

Photosynthetic adjustment after rehydration in *Annona emarginata*

Luís Paulo Benetti Mantoan¹ · Luiz Fernando Rolim de Almeida¹ ·
Ana Claudia Macedo² · Gisela Ferreira¹ · Carmen Silvia Fernandes Boaro¹

Received: 8 December 2015 / Revised: 3 March 2016 / Accepted: 12 May 2016 / Published online: 30 May 2016
© Franciszek Górski Institute of Plant Physiology, Polish Academy of Sciences, Kraków 2016

Abstract Reports indicate that *Annona emarginata* is tolerant to drought and is also used as an alternative rootstock for atemoya under drought conditions. The photosynthetic process can be adjusted after rehydration, resulting on total or partial recovery. The aim of this study was to determine if *A. emarginata* shows adjusts in gas exchange and the chlorophyll *a* fluorescence pattern after rehydration. During water deficits, the gas exchange and water content in the leaf decreased. However, after 5 days of rehydration, the water content in the leaf recovered and rehydrated plants presented the water use efficiency better than irrigated plants. Further remaining gas exchange parameters were lower in relation to irrigated plants. In chlorophyll *a* fluorescence, the rehydrated plants showed higher dissipation of light energy as heat, maintaining high activity of photoprotection. After rehydration, *A. emarginata* shows a positive correlation between transpiration and CO₂ assimilation rate, which optimize the water use efficiency. Thus, *A. emarginata* presents adjustments in gas exchange and photochemical process, resulting on a possible long-term photosynthetic acclimation to water deficiency.

Keywords Water deficit · Photosynthesis · Rootstock · Recovery · Drought · Annonaceae

Introduction

Lack of water is an environmental constraint for agricultural production (Chaves et al. 2003; Endres 2007; Sinclair et al. 2008) and for the development of plant communities (Boyer 1982; Verslues et al. 2006). A better understanding of the drought effect on the physiological processes on plants is a vital importance to improve practices of management and to predict the fate of natural vegetation under climate change (Chaves et al. 2003). Adjustments in the main parameters of the photosynthetic process occur in response to environmental changes. These adjustments regulate the photosynthesis during the day (Geiger and Servaites 1994). Therefore, the evaluation of photosynthesis along the day may provide information about the adaptive potential of plants on the environment (Li et al. 2015), in special under water deficit conditions (Tominaga et al. 2014).

Stomatal conductance is sensitive to water deficit (Yordanov et al. 2000; Medrano et al. 2002), because the reduction in stomatal aperture controls water loss (Chaves et al. 2003; David et al. 2007) and prevents xylem cavitation (David et al. 2007). However, the low stomatal conductance regulates photosynthesis in plants (Yordanov et al. 2000; Medrano et al. 2002), which restricts the entrance of CO₂ and reduces the photosynthetic carbon assimilation (Flexas et al. 2004, 2007). Furthermore, the CO₂ assimilation of plants under a water deficit can also be limited by metabolic issues, such as damage on the enzymes of photosynthesis and on photochemical processes that are responsible for generate energy used to CO₂ reduction (Grassi and Magnani 2005; Galmés et al. 2007; Varone et al. 2012; Ashraf and Harris

Communicated by S. Weidner.

✉ Luís Paulo Benetti Mantoan
luismantoan@hotmail.com

¹ Departamento de Botânica, Instituto de Biociências, Universidade Estadual Paulista “Júlio de Mesquita Filho” (UNESP), Botucatu, SP 18618-689, Brazil

² Departamento de Horticultura, Faculdade de Ciências Agrônômicas, Universidade Estadual Paulista “Júlio de Mesquita Filho” (UNESP), Botucatu, SP 18610-307, Brazil

2013). Thus, photosynthesis plays a key role on plant physiological performance under drought conditions (Pinheiro and Chaves 2011).

It is known that the CO_2 assimilation rate decreases during moderate water deficits or in response of decreased humidity (Chaves et al. 2003). However, few studies reported the photosynthetic response to rehydration (Izanloo et al. 2008; Xu et al. 2009). In general, the recovery of the photosynthetic rate after rehydration can occur quickly and completely or slow and partially (Miyashita et al. 2005; Chaves et al. 2009). In *Populus euphratica* Oliv. under water deficit followed by rehydration, Bogeat-Triboulot et al. (2007) observed partial recovery of CO_2 assimilation and stomatal conductance in rehydrated plants, suggesting a possible long-term acclimation to water deficit, due to an increase of the instantaneous water use efficiency. However, in *Setaria viridis* (L.) Beauv. and *Digitaria ciliaris* (Retz.) Pers., which were also subjected to rehydration after a period of water deficit, Luo et al. (2010) observed a complete recovery of CO_2 assimilation and stomatal conductance. This can result in fast growth resumption on these plants, which would provide an advantage of competition for available water (Luo et al. 2010).

The use of rootstocks tolerant to drought is a strategy used to increase the resistance to water deficiency in commercial plants (Colla et al. 2010; Martínez-Ballesta et al. 2010). The species *Annona emarginata* (Schltdl.) H. Rainer (*araticum-de-terra-fria*) has good compatibility with atemoya (*Annona squamosa* L. \times *Annona cherimola* Mill.), which results in orchards with a higher survival rate and tolerance to root fungus. In addition, reports of farmers indicate that *A. emarginata* species has a tolerance to soil with water shortages (Tokunaga 2000). According to the information above, the hypothesis of this study was that after rehydration, *A. emarginata* presents changes in gas exchange and photochemical process to optimize photosynthesis and water consumption, resulting on a possible photosynthetic acclimation to the water deficit. Therefore, the aim of this study was to evaluate if *A. emarginata* after rehydration shows adjustments in the patterns of gas exchange and chlorophyll *a* fluorescence.

Materials and methods

Plant material and experimental design

The experiment was conducted on the experimental area of the Botany Department at the Universidade Estadual Paulista “Júlio de Mesquita Filho” (UNESP) of Botucatu, State of São Paulo, Brazil (22°53'S, 48°26'W).

The seedlings of *A. emarginata* were obtained from seeds, which were cultivated in 4.5 L polyethylene bags filled with vermiculite and maintained in an environment with a 50 %

reduction in sunlight. During growth, plants were irrigated twice a day until the occurrence of water percolation, and once a week, we applied a Hoagland and Arnon (1950) solution at 50 % ionic strength. After 524 days of sowing, which was the moment of the start of the treatments, the plants were maintained under plastic cover with a 50 % reduction in sunlight to prevent the interruption of the water deficit treatment by the rain. The daytime averages of air temperature, relative humidity, vapor pressure deficit of the air (VPD) and photosynthetic photon flux density (PPFD) during the treatment were 28.5 °C, 48.8 %, 2.2 kPa and 757 $\mu\text{mol m}^{-2} \text{s}^{-1}$, respectively.

The experimental design used was a randomized block with treatments: irrigation, where the vessels were hydrated twice a day, until the observation of water percolation; suspension of irrigation, where the irrigation was stopped; and rehydration, where irrigation was stopped until 38 days after the start of the treatment (DAST), when leaves presented withered, with posterior rehydration. This experiment utilized 24 plants of *A. emarginata*. Of this total, 12 plants were used for the evaluation of gas exchange and chlorophyll *a* fluorescence, with three treatments and four repetitions of one plant. The 12 remaining plants were subjected only to suspension of irrigation treatment with rehydration, to monitor the level of dehydration. For this, was evaluated the relative water content in leaves (RWC) using four repetitions of one plant.

Control of irrigation

The monitoring of the substrate water content (SWC) was performed according to Thameur et al. (2012), where the mass of the vessel during application of the treatments ($\text{MV}_{\text{treatments}}$) was compared with the mass of these same vessels when irrigated until they achieved a maximum water holding capacity ($\text{MV}_{100\%}$). The $\text{MV}_{100\%}$ was obtained through irrigation until the occurrence of percolation of the excess of water, which occurred at night, and for the later weighing vessel, the following morning. The SWC was calculated using the formula: $\text{SWC} = (\text{MV}_{\text{treatments}} / \text{MV}_{100\%}) \times 100$ (Varone et al. 2012).

Relative water content in the leaves

The relative water content in the leaf (RWC) was evaluated once a week in plants submitted to suspension of irrigation followed by rehydration, using four replicates of one plant. For this, a leaf sample of 2 \times 2 cm was removed from the second or third fully expanded leaf of each plant. This sample was weighed to determine the fresh mass (FM) and immersed in deionized water during 4 hours to determine the turgid mass (TM). Then, the sample was placed in a forced air circulation oven at 70 °C until a constant mass was achieved

to determine the dry matter (DM). The RWC was calculated by $RWC = (FM - DM)/(TM - DM) \times 100$ (Weatherley 1949).

Gas exchange

The gas exchange were monitored during the DAST for irrigation and suspension of irrigation treatments at 10 h, and at the 43rd DAST (5 days after rehydration) for the irrigation, suspension of irrigation and rehydration treatments, at 9, 10, 11, 12, 14, and 16 h through an infrared gas analyzer (IRGA) (LI-6400, LI-COR, USA). Four replicate measurements were performed per treatment in the second and third fully expanded leaves. The average of the readings was used to evaluate the gas exchange in these plants. The parameters evaluated were: the CO_2 assimilation rate (A_{Net} , $\mu mol CO_2 m^{-2} s^{-1}$), the stomatal conductance (g_s , $mol H_2O m^{-2} s^{-1}$), the transpiration rate (E , $mmol H_2O m^{-2} s^{-1}$), the internal CO_2 concentration in the substomatal chamber (C_i , $\mu mol CO_2 mol^{-1} ar$), the carboxylation efficiency (A_{Net}/C_i) and the water use efficiency (A_{Net}/E) ($\mu mol CO_2 mmol H_2O^{-1}$).

Chlorophyll *a* fluorescence

The chlorophyll *a* fluorescence was evaluated at 44 DAST (6 days after rehydration) in the irrigation and rehydration treatments at 12 h (period of greatest luminosity) using a modulated pulse fluorometer (JUNIOR-PAM, Walz, Germany). Four replicate measurements were performed per treatment, and these measures were derived from the average of the readings collected in the second and third fully expanded leaves.

Before starting the measurements, the leaves were adapted to the dark during 30 min. After this period, a saturation pulse of $10,000 \mu mol m^{-2} s^{-1}$ of PPFD was applied during 0.6 s to obtain the F_m (maximum fluorescence adapted to darkness) and F_m' (maximum fluorescence adapted to light). The values of F_o (minimal fluorescence adapted to dark) and F_o' (minimal fluorescence adapted to light) were also obtained. After determination of F_m , predefined pulses of 125, 190, 285, 420, 625, 820, 1150, and $1500 \mu mol m^{-2} s^{-1}$ of PPFD were applied for 15 s.

The following parameters were calculated: the fraction of light absorbed by the photosystem II (PSII) antenna used to photochemical electron transport (P , photochemical), the fraction of light absorbed by the PSII antenna that is dissipated as heat (D , heat dissipation), the fraction of excitation energy not dissipated in the antenna that cannot be utilized for photochemistry (E , excess energy) (Demmig-Adams et al. 1996) and the efficiency of excitation energy captured by open PSII reaction centers (ϕ_{exc}) ($(F_m' - F_o')/F_m'$) (Zribi et al. 2009).

Statistical analysis

Statistical analyses were performed using SigmaPlot software (version 12). The data were submitted to the normality test of Shapiro–Wilk ($P < 0.05$). Therefore, to evaluate the differences between treatments in the gas exchange, a one way analysis of variance ANOVA for repeated measures followed by Tukey's test ($P < 0.05$) was conducted. For chlorophyll *a* fluorescence, a two way ANOVA (treatments \times PPFD) was performed followed by Tukey's test ($P < 0.05$). The gas exchange parameters of the rehydration treatment were submitted to the Pearson correlation test ($P < 0.05$).

Results

Monitoring the water deficit

Plants of *A. emarginata* submitted to the suspension of irrigation showed decreased of SWC and RWC, reaching values of 28 and 73 %, respectively, at 38 DAST (last day of water shortage) (Fig. 1a, b). After rehydration, the SWC was 99 % at 45 DAST and the RWC was 90 % at 44 DAST (Fig. 1a, b).

In the gas exchange monitored over the DAST, the plants under suspension of irrigation showed negative A_{Net} from 30 DAST and low g_s from 16 DAST (Fig. 2a, b). At 31 DAST, the SWC and RWC were 36 and 82 %, respectively, and at 17 DAST the SWC and RWC were 52 and 87 %, respectively (Fig. 1a, b).

Gas exchange throughout the day

Under rehydration, *A. emarginata* showed lower A_{Net} at 9 and 10 h (Fig. 3a), lower g_s at 9, 10, 11, and 12 h (Fig. 3b), lower E at 9, 10, 11, and 12 h (Fig. 3c), lower C_i at 9, 10, and 11 h (Fig. 3d) and lower A_{Net}/C_i at 9 h (Fig. 3e), in relation to irrigation treatment at 43 DAST (5 days after rehydration). However, the rehydrated plants had higher A_{Net}/E at 10 and 11 h (Fig. 3f) and higher A_{Net}/C_i at 11 and 14 h (Fig. 3e) compared to the irrigated plants.

A. emarginata subjected to water deficit showed lower g_s and E during the day compared to irrigated and rehydrated plants (Fig. 3b, c). The A_{Net} , A_{Net}/C_i and A_{Net}/E parameters of the suspension of irrigation treatment were not presented because they are negative, indicating that at 43 DAST this treatment was under a severe water deficit.

The air temperature, relative humidity, VPD and PPFD during the evaluations of gas exchange over the hours of 43rd DAST are in the Fig. 4a–d.

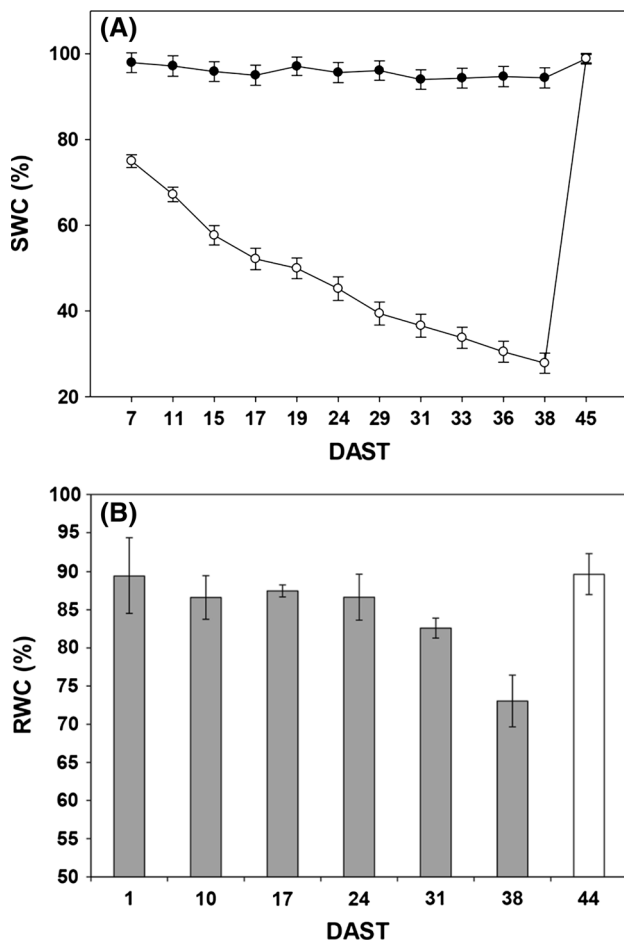


Fig. 1 The substrate water content (SWC; %) ($n = 4$) of the irrigation (black circle) and the suspension of irrigation treatment followed by rehydration (white circle) (a). Relative water content in the leaf (RWC; %) ($n = 4$) of the suspension of irrigation (gray bars) and the rehydration treatment (white bar) (b). Vertical lines indicate the standard deviation

Correlation between gas exchange parameters

At 43 DAST, significant correlations were observed between the gas exchange parameters of the rehydration treatment at 9, 10, 11, and 12 h (Table 1). Positive correlations were observed between A_{Net} and A_{Net}/Ci at 9 and 12 h, between A_{Net} and g_s at 10 and 11 h, between A_{Net} and E at 10 h and between g_s and E at 10, 11, and 12 h (Table 1). Negative correlations were found between Ci and A_{Net}/Ci at 9 h, between A_{Net} and A_{Net}/E at 11 h, between g_s and A_{Net}/E at 10, 11 and 12 h and between E and A_{Net}/E at 10 and 11 h (Table 1).

Chlorophyll *a* fluorescence

At 44 DAST, two-way ANOVA indicated no interaction between treatments and PPFD for P , D , E and ϕ_{exc}

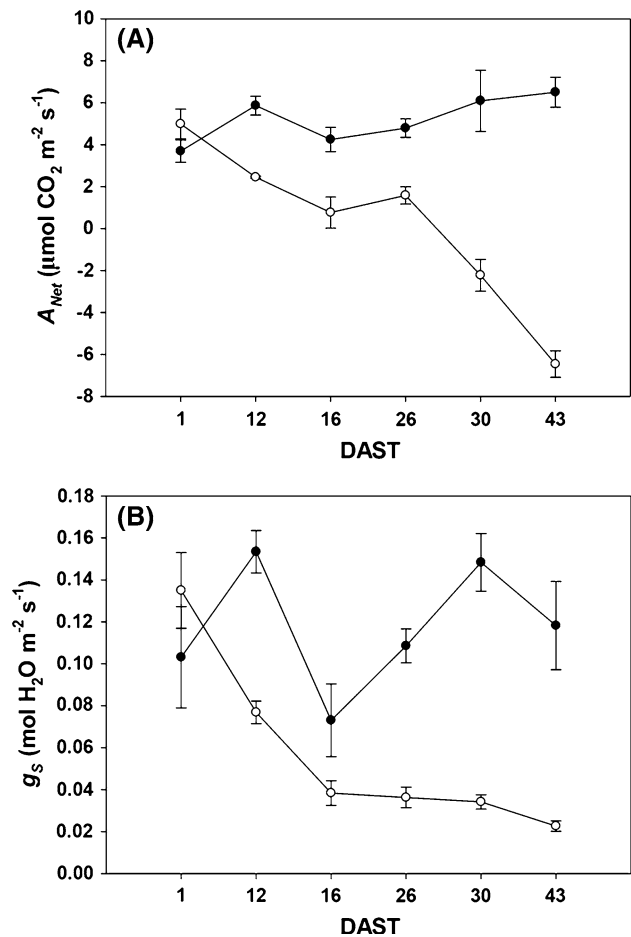


Fig. 2 Monitoring of the gas exchange of the irrigation (black circle) and the suspension of irrigation treatments (white circle) ($n = 4$). CO_2 assimilation rate (A_{Net} ; $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) (a) and stomatal conductance (g_s ; $\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$) (b). Vertical lines indicate the standard deviation

(Table 2), demonstrating that the light intensity did not influence the treatments.

Comparing the treatments, it was observed reduction on the capture of light energy (ϕ_{exc}) and use of light energy (P) in plants subjected to rehydration. However, in this same treatment there was no damage to the PSII, as observed by the low dissipation of energy excess not utilized by photochemical (E) and by the high dissipation of the light energy as heat (D) (Table 2). Comparing the different PPFD levels, it was observed an increase of D and E and reduction of P and ϕ_{exc} with increase of light intensity (Table 2).

Discussion

The cellular volume is determinant to the metabolic activity on the leaf (Sinclair and Ludlow 1985). According to Sinclair and Ludlow (1985), the relative water content in

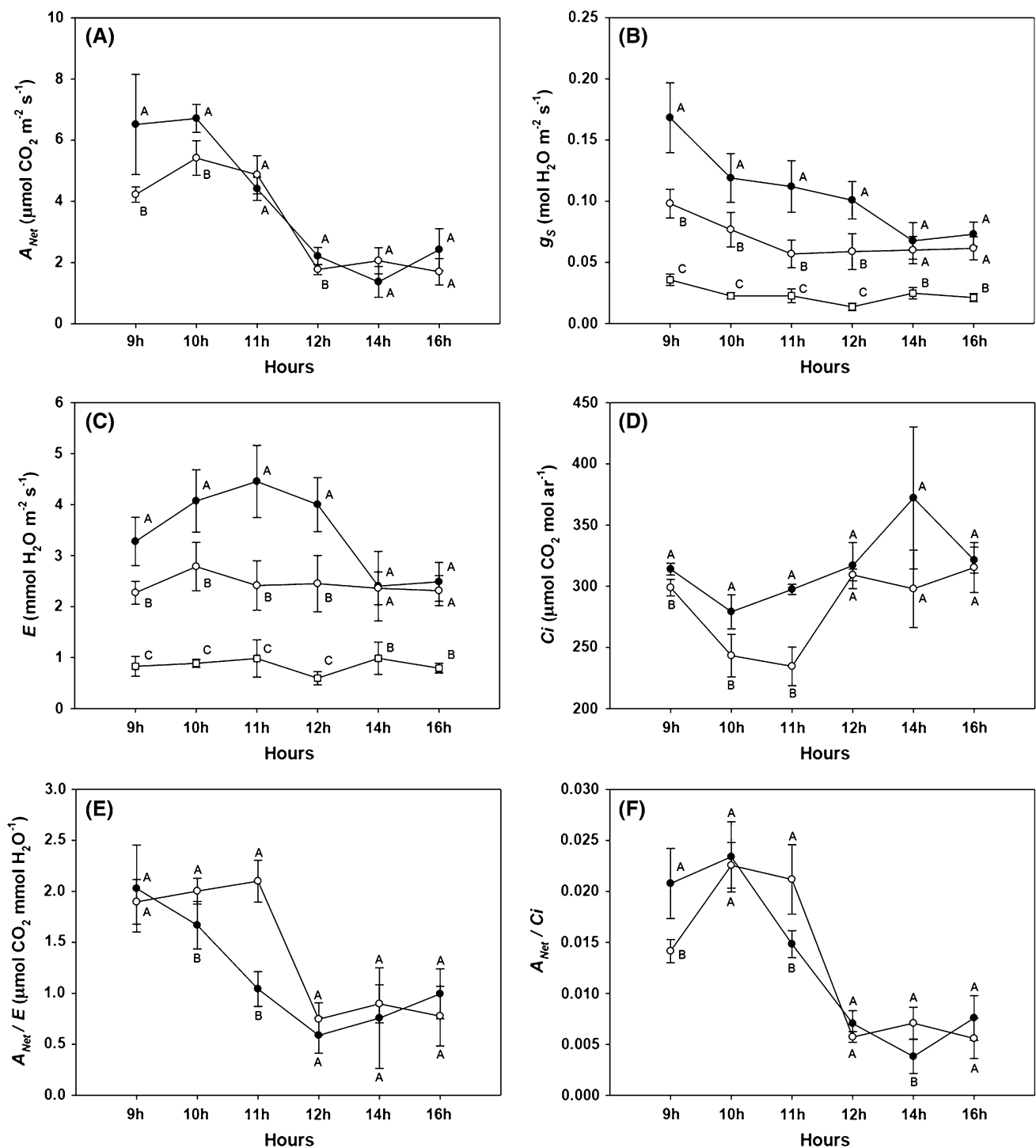


Fig. 3 The gas exchange of *A. emarginata* evaluated at 43 DAST at 9, 10, 11, 12, 14 and 16 h in the irrigation (black circle), rehydration (white circle) and suspension of irrigation treatments (white square) ($n = 4$). CO₂ assimilation rate (A_{Net} ; $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) (a), stomatal conductance (g_s ; $\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$) (b), transpiration rate (E ; $\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$) (c), internal CO₂ concentration in the sub-

stomatal chamber (C_i ; $\mu\text{mol CO}_2 \text{ mol}^{-1} \text{ ar}^{-1}$) (d), water use efficiency (A_{Net}/E) ($\mu\text{mol CO}_2 \text{ mmol H}_2\text{O}^{-1}$) (e) and carboxylation efficiency (A_{Net}/C_i) (f). The different letters indicate significant differences (Tukey's test, $P < 0.05$) between the treatments. The vertical lines indicate the standard deviation

the leaf (RWC) of plants under water deficit tends to have values below 80 %. However, even with reduction on substrate water content (SWC) (Fig. 1a), the RWC of the

suspension of irrigation treatment did not change until 24 DAST (days after the start of the treatment) (Fig. 1b). The reduction observed in the stomatal conductance (g_s) at 12

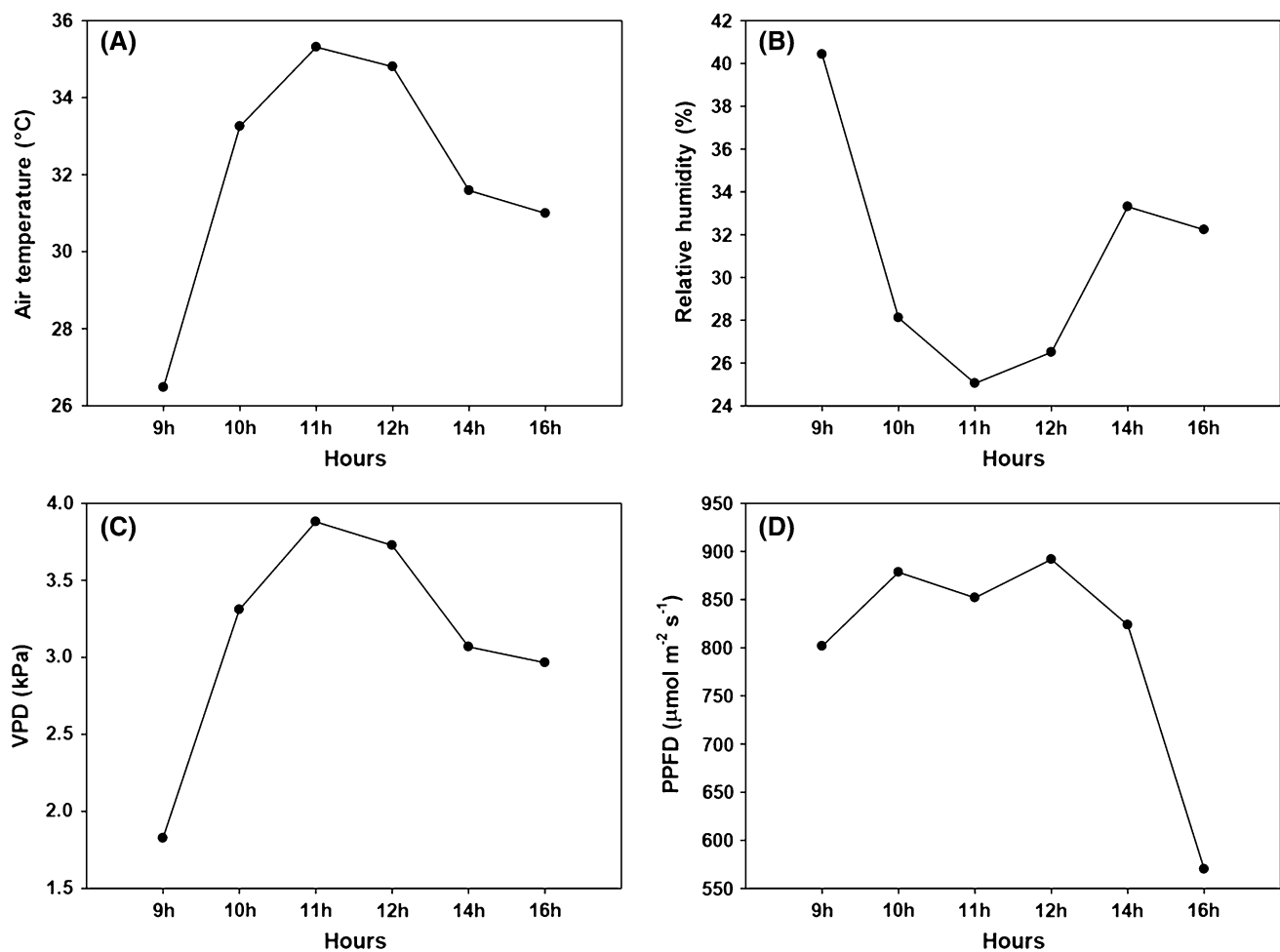


Fig. 4 The mean of air temperature ($^{\circ}\text{C}$) (a), relative humidity (%) (b), vapor pressure deficit (VPD, kPa) (c) and photosynthetic photon flux density (PPFD, $\mu\text{mol m}^{-2} \text{s}^{-1}$) (d) for the hours of the 43rd DAST

DAST in the plants with suspension of irrigation (Fig. 1b) may have influenced the maintenance of RWC and cell turgor pressure (Lawlor and Tezara 2009), because 90 % of a plant's water loss is through stomatal transpiration (Wan et al. 2009).

According to Bartlett et al. (2014), the relative water content at the turgor loss point (RWC_{tlp}), which is analogous to the water potential at the turgor loss point (π_{tlp}), is the point of the occurrence of wilting on leaves in conjunction with the disruption of growth and gas exchange. In *A. emarginata* subjected to suspension of irrigation treatment, the RWC_{tlp} may have been reached at 38 DAST when the CO_2 assimilation rate (A_{Net}) was negative and the leaves presented visual wilting with 73 % of RWC. Any value below the point of turgor loss indicates that there is not enough water absorption by the plant to recover it from wilting (Lenz et al. 2006; Blackman et al. 2010; Bartlett et al. 2012). In many species, photosynthesis becomes irreversibly reduced when the RWC falls from approximately 70–60 % (Lawlor and Cornic 2002). Even with an RWC of 73 % presented by *A.*

emarginata subjected to the water deficit (Fig. 1b), the rehydration treatment showed partial recovery in gas exchange compared with irrigated plants at 43 DAST (5 days after rehydration) (Fig. 3a–f).

The rehydration treatment had lower values of g_s and transpiration rate (E) at 9, 10, 11, and 12 h in relation to the irrigated plants (Fig. 3b–c). Marron et al. (2002), Bogeat-Triboulot et al. (2007) and Flexas et al. (2009) also observed slow recovery of the stomatal opening after rehydration. Positive correlations were found between g_s and E at 10, 11, and 12 h of the rehydration treatment (Table 1), which indicate that in these hours, the loss of water was limited by the stomata. A similar correlation was observed in *Jatropha curcas* L. under water deficit, demonstrating the influence of the stomatal closure to water control (Santos et al. 2013). Endres (2007) found reduction of g_s in *Annona squamosa* L. irrigated under conditions of high vapor pressure deficit of the air (VPD), which is similar to our study for irrigated plants (Figs. 3b, 4c). The same author also suggests that the high VPD has a

Table 1 Pearson correlation ($P < 0.05$) ($n = 4$) between the gas exchange parameters of the rehydration treatment evaluated at 43 DAST at 9, 10, 11 and 12 h

9 h	G_S	E	C_i	A_{Net}/E	A_{Net}/C_i	VPD	Temp air
A_{Net}	0.80	0.01	-0.93	0.42	0.99**	-0.83	-0.81
g_s		0.41	-0.54	-0.05	0.73	-0.74	-0.67
E			0.16	-0.90	-0.05	0.30	0.40
C_i				-0.56	-0.96*	0.68	0.70
A_{Net}/E					0.48	-0.61	-0.70
A_{Net}/C_i						-0.80	-0.80
VPD							0.99**
10 h	g_s	E	C_i	A_{Net}/E	A_{Net}/C_i	VPD	Temp air
A_{Net}	0.99**	0.99**	0.36	-0.99**	0.67	-0.12	0.33
g_s		0.98*	0.47	-0.97*	0.57	-0.19	0.28
E			0.38	-0.98*	0.65	-0.02	0.43
C_i				-0.26	-0.45	-0.23	0.07
A_{Net}/E					-0.74	0.10	-0.33
A_{Net}/C_i						0.12	0.31
VPD							0.89
11 h	g_s	E	C_i	A_{Net}/E	A_{Net}/C_i	VPD	Temp air
A_{Net}	0.96*	0.94	-0.45	-0.88	0.90	0.30	0.73
g_s		0.99**	-0.21	-0.96*	0.75	0.53	0.84
E			-0.15	-0.96*	0.70	0.59	0.87
C_i				0.19	-0.79	0.71	0.28
A_{Net}/E					-0.67	-0.53	-0.74
A_{Net}/C_i						-0.15	0.37
VPD							0.84
12 h	g_s	E	C_i	A_{Net}/E	A_{Net}/C_i	VPD	Temp air
A_{Net}	0.38	0.42	0.25	-0.11	0.98*	-0.03	0.75
g_s		0.99**	0.89	-0.95*	0.23	-0.85	-0.23
E			0.90	-0.94	0.27	-0.83	-0.19
C_i				-0.83	0.07	-0.61	-0.14
A_{Net}/E					0.04	0.95	0.51
A_{Net}/C_i						0.08	0.79
VPD							0.64

The CO_2 assimilation rate (A_{Net} ; $\mu\text{mol } CO_2 \text{ m}^{-2} \text{ s}^{-1}$), stomatal conductance (g_s ; $\text{mol } H_2O \text{ m}^{-2} \text{ s}^{-1}$), transpiration rate (E ; $\text{mmol } H_2O \text{ m}^{-2} \text{ s}^{-1}$), internal CO_2 concentration in the sub-stomatal chamber (C_i ; $\mu\text{mol } CO_2 \text{ mol}^{-1} \text{ ar}$), water use efficiency (A_{Net}/E) ($\mu\text{mol } CO_2 \text{ mmol } H_2O^{-1}$), carboxylation efficiency (A_{Net}/C_i), vapor pressure deficit (VPD; kPa) and air temperature (Temp air; $^{\circ}C$)

* Indicates significant correlation at $P < 0.05$

** Indicates significant correlation at $P < 0.01$

detrimental effect on the stomatal opening in Annonaceae regardless of the plant hydration. However, in *A. emarginata* submitted to rehydration, there was no significant correlation between g_s and VPD (Table 1).

The A_{Net} of the rehydration treatment was partially recovered in relation to the irrigated plants at 9 and 10 h (Fig. 3a). Generally, plants subjected to severe water deficit only recover 40–60 % of the maximum photosynthetic

rate on the day after rehydration, and continue to recover on the following days (Sofo et al. 2004; Bogeat-Triboulot et al. 2007; Chaves et al. 2009). The A_{Net} can be limited by stomatal factors, such as increase of stomatal resistance to CO_2 entry, or by non-stomatal factors, such as decrease of rubisco activity and photochemical efficiency of PSII (Silva and Arrabaca 2004; Flexas et al. 2006; Xu et al. 2009).

Table 2 Mean values ($n = 4$) of the fraction of light absorbed by PSII antenna used in the photochemical electron transport (P), of the fraction of light absorbed by PSII antenna that is dissipated as heat (D), of the fraction of excitation energy not dissipated in the antenna that cannot be utilized for photochemistry (E) and of the efficiency of excitation energy captured by open PSII reaction centers (ϕ_{exc}) of the irrigation and rehydration treatments at 44 DAST in 125, 190, 285, 420, 625, 820, 1150, and 1500 $\mu\text{mol m}^{-2} \text{s}^{-1}$ of PPFD

Treatment	P	D	E	ϕ_{exc}
Irrigation	0.132 a	0.577 b	0.292 a	0.423 a
Rehydration	0.121 b	0.624 a	0.255 b	0.376 b
PPFD				
125	0.192 a	0.506 c	0.303 a	0.494 a
190	0.175 ab	0.557 bc	0.268 a	0.443 ab
285	0.162 bc	0.587 ab	0.250 a	0.413 bc
420	0.141 c	0.607 ab	0.252 a	0.393 bc
625	0.106 d	0.624 ab	0.269 a	0.376 bc
820	0.094 de	0.633 a	0.273 a	0.367 c
1150	0.075 e	0.640 a	0.285 a	0.360 c
1500	0.066 e	0.646 a	0.287 a	0.354 c
Significance				
Treatment	$P < 0.05$	$P < 0.001$	$P < 0.01$	$P < 0.001$
PPFD	$P < 0.001$	$P < 0.001$	ns	$P < 0.001$
Treatment \times PPFD	ns	ns	ns	ns

Different letters indicate significant differences in Tukey's test ($P < 0.05$)

Changes on the patterns of stomatal conductance and carbon concentration inside the mesophyll can provide information regarding to the causes of limitation on the photosynthetic rate (Farquhar and Sharkey 1982; Mielke et al. 2003; Herrera et al. 2008). The carboxylation efficiency (A_{Net}/C_i) also permits differentiation between stomatal and non-stomatal limitations (Ennahli and Earl 2005). Under rehydration, *A. emarginata* showed partial recovery of A_{Net}/C_i at 9 h compared to irrigated plants (Fig. 3e). At this same time, the rehydrated plants showed a positive correlation between A_{Net} and A_{Net}/C_i and a negative correlation between A_{Net}/C_i and internal CO_2 concentration in the sub-stomatal chamber (C_i) (Table 1). These correlations indicate that the increase in A_{Net} is dependent on the increase in A_{Net}/C_i , and the increase in A_{Net}/C_i is not dependent on the increase of C_i . Therefore, it is possible that at 9 h there is a predominance of non-stomatal limitation of A_{Net} in *A. emarginata* submitted to rehydration. In *J. curcas* under water deficit conditions, Santos et al. (2013) observed a negative correlation between A_{Net} and C_i , which suggests that A_{Net} was not limited by CO_2 input.

At 10 h, there was a positive correlation between A_{Net} and g_s in the rehydration treatment (Table 1), which indicate that A_{Net} was influenced by the g_s . The high correlation between these parameters demonstrates strong stomatal control over the rate of photosynthesis (Costa França et al. 2000; Tesfaye et al. 2008; Ninou et al. 2013). In addition, at 10 h, rehydrated plants showed less C_i and no difference on A_{Net}/C_i in relation to the irrigated plants (Fig. 3d–e). Thus, it is possible that, at this time, the rehydration treatment presents a predominance of stomatal limitation of A_{Net} .

Although the lower g_s limits the photosynthetic recovery, the water use efficiency (A_{Net}/E) may increase (Bogeat-Triboulot et al. 2007; Gallé et al. 2007; Galmés et al. 2007) due to a reduction on E (Roelfsema and Hedrich 2005; Nilsson and Assmann 2007). In *A. emarginata*, the A_{Net}/E of the rehydration treatment was higher compared to the irrigation treatment at 10 and 11 h (Fig. 3f). At both time points, negative correlations were observed between A_{Net}/E and g_s and between A_{Net}/E and E , which indicate that g_s and E have an inverse relationship with A_{Net}/E (Table 1).

Interestingly, there was a negative correlation between A_{Net}/E and A_{Net} at 10 h (Table 1), which demonstrates that with an increase of A_{Net} , the A_{Net}/E reduces. This fact may be related to the positive correlation found on the same time between A_{Net} and E (Table 1), where the increase of A_{Net} leads to increases E . This correlation may suggest that in *A. emarginata*, E and A_{Net} are regulated during rehydration to maintain high water use efficiency without major losses on the photosynthetic rate. After rehydration, *Quercus pubescens* Willd. (Miyashita et al. 2005) and *Phaseolus vulgaris* L. (Galle et al. 2007) present the A_{Net} recovery faster than the recovery of g_s , resulting on an increase of instantaneous water use efficiency. It is possible that during the water deficit, plants may present adjustments to avoid water loss. During rehydration, these adjustments could help to mitigate the effect of future deficit events (Gallé et al. 2007).

At 44 DAST, the chlorophyll fluorescence in rehydrated plants has lower efficiency of excitation energy captured by open PSII (ϕ_{exc}) and P (photochemical) in relation to irrigated plants (Table 2), which indicate reduction on both capture of energy and use of energy for the photochemical

process, respectively. However, this reduction did not represent damage on the photochemical process, because rehydrated plants had low values of E (energy not used in photochemical step). The accumulation of this energy excess can result in the formation of the triplet state of chlorophyll, which leads to the formation of singlet oxygen ($^1\text{O}_2$) that causes damage to PSII (Demmig-Adams et al. 1996; Kato et al. 2003). Therefore, the reduction of E indicates the absence of damage to PSII. Mantoan et al. (2015) also did not observe damage to the PSII of *A. emarginata* subjected to a water deficit followed by rehydration.

According to Demmig-Adams et al. (1996), the sum of the parameters P , D and E is one. Therefore, any reduction in one of those parameters results on the increase of the others. Thus, during rehydration, the increase of the dissipation of the excitation energy as heat (D), which is modulated by photoprotection mechanisms (xanthophyll cycle) (Demmig-Adams et al. 1996; Kato et al. 2003), is due to the reduction in the photochemical dissipation (P) and in the dissipation of energy excess (E). According to Baker and Rosenqvist (2004), plants tend to avoid damage to the PSII, reducing the energy for photochemical step (P) and increasing the dissipation of this energy as heat. Therefore, the occurrence of high D after 6 days of rehydration indicates the maintenance of photoprotection, which represents a possible photochemical acclimation to water deficit in *A. emarginata*.

Conclusion

After rehydration, *A. emarginata* shows balance between transpiration and CO_2 assimilation rate to optimize the water use efficiency. Also, in the photochemical process, the rehydrated plants maintain high activity of photoprotection. Thus, *A. emarginata* presents adjustments on gas exchange and photochemical process, resulting in a possible long-term photosynthetic acclimation to the water deficit.

Author contribution statement Luís Paulo Benetti Mantoan conducted the experiment, performed the chlorophyll a fluorescence measurements, analyzed the data and wrote the manuscript. Ana Claudia Macedo performed the gas exchange measurements. Luiz Fernando Rolim de Almeida, Gisela Ferreira and Carmen Silvia Fernandes Boaro helped to review and edit this manuscript.

Acknowledgments We would like to thank the postgraduate program in Biological Sciences (Botany), the Universidade Estadual Paulista “Júlio de Mesquita Filho” UNESP, the Institute of Biosciences, Department of Botany, Botucatu—SP Brazil and the Conselho Nacional de Desenvolvimento Científico e Tecnológico—CNPq for the granted scholarships.

References

- Ashraf M, Harris PJC (2013) Photosynthesis under stressful environments: an overview. *Photosynthetica* 51:163–190. doi:[10.1007/s11099-013-0021-6](https://doi.org/10.1007/s11099-013-0021-6)
- Baker NR, Rosenqvist E (2004) Applications of chlorophyll fluorescence can improve crop production strategies: an examination of future possibilities. *J Exp Bot* 55:1607–1621. doi:[10.1093/jxb/erh196](https://doi.org/10.1093/jxb/erh196)
- Bartlett MK, Scoffoni C, Sack L (2012) The determinants of leaf turgor loss point and prediction of drought tolerance of species and biomes: a global meta-analysis. *Ecol Lett* 15:393–405. doi:[10.1111/j.1461-0248.2012.01751.x](https://doi.org/10.1111/j.1461-0248.2012.01751.x)
- Bartlett MK, Zhang Y, Kreidler N et al (2014) Global analysis of plasticity in turgor loss point, a key drought tolerance trait. *Ecol Lett* 17:1580–1590. doi:[10.1111/ele.12374](https://doi.org/10.1111/ele.12374)
- Blackman CJ, Brodribb TJ, Jordan GJ (2010) Leaf hydraulic vulnerability is related to conduit dimensions and drought resistance across a diverse range of woody angiosperms. *New Phytol* 188:1113–1123. doi:[10.1111/j.1469-8137.2010.03439.x](https://doi.org/10.1111/j.1469-8137.2010.03439.x)
- Bogeat-Triboulot M-B, Brosché M, Renaut J et al (2007) Gradual soil water depletion results in reversible changes of gene expression, protein profiles, ecophysiology, and growth performance in *Populus euphratica*, a poplar growing in arid regions. *Plant Physiol* 143:876–892. doi:[10.1104/pp.106.088708](https://doi.org/10.1104/pp.106.088708)
- Boyer JS (1982) Plant productivity and environment. *Science* 218:443–448. doi:[10.1126/science.218.4571.443](https://doi.org/10.1126/science.218.4571.443)
- Chaves MM, Maroco JP, Pereira JS (2003) Understanding plant responses to drought—from genes to the whole plant. *Funct Plant Biol* 30:239–264. doi:[10.1071/FP02076](https://doi.org/10.1071/FP02076)
- Chaves MM, Flexas J, Pinheiro C (2009) Photosynthesis under drought and salt stress: regulation mechanisms from whole plant to cell. *Ann Bot* 103:551–560. doi:[10.1093/aob/mcn125](https://doi.org/10.1093/aob/mcn125)
- Colla G, Roupheal Y, Leonardi C, Bie Z (2010) Role of grafting in vegetable crops grown under saline conditions. *Sci Hortic (Amsterdam)* 127:147–155. doi:[10.1016/j.scienta.2010.08.004](https://doi.org/10.1016/j.scienta.2010.08.004)
- Costa França MG, Pham Thi AT, Pimentel C, Pereyra Rossiello RO, Zuily-Fodil Y, Laffray D (2000) Differences in growth and water relations among *Phaseolus vulgaris* cultivars in response to induced drought stress. *Environ Exp Bot* 43:227–237. doi:[10.1016/S0098-8472\(99\)00060-X](https://doi.org/10.1016/S0098-8472(99)00060-X)
- David TS, Henriques MO, Kurz-Besson C et al (2007) Water-use strategies in two co-occurring Mediterranean evergreen oaks: surviving the summer drought. *Tree Physiol* 27:793–803
- Demmig-Adams B, Adams WW III, Barker DH, Logan BA, Bowling DR, Verhoeven AS (1996) Using chlorophyll fluorescence to assess the fraction of absorbed light allocated to thermal dissipation of excess excitation. *Physiol Plant* 98:253–264. doi:[10.1034/j.1399-3054.1996.980206.x](https://doi.org/10.1034/j.1399-3054.1996.980206.x)
- Endres L (2007) Daily and seasonal variation of water relationship in sugar apple (*Annona squamosa* L.) under different irrigation regimes at semi-arid Brazil. *Sci Hortic (Amsterdam)* 113:149–154. doi:[10.1016/j.scienta.2007.03.007](https://doi.org/10.1016/j.scienta.2007.03.007)
- Ennahli S, Earl HJ (2005) Physiological limitations to photosynthetic carbon assimilation in cotton under water stress. *Crop Sci* 45:2374–2382. doi:[10.2135/cropsci2005.0147](https://doi.org/10.2135/cropsci2005.0147)
- Farquhar GD, Sharkey TD (1982) Stomatal conductance and photosynthesis. *Annu Rev Plant Physiol* 33:317–345. doi:[10.1146/annurev.pp.33.060182.001533](https://doi.org/10.1146/annurev.pp.33.060182.001533)
- Flexas J, Bota J, Loreto F, Cornic G, Sharkey TD (2004) Diffusive and metabolic limitations to photosynthesis under drought and salinity in C3 plants. *Plant Biol* 6:269–279. doi:[10.1055/s-2004-820867](https://doi.org/10.1055/s-2004-820867)
- Flexas J, Ribas-Carbó M, Bota J, Galmés J, Henkle M, Martínez-Cañellas S, Medrano H (2006) Decreased Rubisco activity

- during water stress is not induced by decreased relative water content but related to conditions of low stomatal conductance and chloroplast CO₂ concentration. *New Phytol* 172:73–82. doi:[10.1111/j.1469-8137.2006.01794.x](https://doi.org/10.1111/j.1469-8137.2006.01794.x)
- Flexas J, Diaz-Espejo A, Galmés J, Kaldenhoff R, Medrano H, Ribas-Carbo M (2007) Rapid variations of mesophyll conductance in response to changes in CO₂ concentration around leaves. *Plant, Cell Environ* 30:1284–1298. doi:[10.1111/j.1365-3040.2007.01700.x](https://doi.org/10.1111/j.1365-3040.2007.01700.x)
- Flexas J, Barón M, Bota J et al (2009) Photosynthesis limitations during water stress acclimation and recovery in the drought-adapted *Vitis* hybrid Richter-110 (*V. berlandieri* × *V. rupestris*). *J Exp Bot* 60:2361–2377. doi:[10.1093/jxb/erp069](https://doi.org/10.1093/jxb/erp069)
- Gallé A, Haldimann P, Feller U (2007) Photosynthetic performance and water relations in young pubescent oak (*Quercus pubescens*) trees during drought stress and recovery. *New Phytol* 174:799–810. doi:[10.1111/j.1469-8137.2007.02047.x](https://doi.org/10.1111/j.1469-8137.2007.02047.x)
- Galmés J, Medrano H, Flexas J (2007) Photosynthetic limitations in response to water stress and recovery in Mediterranean plants with different growth forms. *New Phytol* 175:81–93. doi:[10.1111/j.1469-8137.2007.02087.x](https://doi.org/10.1111/j.1469-8137.2007.02087.x)
- Geiger DR, Servaites JC (1994) Diurnal regulation of photosynthetic carbon metabolism in C3 plants. *Annu Rev Plant Physiol Plant Mol Biol* 45:235–256. doi:[10.1146/annurev.pp.45.060194.001315](https://doi.org/10.1146/annurev.pp.45.060194.001315)
- Grassi G, Magnani F (2005) Stomatal, mesophyll conductance and biochemical limitations to photosynthesis as affected by drought and leaf ontogeny in ash and oak trees. *Plant, Cell Environ* 28:834–849. doi:[10.1111/j.1365-3040.2005.01333.x](https://doi.org/10.1111/j.1365-3040.2005.01333.x)
- Herrera A, Tezara W, Marín O, Rengifo E (2008) Stomatal and non-stomatal limitations of photosynthesis in trees of a tropical seasonally flooded forest. *Physiol Plant* 134:41–48. doi:[10.1111/j.1399-3054.2008.01099.x](https://doi.org/10.1111/j.1399-3054.2008.01099.x)
- Hoagland DR, Arnon DI (1950) The water-culture method for growing plants without soil. *Calif Agric Exp Stn Circ* 347:1–32
- Izanloo A, Condon AG, Langridge P, Tester M, Schnurbusch T (2008) Different mechanisms of adaptation to cyclic water stress in two South Australian bread wheat cultivars. *J Exp Bot* 59:3327–3346. doi:[10.1093/jxb/ern199](https://doi.org/10.1093/jxb/ern199)
- Kato MC, Hikosaka K, Hirotsu N, Makino A, Hirose T (2003) The excess light energy that is neither utilized in photosynthesis nor dissipated by photoprotective mechanisms determines the rate of photoinactivation in photosystem II. *Plant Cell Physiol* 44:318–325
- Lawlor DW, Cornic G (2002) Photosynthetic carbon assimilation and associated metabolism in relation to water deficits in higher plants. *Plant, Cell Environ* 25:275–294. doi:[10.1046/j.0016-8025.2001.00814.x](https://doi.org/10.1046/j.0016-8025.2001.00814.x)
- Lawlor DW, Tezara W (2009) Causes of decreased photosynthetic rate and metabolic capacity in water-deficient leaf cells: a critical evaluation of mechanisms and integration of processes. *Ann Bot* 103:561–579. doi:[10.1093/aob/mcn244](https://doi.org/10.1093/aob/mcn244)
- Lenz TI, Wright IJ, Westoby M (2006) Interrelations among pressure-volume curve traits across species and water availability gradients. *Physiol Plant* 127:423–433. doi:[10.1111/j.1399-3054.2006.00680.x](https://doi.org/10.1111/j.1399-3054.2006.00680.x)
- Li T, Ding Y, Hu Y, Sun L, Jiang C, Liu Y (2015) Diurnal changes in photosynthesis in *Sclerocarya birrea* from South Africa and Israel after introduction and acclimatization in Wenshan, Yunnan Province, China. *South African J Bot* 100:101–107. doi:[10.1016/j.sajb.2015.05.021](https://doi.org/10.1016/j.sajb.2015.05.021)
- Luo Y, Zhao X, Zhou R, Zuo X, Zhang J, Li Y (2010) Physiological acclimation of two psammophytes to repeated soil drought and rewetting. *Acta Physiol Plant* 33:79–91. doi:[10.1007/s11738-010-0519-5](https://doi.org/10.1007/s11738-010-0519-5)
- Mantoan LPB, Ferreira G, Boaro CSF (2015) Chlorophyll a fluorescence in *Annona emarginata* (Schltdl.) H. Rainer plants subjected to water stress and after rehydration. *Sci Hortic (Amsterdam)* 184:23–30. doi:[10.1016/j.scienta.2014.12.019](https://doi.org/10.1016/j.scienta.2014.12.019)
- Marron N, Delay D, Petit J-M, Dreyer E, Kahlem G, Delmotte FM, Brignolas F (2002) Physiological traits of two *Populus* × *euramericana* clones, Luisa Avanzo and Dorskamp, during a water stress and re-watering cycle. *Tree Physiol* 22:849–858
- Martínez-Ballesta MC, Alcaraz-López C, Muries B, Mota-Cadenas C, Carvajal M (2010) Physiological aspects of rootstock–scion interactions. *Sci Hortic (Amsterdam)* 127:112–118. doi:[10.1016/j.scienta.2010.08.002](https://doi.org/10.1016/j.scienta.2010.08.002)
- Medrano H, Escalona JM, Bota J, Gulias J, Flexas J (2002) Regulation of photosynthesis of C-3 plants in response to progressive drought: stomatal conductance as a reference parameter. *Ann Bot* 89:895–905
- Mielke MS, de Almeida A-AF, Gomes FP, Aguilar MAG, Mangabeira PAO (2003) Leaf gas exchange, chlorophyll fluorescence and growth responses of *Genipa americana* seedlings to soil flooding. *Environ Exp Bot* 50:221–231. doi:[10.1016/S0098-8472\(03\)00036-4](https://doi.org/10.1016/S0098-8472(03)00036-4)
- Miyashita K, Tanakamaru S, Maitani T, Kimura K (2005) Recovery responses of photosynthesis, transpiration, and stomatal conductance in kidney bean following drought stress. *Environ Exp Bot* 53:205–214. doi:[10.1016/j.envexpbot.2004.03.015](https://doi.org/10.1016/j.envexpbot.2004.03.015)
- Nilson SE, Assmann SM (2007) The control of transpiration. Insights from *Arabidopsis*. *Plant Physiol* 143:19–27. doi:[10.1104/pp.106.093161](https://doi.org/10.1104/pp.106.093161)
- Ninou E, Tsialtas JT, Dordas CA, Papakosta DK (2013) Effect of irrigation on the relationships between leaf gas exchange related traits and yield in dwarf dry bean grown under Mediterranean conditions. *Agric Water Manag* 116:235–241. doi:[10.1016/j.agwat.2012.08.002](https://doi.org/10.1016/j.agwat.2012.08.002)
- Pinheiro C, Chaves MM (2011) Photosynthesis and drought: can we make metabolic connections from available data? *J Exp Bot* 62:869–882. doi:[10.1093/jxb/erq340](https://doi.org/10.1093/jxb/erq340)
- Roelfsema MRG, Hedrich R (2005) In the light of stomatal opening: new insights into “the Watergate”. *New Phytol* 167:665–691. doi:[10.1111/j.1469-8137.2005.01460.x](https://doi.org/10.1111/j.1469-8137.2005.01460.x)
- Santos CM, Verissimo V, Wanderley Filho HCL, Ferreira VM, Cavalcante PGS, Rolim EV, Endres L (2013) Seasonal variations of photosynthesis, gas exchange, quantum efficiency of photosystem II and biochemical responses of *Jatropha curcas* L. grown in semi-humid and semi-arid areas subject to water stress. *Ind Crops Prod* 41:203–213. doi:[10.1016/j.indcrop.2012.04.003](https://doi.org/10.1016/j.indcrop.2012.04.003)
- Silva JM, Arrabaca MC (2004) Photosynthesis in the water-stressed C4 grass *Setaria sphacelata* is mainly limited by stomata with both rapidly and slowly imposed water deficits. *Physiol Plant* 121:409–420. doi:[10.1111/j.1399-3054.2004.00328.x](https://doi.org/10.1111/j.1399-3054.2004.00328.x)
- Sinclair TR, Ludlow MM (1985) Who taught plants thermodynamics? The unfulfilled potential of plant water potential. *Aust J Plant Physiol* 12:213–217. doi:[10.1071/PP9850213](https://doi.org/10.1071/PP9850213)
- Sinclair TR, Zwieniecki MA, Holbrook NM (2008) Low leaf hydraulic conductance associated with drought tolerance in soybean. *Physiol Plant* 132:446–451. doi:[10.1111/j.1399-3054.2007.01028.x](https://doi.org/10.1111/j.1399-3054.2007.01028.x)
- Sofo A, Dichio B, Xiloyannis C, Masia A (2004) Effects of different irradiance levels on some antioxidant enzymes and on malondialdehyde content during rewetting in olive tree. *Plant Sci* 166:293–302. doi:[10.1016/j.plantsci.2003.09.018](https://doi.org/10.1016/j.plantsci.2003.09.018)
- Tesfaye K, Walker S, Tsubo M (2008) Comparison of water relations, leaf gas exchange and assimilation of three grain legumes under reproductive period water deficit. *J Agron* 7:102–114
- Thameur A, Lachiheb B, Ferchichi A (2012) Drought effect on growth, gas exchange and yield, in two strains of local barley Ardhaoui, under water deficit conditions in southern Tunisia. *J Environ Manage* 113:495–500. doi:[10.1016/j.jenvman.2012.05.026](https://doi.org/10.1016/j.jenvman.2012.05.026)

- Tokunaga T (2000) A cultura da atemóia, 2nd edn. Coordenadoria de Assistência Técnica Integral (CATI), Campinas-SP
- Tominaga J, Inafuku S, Coetzee T, Kawamitsu Y (2014) Diurnal regulation of photosynthesis in *Jatropha curcas* under drought during summer in a semi-arid region. Biomass Bioenergy 67:279–287. doi:[10.1016/j.biombioe.2014.05.010](https://doi.org/10.1016/j.biombioe.2014.05.010)
- Varone L, Ribas-Carbo M, Cardona C, Gallé A, Medrano H, Gratani L, Flexas J (2012) Stomatal and non-stomatal limitations to photosynthesis in seedlings and saplings of Mediterranean species pre-conditioned and aged in nurseries: different response to water stress. Environ Exp Bot 75:235–247. doi:[10.1016/j.envexpbot.2011.07.007](https://doi.org/10.1016/j.envexpbot.2011.07.007)
- Verslues PE, Agarwal M, Katiyar-Agarwal S, Zhu J, Zhu J-K (2006) Methods and concepts in quantifying resistance to drought, salt and freezing, abiotic stresses that affect plant water status. Plant J 45:523–539. doi:[10.1111/j.1365-3113X.2005.02593.x](https://doi.org/10.1111/j.1365-3113X.2005.02593.x)
- Wan J, Griffiths R, Ying J, McCourt P, Huang Y (2009) Development of drought-tolerant canola (L.) through genetic modulation of ABA-mediated stomatal responses. Crop Sci 49:1539–1554. doi:[10.2135/cropsci2008.09.0568](https://doi.org/10.2135/cropsci2008.09.0568)
- Weatherley PE (1949) Studies in the water relations of the cotton plant. I. the field measurement of water deficits in leaves. New Phytol 49:81–97. doi:[10.1111/j.1469-8137.1950.tb05146.x](https://doi.org/10.1111/j.1469-8137.1950.tb05146.x)
- Xu Z, Zhou G, Shimizu H (2009) Are plant growth and photosynthesis limited by pre-drought following rewetting in grass? J Exp Bot 60:3737–3749. doi:[10.1093/jxb/erp216](https://doi.org/10.1093/jxb/erp216)
- Yordanov I, Velikova V, Tsonev T (2000) Plant responses to drought, acclimation, and stress tolerance. Photosynthetica 38:171–186. doi:[10.1023/A:1007201411474](https://doi.org/10.1023/A:1007201411474)
- Zribi L, Fatma G, Fatma R, Salwa R, Hassan N, Néjib RM (2009) Application of chlorophyll fluorescence for the diagnosis of salt stress in tomato “*Solanum lycopersicum* (variety Rio Grande)”. Sci Hortic (Amsterdam) 120:367–372. doi:[10.1016/j.scienta.2008.11.025](https://doi.org/10.1016/j.scienta.2008.11.025)