

Photosynthetic limitations caused by different rates of water-deficit induction in *Glycine max* and *Vigna unguiculata*

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Abstract

Plants are constantly subjected to variations in their surrounding environment, which affect their functioning in different ways. The influence of environmental factors on the physiology of plants depends on several factors including the intensity, duration and frequency of the variation of the external stimulus. Water deficit is one of the main limiting factors for agricultural production worldwide and affects many physiological processes in plants. The aim of this study was to analyse the effects of different rates of induced water deficit on the leaf photosynthetic responses of soybean (*Glycine max* L.) and cowpea (*Vigna unguiculata* L.). The plants were subjected to two types of water deficit induction: a rapid induction (RD) by which detached leaves were dehydrated by the exposure to air under controlled conditions and a slow induction (SD) by suspending irrigation under greenhouse conditions. The leaf gas exchange, chlorophyll (Chl) *a* fluorescence, and relative water content (RWC) were analysed throughout the water-deficit induction. *V. unguiculata* and *G. max* demonstrated similar dehydration as the soil water percentage declined under SD, with *V. unguiculata* showing a greater stomatal sensitivity to reductions in the RWC. *V. unguiculata* plants were more sensitive to water deficit, as determined by all of the physiological parameters when subjected to RD, and the net photosynthetic rate (P_N) was sharply reduced in the early stages of dehydration. After the plants exposed to the SD treatment were rehydrated, *V. unguiculata* recovered 65% of the P_N in relation to the values measured under the control conditions (initial watering state), whereas *G. max* recovered only 10% of the P_N . Thus, the better stomatal control of *V. unguiculata* could enable the maintenance of the RWC and a more efficient recovery of the P_N than *G. max*.

Additional key words: cowpea; photosynthesis; rapid and slow water-deficit induction; recovery; soybean.

Introduction

In agriculture, drought is a major factor that limits the yield of crops by affecting different levels of plant processes, from the expression of genes to effects on photosynthesis and the pattern of growth and accumulation of biomass (Flexas *et al.* 2006, Pinheiro and Chaves 2011).

The effects of water shortage on photosynthesis have been much studied, and some mechanisms of the response are well known (Kaiser 1987, Lawlor and Cornic 2002, Lawlor and Tezara 2009, Pinheiro and

Chaves 2011). In general, water deficiency disturbs the balance between energy capture and energy conversion. Under moderate water stress, the photochemical processes are not significantly affected, whereas the biochemistry of CO₂ fixation is reduced. The causes of the reduced biochemical processes involve different factors, ranging from stomatal limitations for CO₂ entry, mesophyll conductance, a limitation of the synthesis of ribulose-1,5-bisphosphate (RuBP) and an inhibition of the enzymes of the Calvin cycle. Thus, an excess of

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Abbreviations: *E* – transpiration rate; ETR – electron transport rate; F_0 – minimal fluorescence of dark-adapted state; F_0' – minimal fluorescence of light-adapted state; F_m – maximal fluorescence of dark-adapted state; F_m' – maximal fluorescence of light-adapted state; F_s – steady-state fluorescence; F_v – variable fluorescence; F_v/F_m – maximum quantum yield of PSII photochemistry; g_s – stomatal conductance; $H_2O_{soil}\%$ – soil water percentage; NPQ – nonphotochemical quenching; P_N – net photosynthetic rate; q_p – photochemical quenching coefficient; RD – rapid water-deficit induction; RWC – relative water content; SD – slow water-deficit induction; WUE – water-use efficiency; Φ_{PSII} – effective quantum yield of PSII photochemistry.

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energy for CO₂ fixation results in surplus energy that must be dissipated; however, the observed increase in the nonphotochemical quenching (NPQ) is often inadequate, resulting in the production of reactive oxygen species (ROS) that can damage the ATP synthase and the photosystems (particularly the D1 protein of PSII) leading to a reduction of electron transport and photophosphorylation. The decrease in the pool of ATP decreases the synthesis of RuBP, resulting in reductions in the photosynthesis potential, accompanied by decreases in the activity of Rubisco (Parry *et al.* 2002, Chaves and Oliveira 2004, Lawlor and Tezara 2009).

Nevertheless, controversy remains regarding the mechanisms that have the greatest influence in limiting photosynthesis. According to Lawlor and Tezara (2009), the control of photosynthesis is distributed among many metabolic components and processes that may vary in importance, depending on different environmental conditions and plant traits (genetic, physiological, and structural). Therefore, different experimental conditions may lead to different results. Experimental variations of these factors are related to the rate at which water stress is imposed because the effects of water deficit can vary depending on the rate of leaf water potential reductions. Therefore, different responses can be observed in the photosynthetic processes when a plant is exposed to water deficit that is induced slowly or rapidly (Oliveira *et al.* 2002, Silva and Arrabaça 2004). Previously, Ogren (1990) observed that there were mechanistic differences between the effects of rapid dehydration and slowly imposed water stress on Chl fluorescence characteristics. For example, in willow leaves, the nonphotochemical quenching (NPQ) was reduced under conditions of rapid dehydration but remained constant under slowly imposed water stress. Conversely, in *Setaria sphacelata*, Silva and

Arrabaça (2004) observed that the NPQ decreased slightly under rapid water deficit, whereas it was increased at the onset of stress and then decreased significantly under slow water deficit. Therefore, as stated by Lawlor and Tezara (2009) and Reynolds *et al.* (2005), there is a lack of standard experimental conditions that allow the establishment of a single model of photosynthesis and metabolism that will suit all plant-environment relationships.

The objective of this study was to investigate the effect of different rates of water-deficit imposition on the photosynthetic responses of *G. max* and *V. unguiculata* and to analyse the possible causes of photosynthetic limitations using leaf gas exchange and Chl *a* fluorescence techniques. The chosen species show different mechanisms in response to water-stress conditions. The main mechanisms of drought adaptation in *V. unguiculata* are related to a reduction of the leaf area to minimize water loss by transpiration and the development of a root system with a high hydraulic conductivity to maximize water uptake and the control of stomatal opening (Subbarao *et al.* 1995, Souza *et al.* 2004). Such mechanisms confer a high tolerance to drought, allowing the cultivation of *V. unguiculata* in the arid and semiarid regions (Souza *et al.* 2004, Singh and Reddy 2011). In contrast, water availability is particularly important during two developmental periods in *G. max*: between germination and seedling emergence and between flowering and pod filling. During these periods, both water deficit and flooding can be potentially harmful to agricultural profits (Doss and Thurlow 1974, Câmara and Heiffig 2000), and water deficit has caused significant losses in the worldwide soybean yield (Gopefert *et al.* 1993, Catuchi *et al.* 2011).

Materials and methods

Vigna unguiculata L. cv. Pitiúba and *Glycine max* L. cv. CD202 plants were established by initially germinating five seeds per pot, followed by the maintenance of a single plant per pot containing 10 kg of Ultisol red/yellow soil mixed with organic substrate in a 1:1 ratio. The plants were maintained at field capacity until the beginning of the water-deficit treatments when the plants reached the V4 development stage (fourth fully expanded trifoliate leaf).

Two types of independent experiments of water-deficit induction were conducted. Under the rapid water-deficit induction (RD), detached leaves were dehydrated by the exposure to the atmosphere of a sample leaf chamber of a portable gas-exchange meter, according to Silva and Arrabaça (2004), for approx. 50 min under a saturating irradiance of 1,200 $\mu\text{mol}(\text{photon}) \text{m}^{-2} \text{s}^{-1}$, a temperature of 30°C, a relative humidity of 60%, and an external CO₂ concentration of 380 $\mu\text{mol mol}^{-1}$. Under the slow water-deficit induction (SD), water stress was

induced by suspending irrigation under greenhouse conditions (30 \pm 5°C, relative humidity of 60 \pm 10%) until null values were attained for the net photosynthetic rate, followed by a rehydration period of four days.

The soil water percentage (H₂O_{soil}%) was monitored by the volumetric water content and a temperature sensor (Decagon's 5TM, Decagon Devices Inc., Pullman, WA, USA) coupled to a ProCheck device (Decagon Devices Inc., Pullman, WA, USA).

Each experiment consisted of eight replications (one plant per pot), arranged in an entirely randomised design.

The plants in the two experiments of water-deficit imposition (SD and RD) were continuously monitored by simultaneous measurements of gas exchange and Chl *a* fluorescence using a portable gas-exchange meter (LI-6400XTR, LI-COR Inc., Lincoln, NE, USA) coupled to a system for monitoring the Chl fluorescence (LI-6400-40, LI-COR Inc., Lincoln, NE, USA). The measurements were performed under a saturating irradiance of

1,200 $\mu\text{mol m}^{-2} \text{s}^{-1}$, a temperature of 30°C, a relative humidity of 60%, and an external CO_2 concentration of 380 $\mu\text{mol mol}^{-1}$. Under RD, the measurements were continuously collected every 10 min using a single leaf inside a sample chamber until null values were obtained for P_N . Under SD, the measurements were performed daily, between 9:00 and 11:00 h, until null values were obtained for the net photosynthetic rate.

The gas-exchange parameters analysed were as follows: P_N , stomatal conductance (g_s) and transpiration rate (E). The water-use efficiency ($\text{WUE} = P_N/E$) was calculated from these data.

The analysis of the Chl *a* fluorescence was performed by estimating the photochemical activity of photosystem II (PSII) for each repetition of each treatment using the same sequence of leaves subjected to rapid or slow dehydration. The maximal (F_m) and minimal (F_0) fluorescence of dark-adapted state were determined with a modulated irradiation ($<1 \mu\text{mol m}^{-2} \text{s}^{-1}$) shortly after keeping the leaves in the dark for 30 min. A saturating pulse of 7,000 $\mu\text{mol m}^{-2} \text{s}^{-1}$ by 0.8 s was applied for measurements of F_m . The variable fluorescence (F_v) was calculated as F_m minus F_0 . The leaves were continuously illuminated with actinic light at the intensity of 1,200 $\mu\text{mol m}^{-2} \text{s}^{-1}$, the steady-state (F_s) and maximum (F_m') fluorescence were measured by applying a second saturating pulse. Minimal fluorescence of light-adapted state (F_0') were measured after far-red illumination of the previously exposed leaves. Using the fluorescence parameters determined above, the following parameters were calculated: the maximum [$F_v/F_m = (F_m - F_0)/F_m$] and effective (Φ_{PSII}) [$\Phi_{\text{PSII}} = (F_m' - F_s)/F_m'$] quantum yield of PSII photochemistry (Genty *et al.* 1989), the electron transport rate [$\text{ETR} = \Phi_{\text{PSII}} \times \text{PPFD} \times 0.5 \times 0.84$] (Kral

and Edwards 1992), the photochemical quenching coefficient [$q_p = (F_m' - F_s)/(F_m' - F_0')$] and the nonphotochemical quenching [$\text{NPQ} = (F_m - F_m')/F_m'$] (Bilger and Björkman 1990). For estimating the ETR, 0.5 was used as the fraction of excitation energy distributed to PSII, and 0.84 was adopted as the fraction of PPFD absorbed by the leaves (Demmig and Björkman 1987).

RWC was determined at each measurement of the gas exchange in both of the water-deficit treatments by weighing the leaves using an analytical balance (0.0001 g precision). At the end of the treatments, the saturated mass of a leaf was obtained by immersion in distilled water in a dark room for 24 h; the dry mass was obtained by drying in an oven at 60°C until a constant mass was attained. The RWC was calculated according to Čatský (1960), and the determinations of the RWC were performed using eight replicates per sampling for both the RD and SD treatments.

In the plants exposed to the SD treatment, the RWC determinations were conducted using different leaves, with the same visual characteristics and age, for each day sampling. In contrast, all of the measurements were performed using a single leaf for the plants subjected to RD but not the leaf used for the continuous measurements of photosynthesis, albeit with the same morphological and physiological characteristics.

The results of all of the photosynthetic parameters (gas exchange and Chl fluorescence) were analysed by the linear correlation of the RWC values under the SD and RD treatments, and the coefficients of the slope (y) were considered estimates of the rates of response to dehydration: the greater the slope coefficient was, the faster the measured parameters changed in relation to the reduction in the RWC.

Results

During the slow induction of water deficit (SD), both species showed reductions in the RWC with the decreasing soil water percentage ($\text{H}_2\text{O}_{\text{soil}\%}$) (Fig. 1). Both crops showed the same trend until the 8th day of the suspension of irrigation, with similar decreasing slope values and reaching similar values for the RWC (73%) when the $\text{H}_2\text{O}_{\text{soil}\%}$ reached 25%. At the end of the SD experiment, the RWC was reduced by 30% in *G. max* and 17% in *V. unguiculata* compared with the 1st day of treatment (Fig. 1). The average of leaf area (LA) of the plants at the beginning of the water suspension was 0.78 m^2 (± 0.02) and 0.49 $\text{m}^2 \pm 0.03$ for *V. unguiculata* and *G. max*, respectively.

g_s and P_N decreased linearly when both crops were subjected to the SD and RD treatments (Fig. 2). However, the reduction of gas exchange under the SD conditions in *V. unguiculata* was more sensitive during the early stages of dehydration under which we observed that the null values were reached at approx. 70% RWC, whereas the same response was observed at approx. 60% RWC

for *G. max* (Fig. 2).

In the RD experiment, the P_N values for *V. unguiculata* decreased in response to slight RWC changes in comparison to *G. max*, reaching null P_N values at a decrease of 14% and 20% RWC, respectively, during

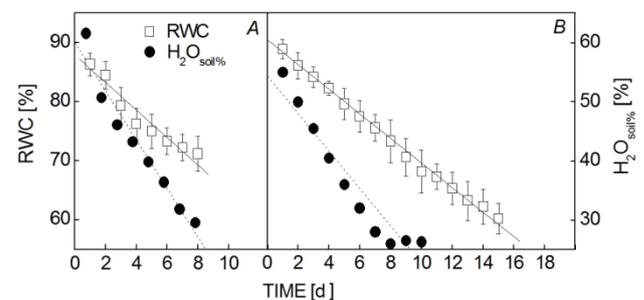


Fig. 1. The soil water percentage ($\text{H}_2\text{O}_{\text{soil}\%}$) and relative water content (RWC) during the slow induction of water deficit (SD). The values were measured every day after the suspension of irrigation in *V. unguiculata* (A) and *G. max* (B). Means, $n = 8$.

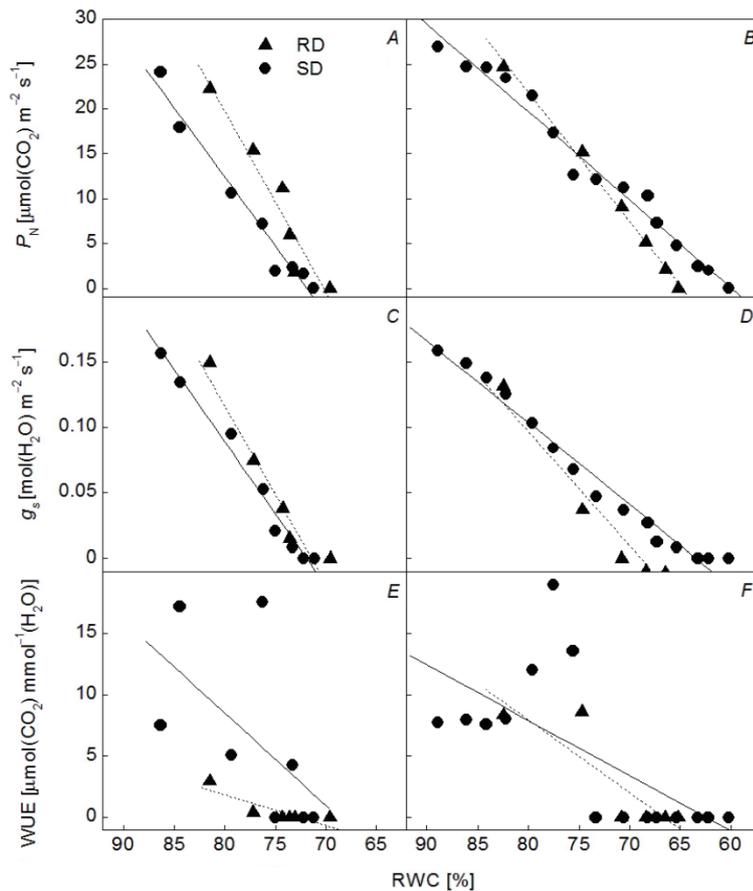


Fig. 2. The effects of the rapid (RD, \blacktriangle) and slow (SD, \bullet) induction of water deficit on the gas-exchange parameters in relation to the relative water content (RWC) in *V. unguiculata* (A,C,E) and *G. max* (B,D,F). Mean values of the net photosynthetic rate (P_N) (A, \blacktriangle $y = -141$, $r = 0.959$, $P = 0.00241$; \bullet $y = -110$, $r = 0.983$, $P < 0.0001$) (B, \blacktriangle $y = -93$, $r = 0.997$, $P < 0.0001$; \bullet $y = -58$, $r = 0.988$, $P < 0.0001$), stomatal conductance (g_s) (C, \blacktriangle $y = -0.968$, $r = 0.957$, $P = 0.00271$; \bullet $y = -0.794$, $r = 0.990$, $P < 0.0001$) (D, \blacktriangle $y = -0.60$, $r = 0.971$, $P = 0.0011$; \bullet $y = -0.398$, $r = 0.987$, $P < 0.0001$), and water-use efficiency (WUE) (E, \blacktriangle $y = -18$, $r = 0.854$, $P = 0.0302$; \bullet $y = -51$, $r = 0.583$, $P = 0.1292$) (F, \blacktriangle $y = -39$, $r = 0.868$, $P = 0.0249$; \bullet $y = -28$, $r = 0.663$, $P = 0.0069$). Means, $n = 8$. Each slope y , expressed in corresponding units of the quantity on y-axis, represents the rate in which the variable on y-axis is changed relating to x-axis.

the same period of dehydration (50 min). The P_N , g_s , and WUE were highly correlated ($P < 0.05$) with the RWC under both of the treatments (SD and RD), with the exception of the WUE in the *V. unguiculata* plants subjected to SD ($P > 0.05$) (Fig. 2). In addition, we observed that the g_s and P_N showed a more pronounced slope (y) for the *V. unguiculata* plants ($y = -141$, $y = -0.96$, respectively) when compared to *G. max* ($y = -93$, $y = -0.60$, respectively), indicating that the responses of these parameters to the RWC in *V. unguiculata* were more sensitive than in *G. max* (Fig. 2).

For *G. max*, the ETR values gradually declined following the reduction of the RWC under the SD treatment, whereas the decline in *V. unguiculata* was more pronounced with smaller variations in the RWC (Fig. 3). The parameters of the Chl *a* fluorescence (ETR, q_p , and NPQ) were strongly correlated ($P < 0.005$) with the RWC (Fig. 3) under both water-deficit induction treatments. As the RWC decreased, the values of the ETR and q_p declined more rapidly in *V. unguiculata* versus *G. max* (Fig. 3), as indicated by high values of the slope (y) for *V. unguiculata* (ETR, $y = -441$; q_p , $y = -1.72$) compared with *G. max* (ETR, $y = -200$; q_p , $y = -0.7$). The nonphotochemical quenching (NPQ) (Fig. 3) showed

a more pronounced increase with the reduction of the RWC when the plants were subjected to RD, and the y values were greater for *G. max* ($y = 4.8$) and *V. unguiculata* ($y = 9.6$) when compared to the SD treatment ($y = 1.9$ for both crops). However, the maximum quantum yield of the PSII photochemistry (F_v/F_m) was correlated with the RWC only when the *V. unguiculata* plants were subjected to SD (Fig. 3), whereas there was significant correlation for *G. max* only when the plants were subjected to RD (Fig. 3). Throughout the SD and RD experiments, there was a strong correlation between the ETR and P_N and between the g_s and P_N for both of the crops (Fig. 4).

After the rehydration period, *V. unguiculata* showed a recovery of 65% when comparing the P_N values measured at the beginning of the experiment, whereas *G. max* recovered only 10% of its initial P_N . Simultaneously, the g_s recovery was more pronounced in *V. unguiculata* (62%) when compared with *G. max* (35%) after four days of rehydration (Fig. 5). The two species recovered approximately 60% of the initial ETR. However, the P_N values for *G. max* did not follow the recovery of the ETR (Fig. 5).

Discussion

Our data indicated that *V. unguiculata* and *G. max* showed a similar dehydration pattern (reduction in the RWC) as the $H_2O_{soil\%}$ declined under the SD treatment (Fig. 1), as indicated by the similar values of the RWC slope coefficient (y values ~ 88). Although *V. unguiculata* had a leaf area that was 40% greater than *G. max*, increasing its capacity of water loss, both species exhibited the same trend of RWC reduction. Thus, likely the faster stomatal closing of *V. unguiculata* under SD could have compensated for the higher potential of water loss leading to a matching of the slope of RWC between both species.

The results of different rates of water-deficit induction showed a greater sensitivity of g_s in response to reductions in the RWC for *V. unguiculata* than *G. max* and a high correlation between the g_s and P_N (Fig. 4) in both of the crops. The more sensitive stomatal response caused a pronounced drop in the g_s in *V. unguiculata*, promoting

a faster decrease in P_N and reaching null values of the P_N seven days before *G. max*. In addition, the significant correlation between the ETR and RWC (Fig. 3) and the ETR and P_N (Fig. 4) under both of the treatments supports the hypothesis of the likely photochemical limitations in both species; the reductions in q_p also support this hypothesis. Therefore, the impairment to photosynthesis caused by the different water-deficit rates was likely influenced by both stomatal and nonstomatal factors (photochemistry). The limitation of CO_2 assimilation imposed by stomatal closure may promote an imbalance between the photosynthetic activity of PSII and the electrons used for photosynthesis, leading to over-excitation and consequent damage to the PSII reaction centres due to photoinhibition (Krause 1988, Raven 2011). Thereby, damage to the photosynthetic machinery may eventually occur, imposing a nonstomatal limitation of photosynthesis.

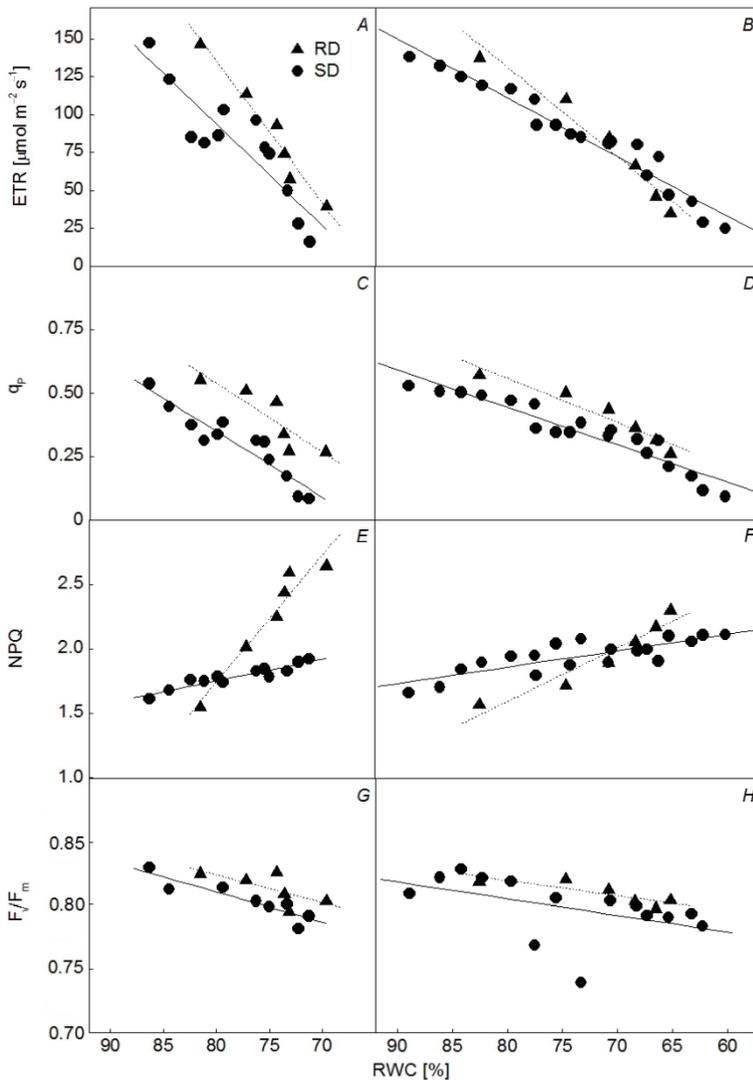


Fig. 3. The effects of the rapid (RD, ▲) and slow (SD, ●) induction of water deficit on the chlorophyll *a* fluorescence parameters in relation to the relative water content (RWC) of *V. unguiculata* (A,C,E,G) and *G. max* (B,D,F,H). Mean values of the electron transport rate (ETR) (A, ▲ $y = -612$, $r = 0.978$, $P = 0.0007$; ● $y = -441$, $r = 0.891$, $P < 0.0001$) (B, ▲ $y = -342$, $r = 0.975$, $P = 0.0009$; ● $y = -200$, $r = 0.972$, $P < 0.0001$), photochemical quenching coefficient (q_p) (C, ▲ $y = -1.62$, $r = 0.883$, $P = 0.0194$; ● $y = -1.72$, $r = 0.939$, $P < 0.0001$) (D, ▲ $y = -0.830$, $r = 0.960$, $P = 0.0023$; ● $y = -0.738$, $r = 0.949$, $P < 0.0001$), nonphotochemical quenching (NPQ) (E, ▲ $y = 9.63$, $r = -0.968$, $P = 0.0014$; ● $y = 3.09$, $r = -0.937$, $P < 0.0001$) (F, ▲ $y = 4.89$, $r = -0.958$, $P = 0.0025$; ● $y = 2.87$, $r = -0.818$, $P < 0.0001$), and maximal quantum yield of the PSII photochemistry (F_v/F_m) (G, ▲ $y = 0.651$, $r = 0.694$, $P = 0.1258$; ● $y = 0.622$, $r = 0.908$, $P = 0.0018$) (H, ▲ $y = 0.723$, $r = 0.832$, $P = 0.0397$; ● $y = 0.701$, $r = 0.482$, $P = 0.0803$). Means, $n = 8$. Each slope y , expressed in corresponding units of the quantity on y -axis, represents the rate in which the variable on y -axis is changed relating to x -axis.

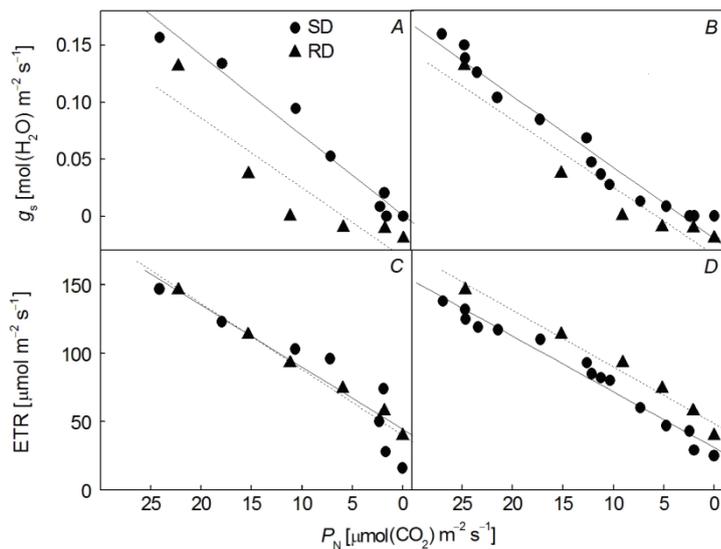


Fig. 4. The effects of the rapid (RD, ▲) and slow (SD, ●) induction of water deficit on the net photosynthetic rate (P_N) in relation to the stomatal conductance (g_s) and electron transport rate (ETR) of *V. unguiculata* (A,C) and *G. max* (B,D). Mean values of stomatal conductance (g_s) (A, ▲ $y = -0.0362$, $r = 0.903$, $P=0.0134$; ● $y = 0.0006$, $r = 0.983$, $P<0.0001$) (B, ▲ $y = -0.034$, $r = 0.953$, $P=0.003$; ● $y = -0.020$, $r = 0.981$, $P<0.0001$) and electron transport rate (ETR) (C, ▲ $y = 44$, $r = 0.995$, $P<0.0001$; ● $y = 40$, $r = 0.919$, $P=0.0012$) (D, ▲ $y = 48$, $r = 0.988$, $P=0.0001$; ● $y = 31$, $r = 0.986$, $P<0.0001$). Means, $n = 8$. Each slope y , expressed in corresponding units of the quantity on y-axis, represents the rate in which the variable on y-axis is changed relating to x-axis.

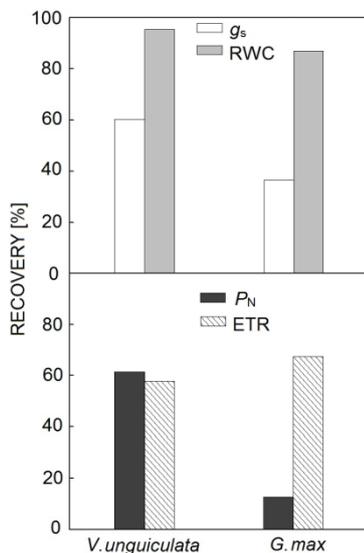


Fig. 5. The recovery percentage of the stomatal conductance (g_s), relative water content (RWC), electron transport rate (ETR) and net photosynthetic rate (P_N) of *V. unguiculata* and *G. max* after a rehydration period in plants exposed to a slow induction of water deficit.

Studies on Chl fluorescence have shown that PSII is resistant to water stress and may (Saccardy *et al.* 1998) or may not (Shangguan *et al.* 2000) be affected under conditions of severe drought. Souza *et al.* (2004) reported that decreases in the F_v/F_m in *V. unguiculata* under water deficit could indicate photoinhibition or the down-regulation of photosynthesis. In contrast, the maintenance of the F_v/F_m values under both dehydration rates in our study indicates that the potential photochemical efficiency was independent of carbon metabolism and, therefore, was not affected by water stress, as was also observed by Silva and Arrabaça (2004) in *Setaria spachelata*. The ability of a plant to increase its capacity for nonphotochemical energy quenching can influence its

survival, development and competition in the field where the irradiance is high (Scholes *et al.* 1997). Therefore, the trend of increasing NPQ values in response to a reduction of the RWC, especially in *V. unguiculata* under RD, may have contributed to the protective effect of photoinhibition observed in our study (Raven 2011).

Overall, our results showed that the two species exhibited different levels of sensitivity to the SD treatment. It was observed that, even with minor variations in the RWC, *V. unguiculata* presented a more effective stomatal control than *G. max* (Fig. 2). Consequently, P_N for *V. unguiculata* declined rapidly compared with *G. max* (Fig. 2). In addition, the data indicate that both species exhibited the same trend in reducing the values of the electron transport rate (ETR) when subjected to SD but with higher sensitivity in *V. unguiculata* during the period of dehydration (Fig. 3). Dehydration can induce changes in the membrane structure at the biophysical level that may be reversible once the environmental conditions become more favourable (Leshem *et al.* 1992). Thus, damage to the structure of the thylakoid membranes may induce changes in the photochemical apparatus, hindering photosynthetic processes (Kaiser 1987, Matos *et al.* 2010). Another finding, which may corroborate this hypothesis, was the responses of the nonphotochemical and, mainly, of the photochemical quenching (NPQ and q_p , respectively), which showed a high sensitivity to the variations in the RWC, indicating that the electron flow may have been diverted from photochemical processes to nonphotochemical energy dissipation mechanisms (Ogren 1990, Tezara *et al.* 2003). If a plant is not able to acclimate and use the extra amount of incident radiation for photosynthesis, the excess energy must be dissipated to prevent the production of reactive oxygen species and the photo-oxidation of Chl (Scholes *et al.* 1997). As observed in our experiments, the electron transport rate under a low RWC and null values for P_N suggest that the residual energy

was allocated to photorespiration, which acted as an alternative electron sink, reducing the possible effects of oxidative stress (Lawlor and Cornic 2002). According to Pinheiro and Chaves (2011), when the limitation of CO₂ assimilation is accompanied by an increase in an alternative electron sink, for example, photorespiration (Harbison *et al.* 1990, Wingler *et al.* 1999) or the Mehler-peroxidase reaction (Biehler and Fock 1996), the non-cyclic electron transport reduction will be proportionally smaller than the observed decrease in the rate of CO₂ assimilation.

Although Silva and Arrabaça (2004) suggest that stomatal limitation is more important than nonstomatal factors in reducing photosynthesis, our data indicate that the P_N in the two studied crops was reduced by both stomatal and nonstomatal factors during the induction of water deficit. Conversely, the recovery limitation of photosynthesis displayed different causes in each species after the rehydration period. The partial P_N recovery in *V. unguiculata* was mainly due to both photochemical and stomatal constraints (Fig. 5). In *G. max*, however, the limited recovery of P_N was probably caused by biochemical factors. The recovery of the RWC, g_s and ETR (Fig. 5) was more effective than the recovery of the P_N after rehydration, indicating that those parameters were not limiting factors for P_N recovery. Therefore, we suggest that the P_N values for *G. max* may have been influenced by a decrease in both the synthesis of RuBP (Bota *et al.* 2004) and the activity of Rubisco (Parry *et al.* 2002).

Our results showed a more efficient recovery of the values of the P_N in *V. unguiculata* than in *G. max*. This improved performance in the recovery of *V. unguiculata* may have been provided by more effective stomatal control during the slow water-deficit induction, which prevented the photosynthetic apparatus from suffering extensive damage; thus, when the plants were rehydrated, it was possible to improve the efficiency of the recovery of carbon assimilation (Souza *et al.* 2004). According to Cornic (2000), a rapid increase in photosynthesis of plants rehydrated after a period of water stress indicates

that the photochemical and biochemical mechanisms may not have been damaged by the water shortage, suggesting that the decrease in carbon assimilation during dehydration is a result of stomatal limitation. Generally, the recovery of photosynthesis after a period of moderate water stress is very fast (one day after rehydration) and nearly complete (Flexas *et al.* 2006). In contrast, after a period of severe stress, the recovery of photosynthesis is progressive, slow (lasting days to weeks) and sometimes incomplete (Souza *et al.* 2004, Miyashita *et al.* 2005, Flexas *et al.* 2006). Therefore, our results for the P_N recovery indicated that the water restriction imposed by slow dehydration was moderate for *V. unguiculata* and more severe for *G. max*, indicating that the mechanisms of drought tolerance in *V. unguiculata*, particularly the efficient stomatal responses observed in our experiments under RD and SD conditions, were effective to allow the maintenance of the RWC by reducing the water lost *via* transpiration, despite its higher leaf area compared with *G. max*. However, it is important to consider that if the null values of photosynthesis were maintained for long periods, this could cause an increase in the excess energy that could induce the formation of ROS and cause significant long-term damage to the photosynthetic apparatus (Lawlor and Tezara 2009). Therefore, this protective mechanism in response to water shortage based on the closing of stomata in *V. unguiculata* may not be as efficient during long-term drought effects.

To summarise, our results showed that *V. unguiculata*, a drought-tolerant crop (Souza *et al.* 2004), presents more prompt stomatal responses to RWC reductions when compared with *G. max*. In both species, the P_N impairment induced by water deficit was caused by stomatal and nonstomatal factors. However, the photosynthetic recovery following rehydration was dominated by biochemical limitations in *G. max* but due to photochemical and stomatal causes in *V. unguiculata*. These results suggest that biochemical damages in the photosynthetic apparatus are more difficult to recover than the stomatal and photochemical limitations caused by water deficit.

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