, and water use efficiency (WUE) was calculated by dividing A by the transpiration rate (E).

Leaves were dark adapted for 30 min to determine the minimum chlorophyll fluorescence (F_o). Then, the maximum chlorophyll fluorescence (F_m) was obtained by a saturation pulse at ~7800 μmol m^{-2} s^{-1}. The fluorescence emission at a steady state (F_t) and the maximum fluorescence (F_m') were determined for light-adapted leaves undergoing stable photosynthesis. The photochemical parameters were calculated according to Baker (2008): maximum quantum efficiency of PSII ($F_v/F_m = (F_m - F_o)/F_m$), PSII maximum efficiency ($F_v'/F_m' = (F_m' - F_o')/F_m'$, where F_o' is the minimal fluorescence of a light adapted leaf that has momentarily been darkened). Measuring minimal fluorescence of a light adapted leaf involves turning off the actinic light briefly while using a pulse of weak far-red light (740 nm centre wavelength) to maximally Q_A oxidation. The far-red radiation drives PSI shortly to help drain PSII of electrons. Electron transport rate ($ETR = (F_q'/F_m') \times PPFD \times 0.5 \times 0.84$, where 0.5 was used as the fraction of excitation energy distributed to PSII and 0.84 was used as the fraction of incident light absorbed by the leaves), photochemical quenching ($qP = (F_m' - F_s)/(F_m' - F_o')$) and non-photochemical quenching ($NPQ = (F_m - F_m')/F_m'$) were calculated. The relative energy excess at the PSII level ($EXC = (F_v/F_m) - (F_q'/F_m')/(F_v/F_m)$) was calculated according to Bilger et al. (1995). Vapor pressure deficit (VPD) was measured at 0800 h, 1400 h and 1700 h according