Different physiological responses under drought stress result in different recovery abilities of two tropical woody evergreen species

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ABSTRACT

The effects of water deficit on physiological and biochemical variables of young plants of two tropical woody species, *Pachira aquatica* and *Sterculia foetida*, and their recovery abilities were measured. Leaf water potential, gas exchange and selected carbon metabolism components were measured in a greenhouse experiment with control, moderate and severe water deficit treatments. Under severe drought stress, gas exchange and chlorophyll fluorescence decreased in both species compared to their respective controls. After rehydration, *P. aquatica* and *S. foetida* showed a partial and full recovery of the measured variables, respectively. In addition, a decrease and an increase in photosynthetic pigments were observed for *P. aquatica* and *S. foetida*, respectively, compared to their controls. In conclusion, the two species showed differing responses regarding photosynthetic pigment content dynamics for tolerating water deficit. Individuals of *P. aquatica* in the severe water deficit treatment showed a decrease in pigments, which may have impaired the recovery of metabolism (gas exchange) after rehydration, while *S. foetida* experienced an increase, favoring a full recovery of gas exchange and biochemical metabolism after rehydration.

Keywords: chlorophyll fluorescence, climate changes, gas exchange, semiarid, water deficit

Introduction

In tropical regions, which present a strong seasonality and water restriction, woody and evergreen species can be a viable alternative for sustainability of the local biodiversity. Climate changes increase rainfall irregularities and average temperatures in semiarid regions (Donohue *et al*. 2013), such as the Brazilian Northeast. It is thus essential to understand how tree species behave under water restriction and which strategies they use to tolerate low water availability in the soil (Souza *et al*. 2010; Flexas *et al*. 2013).

In general, under water deficit, the leaf metabolism of woody tropical species changes, resulting in changes in water potential, gas exchange, chlorophyll fluorescence, organic solute contents and photosynthetic pigment (Frosi *et al*. 2013; Rivas *et al*. 2013; Oliveira *et al*. 2014). Some species decrease their photosynthetic pigments and accumulate organic solutes without showing a faster recovery of the photosynthetic activity, while other species show the opposite behavior (Souza *et al*. 2010; Rivas *et al*. 2013; Oliveira *et al*. 2014). This variation in plant responses, when subjected to water deficit and rehydration, is relevant

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especially for evergreen species in semiarid environments due to the seasonality of water availability.

Pachira aquatica and *Sterculia foetida* are tropical woody evergreen species belonging to the Malvaceae family. They may have a potential for biodiesel production under semiarid conditions due to the high content and quality of oil in the seeds (Oliveira *et al*. 2000). Studies on these species focus on germination and characterization of oilseeds for biodiesel production. Ecological uses are also studied, such as the ability to recover degraded areas (Santos *et al*. 2004; Infante-Mata *et al*. 2014). However, ecophysiological aspects such as drought tolerance are unknown, especially those related to photosynthetic activity and recovery after periods of low water availability.

The maintenance of the integrity of the photosynthetic machinery is a most desirable ability in evergreen species, enabling their growth in semiarid regions. Thus, this study aimed to evaluate the effects of water deficit and recovery capacity of two tropical woody evergreen species, *P. aquatica* and *S. foetida*, in a greenhouse, on physiological and biochemical variables. We hypothesize that both species will present a similar response regarding photosynthetic and biochemical adjustments during a water restriction period and also during rehydration, since both species belong to the same family and are widely found in the Brazilian Northeast.

Materials and methods

Plant material, growth conditions and experimental design

Paquira aquatica Aubl. and *Sterculia foetida* L. seeds were collected in Pernambuco State, Brazil. The experiment was conducted in a greenhouse (8°08′58″S, 34°56′55″W). Seeds were previously disinfected in 1% sodium hypochlorite for five minutes, followed by washing three times in distilled water. Disinfected seeds were subsequently planted in plastic pots with a 10 kg capacity (0.27 x 0.22 m) of substrate. They contained a mixture of topsoil (organic), red clay and sand at a ratio of 3:1:1. They were then transferred to the greenhouse. *P. aquatica* seeds germinated in three days, while *S. foetida* seeds germinated in six days.

P. aquatica and *S. foetida* plants were kept in the greenhouse under an average temperature of 35 ± 2 °C and 45-60% relative humidity, watered daily with 600 mL (capacity of soil in pot) for nine and seven months, respectively, until the beginning of the treatments. The design was completely randomized with the following different hydration levels: control (100% capacity of soil in pot), moderate (50% capacity of soil in pot) and severe (interruption of irrigation), with four plants per treatment (one plant per pot). The maximum stress occurred 15 days after the interruption of irrigation, when gas exchange reached almost zero, and rehydration took place over the

subsequent four days for all plants subjected to selected water deficit treatments (moderate and severe).

Leaf water potential (Ψ_l) and soil moisture

Leaf water potential (Ψ_l) was measured using a Scholander pressure chamber (Soil moisture Equipment Corp., Santa Barbara, CA, USA) on the third fully expanded and not senescent leaf pair (Scholander *et al*. 1965). Measurements were performed at 6:00 am during maximum stress and rehydration. Soil moisture was obtained at a depth of 20 cm (sensor reach) using a soil moisture meter (v/v) (Falker HFM 2030, Porto Alegre, BR).

Leaf gas exchange, chlorophyll fluorescence and vapor pressure deficit (VPD)

Leaf gas exchange was performed from 9:00 am to 11:00 am on the third fully expanded and not senescent leaf pair on alternate days during the experiment. Chlorophyll fluorescence variables were measured at the same time of the gas exchange leaf pair. The measurements were performed using a LI-COR 6400XT portable photosynthesis system (Li-COR - Lincoln, Nebraska, USA) attached to a modulated fluorometer (6400-40). The photosynthetic photon flux density was fixed at 2,000 mmol m^{-2}s^{-1} according to the incident radiation at the time of measurement of gas exchange. Stomatal conductance (g_s), net photosynthetic rate (*A*) and transpiration rate (*E*) were measured. In order to evaluate the variable chlorophyll fluorescence, leaves were adapted to dark for 30 min prior to the measurements of minimal fluorescence (F$_o$). Maximum fluorescence on the dark-acclimated leaf (F$_m$) was measured after applying a saturation pulse of ~7,000 µmol m^{-2} s^{-1}. Steady state fluorescence emission (F′) and maximum fluorescence (F$_m$′) were determined using a light-adapted leaf under a stable photosynthesis obtained with the exposition of leaves to a light intensity of 2,000 µmol m^{-2} s^{-1} for 1.0 to 1.5 min. The data were used to calculate the following fluorescence variables: maximum quantum efficiency of PSII photochemistry (F$_v$/F$_m$), PSII operating efficiency (F$_q$′/F$_m$′), photochemical quenching (qP), electron transport rate (ETR), and non-photochemical quenching (NPQ) (Baker 2008).

Vapor pressure deficit (VPD) was calculated using the formula $e_s - e_a$, where e_s is the saturated vapor pressure and e_a is the ambient pressure deficit (Campbell & Norman 1998), using temperature and relative humidity obtained with a digital thermohygrometer (Termo-Higro SH 122, J Prolab. São José dos Pinhais, BR).

Biochemical analysis

The leaves of third leaf pair used for gas exchange and chlorophyll fluorescence measurements were collected

at 3:00 pm, during maximum stress and on the last day of rehydration for biochemical analysis. Samples were immediately frozen in liquid nitrogen and stored at -20 °C. The analyses were performed soon after the end of the experiment. For the analyses, 50 mg of fresh leaves were used to determine total soluble sugar (SS) (Dubois *et al.* 1956), leaf free amino acid (FAA) (Moore & Stein 1948), total soluble protein (TSP) (Bradford 1976), chlorophyll *a* and *b* (Chl *a* and Chl *b*), and carotenoid (Car) contents (Lichtenthaler & Buschmann 2001). All readings were performed using a double beam spectrophotometer (Genesis 10S UV-Vis, Thermo Scientific, Waltham, USA). The insoluble fraction from carbohydrate extraction was used to determine the starch content. The pellet was hydrolyzed for 1 h with 10 units of amyloglucosidase, and the resulting sugars were analyzed one more time (Dubois *et al.* 1956).

Statistical analysis

The experiment was conducted in a 2x3 factorial design considering two species (*P. aquatica* and *S. foetida*) and three water levels (control, moderate and severe water deficit). The mean and standard error were calculated for all attributes (n=4 plants per treatment). Leaf water potential, soil moisture, gas exchange, chlorophyll fluorescence and biochemical data were analyzed using two-way analysis of variance (ANOVA) (species x water levels), and significant differences were compared using the Newman Keuls test at a 5 % probability. The data were analyzed using the Statistica 8.0 software (StatSoft. Inc., Tulsa, Oklahoma, USA). The statistical summary is shown in Tab. 1. A principal component analysis (PCA) was performed to verify possible groupings among dependent variables, eliminating redundancies and defining the most important variables to separate groups with the highest stress and rehydration. For PCA analysis, the data were transformed (ranging) in order to be standardized due different magnitudes of scale and level of importance of each variable, determined by the order of eigenvectors (McGarigal *et al.* 2000). The

level of importance of each principal component (PC) was determined using the Broken-stick method, in which eigenvalues that exceeded what was expected were kept for interpretation. Analyses were performed using the Fitopac 2.1.2.85 software (Shepherd 2010).

Results

Leaf water potential (Ψ_1) and soil moisture (SM)

Under maximum stress, the Ψ_1 was higher in *P. aquatica* plants compared to *S. foetida* plants, showing a decrease in the severe treatment in both species compared to the control. Moderate treatments did not differ (Tab. 2). The soil moisture, for the control and moderate treatments, was 20 % for both species, whereas under severe water deficit it was 4 %. After rehydration, Ψ_1 and soil moisture were recovered in the severe treatment and did not differ from control plants for both species. In this period, AS maximum stress, higher values of Ψ_1 were observed for *P. aquatica* compared to *S. foetida* plants.

Leaf gas exchange, chlorophyll fluorescence and vapor pressure deficit (VPD)

The VPD was 3 and 4 kPa in maximum stress and rehydration days, respectively. Regarding gas exchange, the control and moderate treatments presented similar g_s, *A* and *E* values during the experiment for both species. However, the severe treatment, under maximum stress, showed significant decreases in all variables compared to the control treatment for each species, i.e., more than 80 %, with a smaller decrease in *A* in *S. foetida* plants (Tab. 2). After rehydration, *P. aquatica,* under severe water deficit, had a partial recovery of g_s, *A* and *E* compared to the control, while *S. foetida*, after a severe water deficit, had a full recovery of gas exchange rates.

Table 1. Statistical summary of two-way ANOVA for each period of analysis (maximum stress and rehydration). Significant levels are indicated as follows: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ns, not significant for leaf water potential (Ψ_1), stomatal conductance (g_s), net photosynthetic rate (*A*), transpiration rate (*E*), maximum quantum efficiency of PSII (F_v/F_m), PSII operating efficiency (F_q'/F_m'), photochemical quenching (qP), electron transport rate (ETR), non-photochemical quenching (NPQ), starch, soluble sugars (SS), free amino acids (FAA), total soluble protein (TSP), chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*) and carotenoids (Car).

Maximum stress	Attributes															
	Ψ_1	g_s	A	E	F_v/F_m	F_q'/F_m'	qP	ETR	NPQ	Starch	SS	FAA	TSP	Chl a	Chl a	Car
S (species)	*	ns	***	ns	ns	ns	ns	ns	*	***	***	ns	***	***	***	***
W (water)	***	***	***	***	***	***	***	***	***	***	***	ns	ns	ns	ns	**
S x W	***	ns	***	ns	ns	***	**	***	ns	***	*	*	ns	***	***	**
Rehydration																
S (species)	***	**	***	***	***	ns	ns	ns	**	***	*	**	ns	***	***	***
W (water)	ns	**	***	***	**	*	ns	***	**	***	***	*	***	ns	**	*
S x W	ns	**	***	***	**	***	ns	***	***	***	*	ns	ns	ns	*	***

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Table 2. Leaf water potential (Ψ_l), stomatal conductance (g_s), net photosynthetic rate (A), transpiration rate (E), maximum quantum efficiency of PSII (F_v/F_m), PSII operating efficiency (F_q'/F_m'), photochemical quenching (qP), electron transport rate (ETR) and non-photochemical quenching (NPQ) of *P. aquatica* and *S. foetida* leaves of young plants under greenhouse conditions subjected to the following water restriction levels: control (100 % capacity of soil in pot), moderate (50 % capacity of soil in pot) and severe (suspension of irrigation). Values represent the mean values of experimental replications followed by standard error (n=4±S.E.). Different letters indicate differences by Newman Keuls test.

Attributes	Maximum Stress					
	P. aquatica			*S. foetida*		
	Control	Moderate	Severe	Control	Moderate	Severe
Ψ_l (MPa)	-0.21±0.01^A	-0.25±0.02^A	-0.91±0.07^C	-0.42±0.01^B	-0.38±0.01^B	-1.06±0.02^D
g_s (mol m^{-2}s^{-1})	0.12±0.01^A	0.11±0.01^A	0.01±0.00^B	0.11±0.01^A	0.11±0.01^A	0.01±0.0^B
A (mmol m^{-2}s^{-1})	9.4±0.71^A	9.1±0.56^A	0.14±0.01^C	7.7±0.41^A	7.5±0.42^A	1.19±0.08^B
E (mmol m^{-2}s^{-1})	3.9±0.10^A	4.0±0.20^A	0.4±0.01^B	3.7±0.34^A	4.0±0.37^A	0.5±0.01^B
F_v/F_m	0.78±0.01^A	0.77±0.01^A	0.69±0.02^B	0.75±0.00^A	0.80±0.03^A	0.67±0.03^B
F_q'/F_m'	0.10±0.01^A	0.11±0.01^A	0.02±0.00^D	0.07±0.00^B	0.08±0.01^{AB}	0.03±0.00^C
qP	0.29±0.01^A	0.31±0.04^A	0.07±0.01^C	0.21±0.02^A	0.21±0.01^A	0.10±0.01^B
ETR	87.6±7.3^A	94.4±11.8^A	15.5±1.4^D	64.1±3.47^B	69.6±5.23^{AB}	21.81±1.40^C
NPQ	1.95±0.17^B	2.20±0.29^B	3.22±0.20^A	1.63±0.25^B	1.48±0.17^B	3.02±0.24^A
	Rehydration					
Ψ_l (MPa)	-0.29±0.01^A	-0.25±0.03^A	-0.26±0.03^A	-0.44±0.03^B	-0.39±0.03^B	-0.45±0.03^B
g_s (mol m^{-2}s^{-1})	0.11±0.00^A	0.11±0.01^A	0.03±0.00^B	0.12±0.02^A	0.11±0.01^A	0.11±0.01^A
A (mmol m^{-2}s^{-1})	10.0±0.28^A	9.0±0.56^A	2.7±0.25^B	9.0±0.74^A	8.5±0.44^A	10.3±0.36^A
E (mmol m^{-2}s^{-1})	4.2±0.15^A	4.3±0.34^A	1.7±0.15^B	4.5±0.63^A	4.6±0.13^A	4.6±0.28^A
F_v/F_m	0.70±0.01^A	0.63±0.01^B	0.74±0.02^A	0.76±0.02^A	0.73±0.01^A	0.73±0.01^A
F_q'/F_m'	0.11±0.02^A	0.11±0.01^A	0.05±0.01^C	0.09±0.01^{AB}	0.07±0.01^{BC}	0.09±0.01^{AB}
qP	0.32±0.01^A	0.30±0.01^A	0.22±0.02^A	0.25±0.01^A	0.24±0.02^A	0.25±0.01^A
ETR	86.8±10.60^{AB}	101.4±4.51^A	46.8±4.33^C	81.2±4.76^{AB}	73.6±3.91^B	82.0±2.24^{AB}
NPQ	2.12±0.10^{BC}	1.45±0.29^C	3.42±0.32^A	1.61±0.16^{BC}	2.33±0.10^B	1.59±0.11^{BC}

As for chlorophyll fluorescence, the severe treatment showed decreases in F_v/F_m, F_q'/F_m', qP and ETR and increases in NPQ, under maximum stress, for both species compared to control treatments (Tab. 3). Comparing the species, *P. aquatica* plants under severe water deficit had higher decreases in F_q'/F_m', qP and ETR compared to *S. foetida* plants under the same water restriction conditions. After rehydration, the recovery of photochemical attributes in severe treatments was similar to the recovery of gas exchange for both species. *P. aquatica* had a partial recovery of F_q'/F_m', qP and ETR in the severe treatment compared to the control, except for F_v/F_m, which had a full recovery in this treatment and a low recovery in moderate water deficit plants. Furthermore, the NPQ in the severe treatment remained with high values for this species under maximum stress. In *S. foetida* plants of the severe treatment, a full recovery of all photochemical variables, compared to control plants, was observed.

Biochemical analysis

Under maximum stress, starch was superior in *S. foetida* plants compared to *P. aquatica* in all treatments. *S. foetida* showed the leaf starch content higher in moderate water

deficit plants and decreased in severe treatment compared to control, without differences among *P. aquatica* treatments (Tab. 3). The SS, in general, was higher in *S. foetida* plants than *P. aquatica* plants, with reductions in the severe treatment for both species in comparison with control treatments. However, this reduction was more pronounced in *P. aquatica*. The FAA decreased only in the control treatment of *S. foetida*, while the TSP differed between species, with higher concentrations in *S. foetida* plants compared to *P. aquatica* plants. Regarding photosynthetic pigments, *S. foetida* plants had a higher concentration compared to *P. aquatica* plants. In addition, the dynamics of such pigments regarding water levels were different for each species. *S. foetida* plants of the severe treatment increased their concentrations, while *P. aquatica* plants decreased compared to control plants.

After rehydration, *S. foetida* plants had a higher concentration of starch compared to *P. aquatica*. In relation to water levels, the severe treatment of *P. aquatica* had a higher concentration of starch compared to control plants, while *S. foetida* plants of the severe water deficit treatment did not differ from the control, with higher concentrations when compared to *P. aquatica*. As for SS, the severe water deficit treatment had a lower concentration compared to their respective control for both species. The FAA differed between species, with higher concentrations observed for

Table 3. Starch, soluble sugars (SS), free amino acids (FAA), total soluble protein (TSP), chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*) and carotenoids (Car) of *P. aquatica* and *S. foetida* leaves of young plants under greenhouse conditions subjected to the following water restriction levels: control (100 % capacity of soil in pot), moderate (50 % capacity of soil in pot) and severe (suspension of irrigation). Values represent the mean values of experimental replications followed by standard error (n=4±S.E.). Different letters indicate differences by Newman Keuls test.

Attributes	Maximum Stress					
	P. aquatica			*S. foetida*		
	Control	Moderate	Severe	Control	Moderate	Severe
Starch (mmol kg^{-1} DW)	406.6±32.2^D	405.9±13.7^D	463.0±16.0^D	1510.2±18.8^B	1673.2±14.7^A	1314.1±25.3^C
SS (mmol kg^{-1} DW)	1118.2±30.1^B	1083.7±18.0^B	703.8±31.9^D	1221.3±24.7^A	1097.8±19.4^B	852.2±24.8^C
FAA (mmol kg^{-1} DW)	66.7±2.6^A	68.2±3.8^A	61.4±3.8^A	55.3±2.1^B	61.4±3.1^A	69.4±3.7^A
TSP (g kg^{-1}DW)	80.8±4.9^B	101.5±6.4^B	93.2±4.2^B	106.7±4.2^A	108.2±5.0^A	115.6±7.8^A
Chl *a* (g kg^{-1} DW)	2.8±0.3^D	2.3±0.1^E	1.3±0.1^F	4.2±0.1^C	4.7±0.1^B	5.5±0.2^A
Chl *b* (g kg^{-1} DW)	0.9±0.1^D	0.8±0.0^D	0.5±0.0^E	1.4±0.0^C	1.6±0.0^B	1.8±0.1^A
Car (g kg^{-1} DW)	0.8±0.0^C	0.8±0.0^C	0.5±0.0^D	1.5±0.0^B	1.7±0.0^A	1.6±0.0^{AB}
	Rehydration					
Starch (mmolkg^{-1} DW)	388.7±12.4^D	377.2±21.4^D	648.9±31.2^C	1743.7±21.8^A	1543.9±30.1^B	1681.4±38.0^A
SS (mmol kg^{-1} DW)	987.8±11.6^{AB}	882.3±19.6^{BC}	746.3±25.1^D	962.7±4.1^{AB}	1052.9±3.7^A	806.4±18.0^{CD}
FAA (mmol kg^{-1} DW)	53.6±1.1^B	55.5±1.8^B	65.1±3.5^B	89.0±8.5^A	79.3±4.5^A	93.7±5.3^A
TSP (g kg^{-1}DW)	103.3±8.8^B	141.6±2.8^A	154.9±4.2^A	104.7±11.8^B	132.9±4.7^A	127.7±5.7^A
Chl *a* (g kg^{-1} DW)	1.8±0.3^B	2.5±0.2^B	1.6±0.1^B	3.9±0.2^A	3.6±0.2^A	3.6±0.1^A
Chl *b* (g kg^{-1} DW)	0.4±0.0^C	0.8±0.1^B	0.7±0.0^B	1.1±0.1^A	1.1±0.0^A	1.1±0.1^A
Car (g kg^{-1} DW)	0.5±0.0^C	0.8±0.1^B	0.6±0.0^B	1.3±0.0^A	1.1±0.0^A	1.2±0.0^A

S. foetida plants compared to *P. aquatica* plants, while TSP differed regarding water levels, with low values in control plants for both species. Regarding photosynthetic pigments, high concentrations were observed in *S. foetida* plants when compared to *P. aquatica*. In relation to water levels, *S. foetida* did not differ among treatments, while *P. aquatica* plants had high values in the moderate and severe treatments when compared to the control.

Principal component analysis (PCA)

The PCA analysis performed on *P. aquatica* showed that all variables accounted for 70.25 % of the total data variation (Fig. 1A), evidencing a separation among treatments via PC1 and between periods (maximum stress and rehydration) via PC2. The variables that contributed the most to the differences between the groups had eigenvalues > 0.23; their respective substantial correlation coefficients are detailed below. For PC1, the most relevant attributes were A (0.97), E (0.96), g_s (0.96), ETR (0.91), qP (0.89), F_q'/F_m' (0.88), NPQ (-0.84), SS (0.83), Chl *a* (0.76), and Ψ_l (-0.75). For PC2, the most relevant attributes were FAA (0.77), Car (0.75), Chl *b* (0.69), and F_v/F_m (0.68).

Regarding *S. foetida*, the PCA showed that 80.25 % of the total data variance can be explained by the set of variables evaluated (Fig. 1B). The variables that contributed the most to the differences between groups had eigenvalues > 0.22; their respective substantial correlation coefficients are detailed below. In the graphical analysis, a separation

between the different periods (maximum stress and rehydration) was observed for PC1. Meanwhile, a separation between the treatments was observed for PC2 mainly during the maximum stress period. In that analysis, the control and the moderate treatments were grouped together and the severe treatment was distanced. For PC1, the most significant variables were A (0.98), ETR (0.96), E (0.96), F_q'/F_m' (0.94), qP (0.93), g_s (0.92), Chl *a* (-0.88), Ψ_l (0.88), starch (0.84), Chl *b* (-0.84), and NPQ (-0.77). For PC2, the most relevant attributes were SS (0.77), FAA (-0.74), F_v/F_m (0.66), TSP (-0.63), and Car (0.57).

Discussion

The stomatal closure observed for both *P. aquatica* and *S. foetida* under severe water deficit conditions might be related to a decrease in leaf water potential in these plants. Therefore, stomatal closure led to decreases in gas exchange and photochemical variables. Under severe water restriction, plants typically show a marked photoinhibitory effect characterized by decreases in F_v/F_m, F_q'/F_m', qP and ETR and increases in NPQ. Such responses suggested that plants invested in energy dissipation as heat, avoiding damage to the photosynthetic apparatus (Ribeiro *et al*. 2004). Similar results for light energy use in two tree species (*Piptadenia moniliformes* and *Trischidium molle*) under water stress were found by Souza *et al*. (2010). However, both species, also from the semiarid region of Brazil, did not
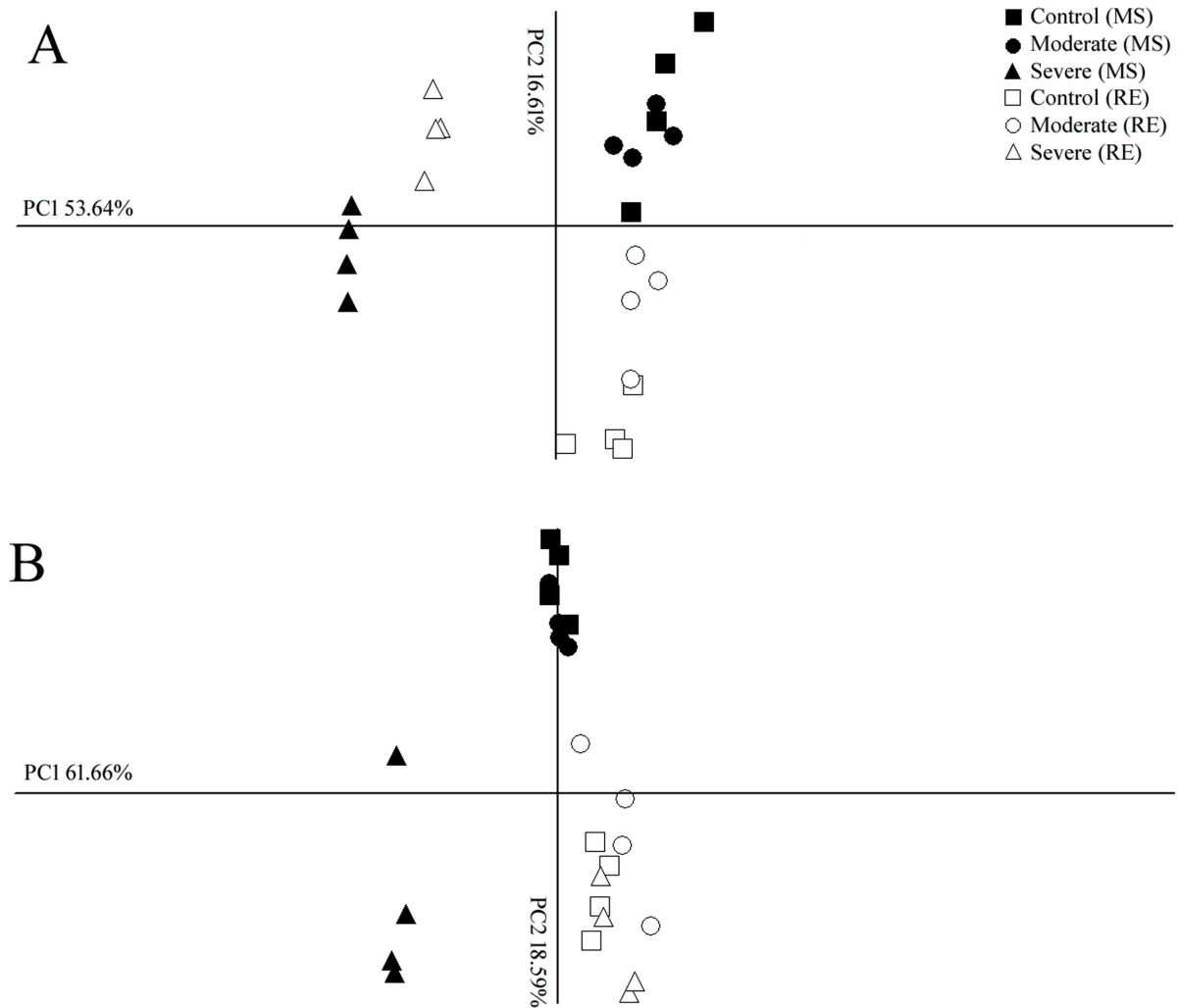
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Figure 1. The first (PC1) versus the second (PC2) principal component analysis of the ordination based on the whole dataset of the study of *P. aquatica* (A) and *S. foetida* (B). Closed symbols represent maximum stress (15th) and open symbols represents rehydration (19th) days.

present a permanent photochemical damage, recovering F_v/F_m values after rehydration. Furthermore, similar responses were also observed for *Moringa oleifera* (Rivas *et al*. 2013) and *Anadenanthera columbrina* (Oliveira *et al*. 2014), both common species in the Brazilian northwest region.

The responses of physiological variables after re-watering have received less attention than photosynthetic responses during the evolution of drought stress (Rivas *et al*. 2016). After a severe water deficit, the recovery of photosynthesis may be progressive and incomplete. In general, plants subjected to a severe water deficit present 40-60 % of the maximum photosynthetic rate one day after re-watering, and the recovery continues over the following days, although maximum photosynthesis rates are not always recovered (Gallé *et al*. 2007). This pattern was observed for *P. aquatica* plants subjected to severe water deficit. However, a full

recovery of gas exchange and photochemical variables could be achieved with more rehydration days for this species.

Under maximum stress, the decrease in chlorophylls and carotenoids of *P. aquatica* plants under severe water deficit probably contributes to a slower recovery after rehydration. Similar responses in photosynthetic pigments, such as those observed for *P. aquatic*a under water restriction, were present in *Olea europaea* (Guerfel *et al*. 2009).

The decrease in photosynthetic pigment concentrations could contribute to avoid severe damages to the photosynthetic machinery, since under low water availability there would be lower CO_2 concentrations inside the chloroplast caused by stomatal closure (Hörtensteiner 2009; Oliveira *et al*. 2014). On the other hand, increases in chlorophylls and carotenoids observed for *S. foetida* plants subjected to severe water deficit, under maximum stress,

could provide a faster recovery of the photosynthetic activity after rehydration. A similar response was observed for young *Anadenanthera columbrina* and *Moringa oleifera* plants (Rivas *et al.* 2013; Oliveira *et al.* 2014). Such different responses regarding the dynamic content of leaf photosynthetic pigments may have influenced the performance of the photosynthetic machinery during the recovery period for both species.

The low concentration of starch and SS in plants of *P. aquatica* and *S. foetida* under severe treatments may be related to the low photosynthesis observed in these treatments when compared to the control. Other studies reported that water deficit may promote a decrease in SS (Frosi *et al.* 2013). The maintenance of FAA and TSP in *P. aquatica* plants of the severe treatment, under maximum stress, suggests a decrease in growth. Similar results for FAA and TSP were obtained by Oliveira *et al.* (2014) in *Anadenanthera columbrina* plants under drought. Different from *P. aquatica*, the high FAA in *S. foetida* plants under severe water deficit may have increased their tolerance to stress, favoring a full recovery after rehydration. However, the increase in starch and TSP in the severe water deficit treatment for both species after rehydration suggested that SS was directed to accumulate energy due to the recovery of photosynthesis and a re-growth thereafter. Such results indicated that changes in photosynthetic pigments are a strategy to tolerate water deficit in this species (Tab. 3).

The different responses of these species under limited water availability were clearly shown by the PCA. Due to the partial recovery of gas exchange and photochemical and biochemical attributes of *P. aquatica* plants subjected to severe water deficit, this treatment was formed a group separated from the other treatments under maximum stress and rehydration (Fig. 1A). *S. foetida* plants under a severe water deficit have a pattern similar to *P. aquatica* under maximum stress. However, after rehydration, all *S. foetida* treatments formed a single group due to the full recovery mainly of gas exchange and photochemical attributes in plants from the severe water deficit treatment (Fig. 1B).

This study evidenced that both species, under water restriction, showed different leaf responses to tolerate drought stresses, although both are perennial and tropical woody species, which leads us to reject the hypothesis of this study. Decreases in stomatal conductance and the expected consequences were observed. However, the species behaved differently regarding FAA, TSP and dynamic responses of photosynthetic pigments to tolerate water deficits. *P. aquatica* did not change its FAA and TSP, and degraded the pigments under severe water restriction. *S. foetida* increased its FAA and photosynthetic pigments concentrations in this condition. Thus, this reflects in a differential metabolism recovery for both species. *P. aquatica* had a partial recovery of gas exchange rates, while *S. foetida* showed a full recovery after rehydration. On the other hand, such differences between *P. aquatica* and *S. foetida* regarding recovery speed may be related to different times of response for each species in this study.

Although both species are able to tolerate drought stress using different leaf metabolism responses, *S. foetida* plants showed a better performance after re-watering when compared to *Pachira aquatica* plants. This indicates that a faster recovery may enable a competitive advantage by resuming growth after a period of drought, which is a cyclical event in semiarid environments.

Acknowledgements

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