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Stomatal conductance and foliar phytohormones under water status changes in *Annona leptopetala*, a woody deciduous species in tropical dry forest

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A R T I C L E I N F O

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A B S T R A C T

Stomatal conductance is influenced by environmental variables and phytohormonal balance. However, the control of phytohormones on stomatal behavior is still unclear for woody species in seasonally dry tropical forests during the year. This study correlated stomatal behavior, environmental parameters and phytohormones levels in rainy and dry seasons, and the transition between them. We used the woody deciduous species *Annona leptopetala*, from a Brazilian dry tropical forest, as model, and to test our central question we measured plant and soil water status, stomatal conductance (g_s), and hormones levels. Principal Component Analysis (PCA) identified clustering patterns of seasons related to measured variables. The trees begin regrowth of leaves in the first rains of January with decreases of ABA foliar level, which begins to stimulate a greater stomatal opening. Stomatal conductance and leaf water status were related to vapor pressure deficit, photon flux density, and soil water balance. Abscisic acid (ABA) level was higher in dry months, favoring small stomatal aperture, and limiting ethylene precursor (ACC) production, which was higher in rainy season. Jasmonic acid levels were higher in rainy months, and showed a positive relation with g_s. Salicylic acid levels were not related to g_s. Our analysis concluded that two main phytohormones, ABA and ethylene precursor, influenced directly on stomatal behavior during the year; moreover, the present study showed that *A. leptopetala* phenology is associated with phytohormones balance.

1. Introduction

Plants are often exposed to severe water deficiency in Seasonally Dry Tropical Forests (SDTF) and consequently show different strategies of drought tolerance (Lima et al., 2012; Oliveira et al., 2014; Figueiredo et al., 2015). One of these strategies is the phenological pattern shown by deciduous species, which lose their leaves as soil water availability decreases, to escape from water deficit effects (Lima et al., 2012).

Stomatal closure is the plant's first response to water deficit (Schroeder et al., 2001). The mechanism regulating stomatal behavior is complex and involves, among other factors, the interaction of different phytohormones (Skelton et al., 2017). The most known signaling pathway is that of Abscisic Acid (ABA) (McAdam and Brodribb, 2015), although it acts synergistically and antagonistically with jasmonic acid, auxins, ethylene, and cytokinins (Nemhauser et al., 2006; Daszkowska-Golec and Szarejko, 2013). In general, ABA acts negatively on stomatal control, causing stomatal closure under water deficit conditions. However, the same environmental and genetic factors conferring high ABA foliar level also increase stomatal sensitivity to closure signals, such as low soil moisture or high vapor pressure deficit (Giday et al., 2014; McAdam and Brodribb, 2016). Thus, the relationship among phytohormones concentration, environmental variables and stomatal closure seems to be species-specific (Wilkinson and Davies, 2010; McAdam and Brodribb, 2015).

Woody deciduous species from semiarid regions have shown ability to adjust control of stomatal opening, foliar primary biochemistry, activity of antioxidant enzymes, and changes in anatomical traits, such as decrease in stomata density during periods of limited water availability (Oliveira et al., 2014; Santos et al., 2014; Falcão et al., 2015; Figueiredo et al., 2015). Most studies on deciduous species under field conditions have focused on the stomatal response to soil drought and other environmental factors that may affect stomatal opening (Thomas and

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Eamus, 2002; McAdam and Brodribb, 2015). However, changes in stomatal conductance and its relationship to the plant water status and phytohormonal balance were little evaluated yet, especially throughout annual variation in rainfall regime (Skelton et al., 2017).

Annona leptopetala is a woody, deciduous and highly representative species of the Brazilian semiarid region. It has high wood density, and its vegetative and reproductive development is strongly correlated with water availability in the environment (Lima et al., 2012). Although secondary metabolites composition has been measured in this species (Feitosa et al., 2009; Costa et al., 2012), no studies were carried out on ecophysiological aspects, such as phytohormonal balance, that coordinate stomatal response, especially in areas with marked seasonality of water availability. In this study we tested the hypothesis that this woody deciduous species has high foliar ABA level under low soil water balance, at the same period low xylem water potential and stomatal conductance would be noted. Thus, this study evaluated changes in stomatal behavior of *A. leptopetala* in response to soil water availability, and the relationship among stomatal, phytohormones, leaf water status and environmental variables.

2. Material and methods

2.1. Study site and plant material

The study was carried out in a 300 ha site of a woody Seasonally Dry Tropical Forest (SDTF) fragment located in the Mata da Pimenteira State Park (7° 59′ 31" S, 38° 17′ 59" W), municipality of Serra Talhada, Brazil. Soil is sandy loam type, climate is BSh type, hot semiarid, according to Köppen-Geiger's classification (Alvares et al., 2013), with 750 mm average annual rainfall. Data collection covered dry–rainy transition period (January 2014); the rainy season (March and May 2014); rainy–dry transition (July 2014); and a rainfall event during the dry season (December 2014). Data on rainfall, the mean air temperature and water balance were obtained from a meteorological station located nearby the study site. For measurements of gravimetric water content, soil samples were collected at 30 cm depth near plant individuals, packed in airtight zip lock bags, and referred to laboratory for weighing. Soil water content (SWC) was calculated by the formula: SWC (%) = [(FW-DW)/DW]*100, where FW is fresh weight and DW is dry weight, obtained after 48 h at 70 °C in a forced ventilation oven. The vapor pressure deficit (VPD) at the moment of measurements was calculated using air relative humidity and air temperature at the moment of gas exchange measurements, obtained with a portable thermohygrometer, through the formula: VPD = e_s-e_a, where e_s is saturated vapor pressure, and e_a is environmental vapor pressure (Campbell and Norman, 1998). All ambient data are shown in Fig. 1.

The plant model used in this study was *Annona leptopetala* (R. E. Fr.) H. Rainer, a deciduous tree, native and endemic species from Caatinga, the Brazilian SDTF (Lemos and Zappi, 2012; Albuquerque et al., 2012; Santos et al., 2014). Five individuals were tagged for measurements. Only adult individuals with healthy foliage were selected, without significant damage caused by herbivory or diseases, located at the lower border of the canopy, but totally exposed to the sun. Measurements were performed on a single day in each month of collection. In December 2014, the measurements were carried out a few days after the singular rain event.

2.2. Water status and stomatal conductance

Xylem water potential (Ψ_x) was measured using a pressure chamber (Soil Moisture Equipment Corp., Santa Barbara, CA, USA), according to Scholander et al. (1964). Measurements were performed on fully expanded, non-senescent and free of damage leaves, in the period of the day with the highest VPD values (13:00 h).

Stomatal conductance (g_s) was measured using Infrared Gas Analyzer (IRGA, ADC, model LCi-pro; Hoddesdon, UK). Measurements were carried out in fully expanded, not senescent and free from damage leaves on sunny and cloudless days, between 13:00 h and 13:30 h. We defined the time of adaptation to the conditions of the IRGA chamber in a maximum of two minutes, and the measurements were saved when the plant presented three similar measures in sequence (CV < 10%). Equipmenťs light chamber was set with constant photosynthetic photon flux density (PPFD), adapted to the light radiation of the day and moment of collection.

2.3. Phytohormones analysis

After the stomatal conductance measurements, leaf samples of approximately 3 g of fresh weight were collected in the same leaf at 14:00 h and immediately frozen in liquid nitrogen, and then stored in a freezer at −80 °C until analysis. Abscisic acid (ABA), salicylic acid (SA), jasmonic acid (JA), *trans*-zeatin (Tz), and ethylene precursor 1-aminocyclopropane-1-carboxylic acid (ACC) were analyzed according to Albacete et al. (2008). We submitted about 0.1 g of lyophilized plant material to a hydro-alcoholic extraction (methanol/water, 80:20 v/v). Solids were separated by centrifugation (20.000g, 15 min), and re-extracted for 30 min at 4 °C with 0.5 mL of the same extraction solution. Supernatant fraction was passed through Sep-Pak Plus C18 columns (SepPak Plus, Waters, USA) for removal of any lipid or leaf pigments interference, and vacuum evaporated at 40 °C until organic solvent total removal. The residue was solubilized in 1 mL of methanol/water (20:80 v/v) using ultra-sonic bath. Dissolved samples were filtered in a Millex filter with 13 mm of diameter and a 0.22-μm membrane (Millipore, Bedford, MA, USA).

We injected 10 μL of filtered extract in a U-HPLC–MS, consisting of a series Accela U-HPLC linked to a mass spectrometer (ThermoFisher

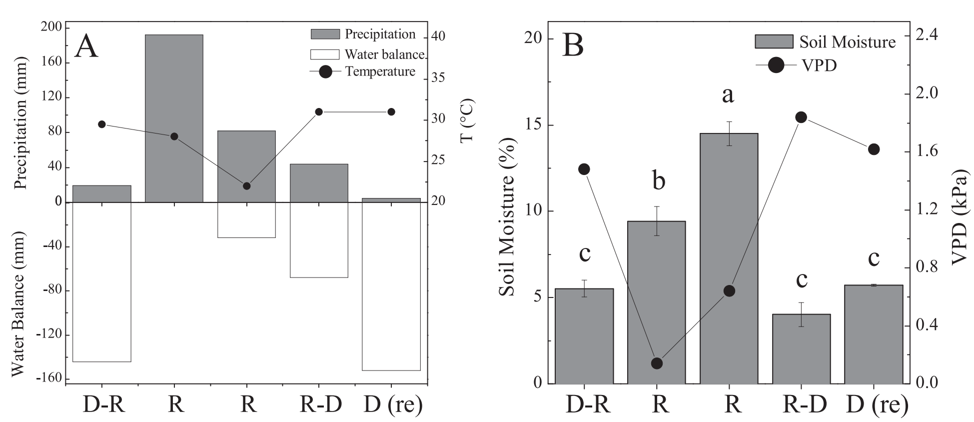

Fig. 1. Environmental parameters for the months of collection in Serra Talhada, Brazil. (A) Precipitation (mm), air temperature (°C) and soil water balance (mm); (B) Soil moisture (%) and vapor pressure deficit (kPa). D-R − dry–rainy season transition; R − rainy season; R-D − rainy–dry season transition; D (re) − dry season with a rain event.