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Photosynthetic parameters and leaf water potential of five common bean genotypes under mild water deficit

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Abstract

The leaf water potential, gas exchange and chlorophyll fluorescence were evaluated in five common bean (*Phaseolus vulgaris*) genotypes A222, A320, BAT477, Carioca and Ouro Negro subjected to moderate water deficit. At the maximum water deficit (10 d of water withholding), the leaf water potential of genotypes A320 and A222 was higher (-0.35 and -0.50 MPa) when compared to the other genotypes (-0.67 to -0.77 MPa). The stomatal conductance and net photosynthetic rate were significantly reduced in all genotypes due to the water deficit. The greater reduction in stomatal conductance of A320 under drought resulted in high intrinsic water use efficiency. Mild water deficit affected the photochemical apparatus in bean genotypes probably by down-regulation since plants did not show photoinhibition. The photochemical apparatus of A222 and A320 genotypes was more sensitive to drought stress, showing reduced apparent electron transport even after the recovery of plant water status. On the other hand, even after 10 d of water withholding, the maximum efficiency of photosystem 2 was not affected, what suggest efficiency of the photoprotection mechanisms.

Additional key words: chlorophyll fluorescence, drought, *Phaseolus vulgaris*, water stress tolerance.

Introduction

The nature and extent of the effects of water deficit on plants are a function of the intensity and duration of the stress, as well as of the genetically-determined capacity of a given species to cope with environmental constraints (Chaves *et al*. 2002). Even moderate drought stress can reduce the net photosynthetic rate (P_N) in water stress sensitive plants, such as common beans (Vassey and Sharkey 1989, Santos *et al*. 2004, 2006). When water stress occurs at the pollination stage, the drought effects can be enhanced (Pimentel *et al*. 1999a).

Water deficit effects on leaf photosynthetic parameters have been debated over the last three decades. Some authors have reported that reductions in bean leaf photosynthesis caused by mild water stress are primarily the result of stomatal closure (Sharkey and Seemann 1989, Cornic and Briantais 1991). However, Boyer's group suggested a metabolic impairment of bean photosynthesis by evaluating intercellular CO_2 partial pressure, not calculated from gas exchange measurements, but measured directly in the leaves (Lauer and Boyer 1992). In addition, Tang *et al*. (2002) argued that a combination of stomatal and non-stomatal effects on photosynthesis exists, depending on the extent of drought stress and even in plants well hydrated (Yu *et al*. 2009). Tezara *et al*. (1999) concluded that water stress inhibits photosynthesis through diminished ribulose-1,5-bisphosphate (RuBP) supply caused by low ATP synthesis. Considering the biochemical reactions, water deficit can also increase the oxygenase activity of the RuBP carboxylase/oxygenase (Rubisco), reducing carboxylation efficiency.

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Abbreviations: AES - alternative electron sink; c_i - intercellular CO_2 concentration; ETR - apparent electron transport rate; F_v/F_m - variable to maximum fluorescence ratio (potential quantum efficiency of PS 2); g_s - stomatal conductance; IWUE - intrinsic water use efficiency; NPQ - non-photochemical quenching; P_N - net photosynthetic rate; PS 2 - photosystem 2; PPFD - photosynthetic photon flux density; q_P - photochemical quenching; $\Delta F/F_m$' - effective quantum efficiency of PS 2; ΦCO_2 - instantaneous quantum efficiency of CO_2 fixation; Ψ_w - predawn leaf water potential.

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Therefore, decreases in the rate of photosynthesis in drought-stressed plants can be caused by stomatal closure (*i.e*. reduction of CO_2 availability) and/or impairments in photochemical (*i.e*. decrease in NADPH and ATP supply) and/or biochemical (*i.e*. reduced RuBP regeneration and carboxylation efficiency) reactions. It is also important to consider that low biochemical activity may cause photochemical down-regulation, decreasing the demand for photochemical products under drought stress.

Bean plants are commonly exposed to high irradiance during the diurnal period (Ribeiro *et al*. 2004), which may cause additional disturbances in the photosynthetic apparatus under water deficit. At the leaf level, the dissipation of available energy through processes other than photosynthetic carbon metabolism is an important defense mechanism under drought stress (Chaves *et al*. 2002) or natural conditions. Non-photochemical quenching (NPQ) is one of the principal plant mechanisms used to prevent or to alleviate damage caused by excessive light energy reaching the photosynthetic apparatus (Ruban and Horton 1995, Maxwell and Johnson 2000). Basically, NPQ is related to the dissipation of excess light energy as heat at the photosystem 2 (PS 2) level (Ruban and Horton 1995). Regarding alternative electron sinks, photorespiration and pseudocyclic photophosphorylation (Mehler reaction) also collaborate with NPQ to alleviate the effects of excess photons on photosynthesis (Niyogi 2000). Indeed, increases in non-photochemical quenching and decreases in the efficiency of excitation capture by open centers were observed in cowpea plants under water deficit (Souza *et al*. 2004).

This work aimed to evaluate the responses of leaf gas exchange and chlorophyll fluorescence to moderate water deficit in five common bean genotypes, and to discuss the possible differences between genotypes in relation to the physiological mechanisms triggered by drought.

Materials and methods

Plants, growth conditions and drought treatment: *Phaseolus vulgaris* (L.) genotypes A222, A320, BAT477, Carioca and Ouro Negro were compared. The A222 and A320 are genotypes that maintain high leaf water potential under drought (Pimentel *et al*. 1999a). Ouro Negro is a black seeded genotype commonly cultivated in Brazil (Pimentel *et al*. 1999b, Santos *et al*. 2004); Carioca is one of the most often cultivated genotypes in Brazil, due to its high productivity and stability under field conditions (Vicente *et al*. 2000), and BAT477 shows a reasonable performance under water deficit (Pimentel *et al*. 1999b).

The plants were sown in pots, two plants per pot, containing 8 kg of a growth medium consisting of a soilless mixture (*Plantimax*, *Eucatex*, São Paulo, SP, Brazil). The plants were sufficiently fertilized and grown under natural irradiance in a greenhouse, where the daily mean air temperature varied from 11 to 28 °C. The pots were irrigated daily until the onset of the drought treatment, imposed 34 d after seedling emergence, at the pre-flowering stage. The plants were irrigated again after ten days of water withholding and continued to be irrigated until the end of their cycle.

At the harvest, the effects of water deficit on yield components such as number of pods (NP), number of seeds (NS) and seed mass (SM) per plant were evaluated.

Leaf water status: The predawn water tension in the xylem was measured every two days, in the predawn, using a Scholander pressure chamber (*Soil Moisture Equipment Corp*., Santa Barbara, CA, USA) on the fourth trifoliolate leaf from the base of the plant, a mature but not senescent leaf. These measurements were assumed to represent the leaf water potential (Ψ_w).

Leaf gas exchange: Net photosynthetic rate (P_N), intercellular CO_2 concentration (c_i) and stomatal conductance (g_s) were assessed using an open gas exchange system with a 6 cm^2 clamp-on leaf cuvette (*LI-6400, LICOR*, Lincoln, NE, USA). The intrinsic water use efficiency (IWUE) was calculated by the relationship P_N/g_s (Osmond *et al*. 1980). Leaf gas exchange was evaluated in the middle leaflet of the fifth trifoliolate leaf. These measurements were taken between 10:00 and 11:30, during 10 d after water withholding at intervals of 2 d. After rehydration, daily measurements were taken in order to evaluate plant recovery. Photosynthetic photon flux density (PPFD) was fixed at 600 $\mu mol\ m^{-2}\ s^{-1}$, using a red-blue LED light source built into the leaf cuvette, though other environmental factors, such as air humidity and temperature, were not controlled, *i.e*. natural variation was permitted. The vapor pressure deficit in the cuvette was maintained below 2.5 kPa to prevent stomatal closure due to the low air humidity effect. The air collected outside the greenhouse was passed through a buffering gallon and then pumped into the system, with mean CO_2 concentration of 380 $\mu mol\ mol^{-1}$.

Chlorophyll fluorescence: Chlorophyll fluorescence was measured using a *PAM-2000* fluorometer (*Heinz Walz*, Effeltrich, Germany). In dark-adapted leaves (30 min), the basal fluorescence (F_0) was recorded under PPFD < 5 $\mu mol\ m^{-2}\ s^{-1}$ and the maximum fluorescence (F_m) was attained during a 0.8 s saturation pulse 15 000 $\mu mol\ m^{-2}\ s^{-1}$. The steady-state (F_s) and maximum (F_m') fluorescence were also determined in light-adapted leaves under steady-state photosynthesis. The variable fluorescence was calculated in both dark ($F_v = F_m - F_0$) and light-adapted ($\Delta F = F_m' - F_s$) samples. Minimum fluorescence (F_0') was measured after exciting photosystem 1 with far-red radiation. Chlorophyll fluorescence and leaf gas exchange measurements were taken simultaneously.

Based on the measured chlorophyll fluorescence parameters, some photochemical variables were calculated: potential (F_v/F_m) and effective ($\Delta F/F_m'$) quantum efficiency of PS 2, apparent electron transport rate ($ETR = \Delta F/F_m' \times PPFD \times 0.5 \times 0.84$), photochemical [$q_P = (F_m'-F_s)/(F_m'-F_0')$] and non-photochemical [$NPQ = (F_m-F_m')/F_m'$] fluorescence quenching (Schreiber *et al.* 1994, Maxwell and Johnson 2000). For ETR estimation, 0.5 was used as the fraction of excitation energy distributed to PS 2, and 0.84 as the fraction of light absorption.

The alternative electron sinks (*AES*) were estimated by the relationship between the effective quantum efficiency of PS 2 and the quantum efficiency of CO_2 assimilation, as $AES = [(\Delta F/F_m')/\Phi CO_2]$. The quantum efficiency of P_N was calculated as $\Phi CO_2 = [P_N/(PPFD \times 0.84)]$, adapted from Edwards and Baker (1993) by Ribeiro *et al*. (2004).

Statistical analysis: The experiment was arranged in a random block design with four replications. The data were subjected to analysis of variance (*ANOVA*), and means were compared by the Student-Neuman-Keuls test when significance ($P < 0.05$) was detected. Data obtained at the maximum water deficit (at the 10th day of water withholding) and after the rehydration are shown.

Results

Leaf water status: The predawn leaf water potential (Ψ_w) in the well-watered plants of all genotypes showed similar values (Fig. 1*A*,*B*). However, significant differences among genotypes were observed under water deficit. The genotypes A320 and A222 showed higher Ψ_w when compared to the other genotypes at maximum water deficit. Values of Ψ_w lower than -0.6 MPa were observed in the BAT477, Carioca and Ouro Negro (Fig. 1*A*). After recovery (two days after rehydration), the Ψ_w of all plants subjected to water deficit returned to the values observed in well-watered plants (Fig. 1*B*).

Leaf gas exchange: In relation to the control treatment (well-watered plants), stomatal conductance (g_s) was reduced by 40 and 48 % in the A222 and A320 while BAT 477, Carioca and Ouro Negro had reductions varying between 16 and 23 % after 4 d of water withholding (data not shown). After 6 d of water withholding, a large decrease in g_s occurred in all genotypes, with the exception of Carioca. This genotype exhibited a reduction of g_s around 43 % when comparing plants under water deficit with the well-watered plants. Those decreases in g_s caused a reduction in intercellular CO_2 concentration (data not shown). However, Carioca genotype did not show significant ($P > 0.05$) difference in c_i and P_N between control and treated plants even after 6 d of water deficit, but after 10 d the stressed plants showed low values of g_s in all genotypes (Fig. 1*C*).

The lowest g_s and P_N values were observed between 8 d (A320) and 10 d (A222, BAT477, Carioca and Ouro Negro) of water withholding. On the first day of rehydration, only A222 and Ouro Negro showed g_s and P_N values similar to well-watered plants. Both BAT477 and Carioca recovered g_s, c_i (data not shown) and P_N only after 2 d of rehydration (Fig. 1*F*), with the exception of A320.

The IWUE increased from the 4th day of water deficit in all genotypes evaluated, with exception of A320 that showed an increase in IWUE since the second day of water withholding (data not shown). The highest IWUE values were found between 6 and 10 d of water withholding, depending on the genotype (Fig. 1*G*). All genotypes reached the IWUE similar to well-watered plants after 2 d of rehydration, with the exception of Ouro Negro (Fig. 1*H*).

Chlorophyll fluorescence: The maximum quantum efficiency of PS 2 photochemistry (F_v/F_m) did not show difference at maximum stress and recovery (Fig. 2*A*,*B*). The effective quantum efficiency of PS 2 ($\Delta F/F_m'$) began to decrease after 8 d of water withholding in all genotypes (data not shown). At the maximum water deficit (at the 10th day), all genotypes showed decreases in $\Delta F/F_m'$ between 35 and 47 % (Fig. 2*C*).

The ETR showed a different trend during water deficit when comparing genotypes. The first genotype that presented a reduction in ETR was A320, on the 6th day of water withholding. Afterwards, the A222 genotype began to show reduced ETR from the 8th day of water withholding (data not shown). A significant reduction in ETR was only observed at the maximum water deficit in BAT477, Carioca and Ouro Negro (Fig. 2*E*). After rehydration, both $\Delta F/F_m'$ and ETR values were similar to those found in well-watered plants for the BAT477, Carioca and Ouro Negro (Figs. 2*D*,*F*). The genotypes A222 and A320 did not show full recovery of ETR values after plant rehydration (Fig. 2*F*).

Photochemical fluorescence quenching (q_P) decreased at the maximum water deficit in all genotypes evaluated (Fig. 3*A*). The greatest q_P decrease in relation to the well-watered plants was observed in Carioca (29 %) when compared to the other genotypes. All genotypes recovered the q_P values of well-watered plants after rehydration (Fig. 3*B*). NPQ increased in all genotypes at the maximum water deficit (Fig. 3*C*), with increases ranging from 54 (Carioca) to 191 % (Ouro Negro). Plant rehydration caused a reduction in NPQ for all genotypes, with exception of A320 (Fig. 3*D*). The AES increased from the 8th day of water withholding for A222, A320, BAT477 and Carioca water-stressed plants (data not shown). However, the highest AES values were observed at the maximum water deficit (Fig. 3*E*). One day after rehydration, all genotypes showed similar AES when comparing treated and control plants (Fig. 3*F*).

Yield components: The number of pods, number of seeds and seed mass per pot were not significantly affected by the water deficit treatment (data not shown).

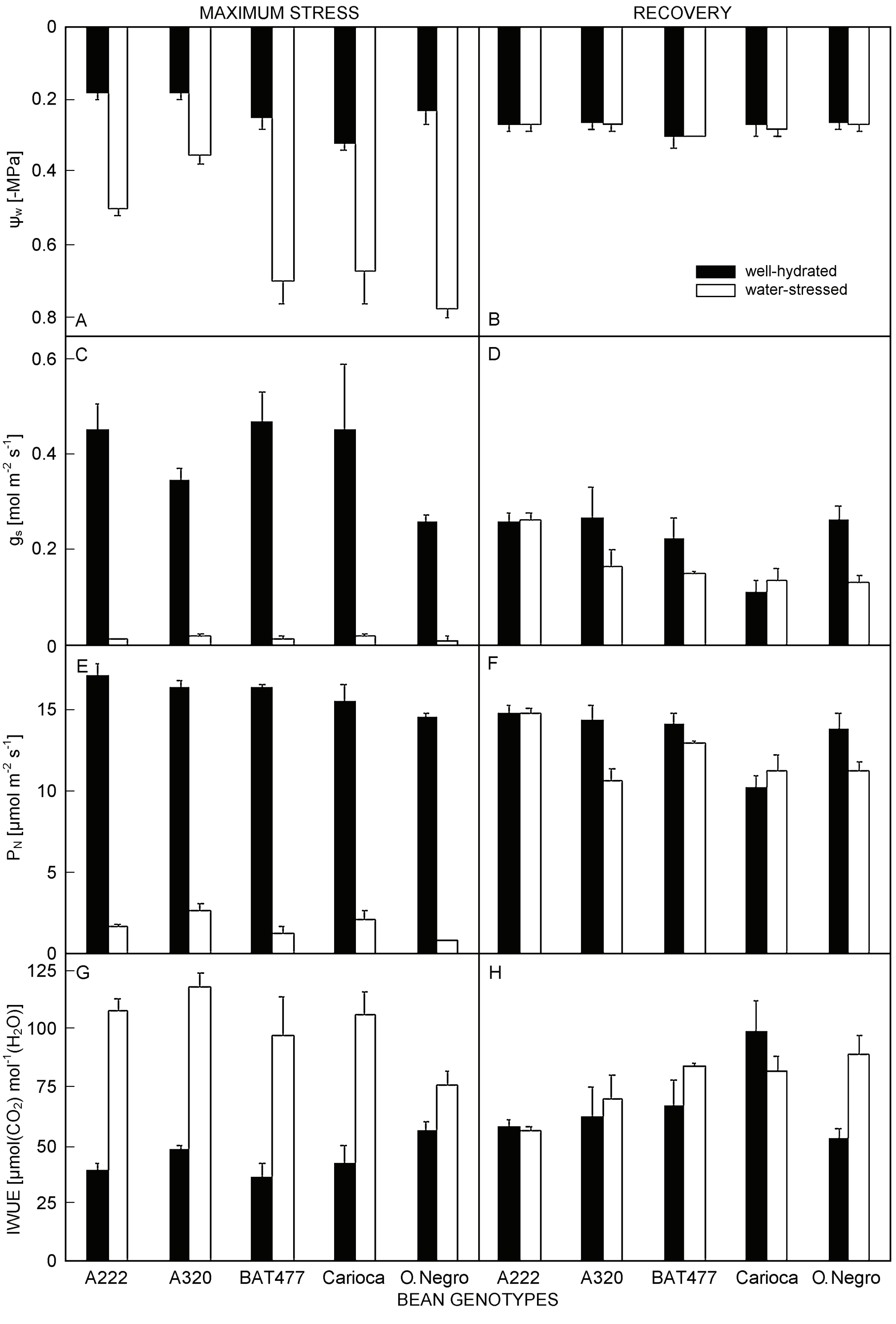

Fig. 1. Changes in predawn leaf water potential (Ψ_w - *A,B*); stomatal conductance (g_s - *C,D*), net photosynthetic rate (P_N - *E,F*) and intrinsic water use efficiency (IWUE - *G,H*) of five common bean genotypes (A222, A320, BAT477, Carioca and Ouro Negro) at maximum water deficit (10 d of water withholding) and 2 d after rehydration (recovery). The values presented are means (*n* = 4) and vertical bars represent ± SE.

Discussion

The water deficit treatment induced different patterns among bean genotypes. The A320 genotype differed from the others, maintaining high Ψ_w even after 10 d of water withholding (Fig. 1*A*), which is in agreement with Pimentel *et al*. (1999b). Since water deficiency causes strong photosynthesis impairment in *Phaseolus vulgaris* even under mild water deficit (Vassey and Sharkey 1989, Santos *et al*. 2004, 2006), the maintenance of shoot hydration (given by high Ψ_w) may alleviate the harmful effects of drought on photosynthesis.

Stomatal and non-stomatal limitation of photosynthesis has been reported under mild drought stress (Sharkey and Seemann 1989, Vassey and Sharkey 1989, Lauer and Boyer 1992, Pimentel *et al*. 1999b, Tezara *et al*. 1999, Chaves *et al*. 2002). Photosynthesis can be inhibited even when the stomatal influence is eliminated (leaf discs without epidermis), suggesting that factors other than low CO_2 availability affect photosynthesis (Tang *et al*. 2002). Under moderate water deficit, the decrease in P_N is related to stomatal closure (Chaves *et al*. 2002), with none indication of damage to chloroplast reactions (Sharkey and Seemann 1989, Cornic and Briantais 1991). However, Tang *et al*. (2002) argued that P_N was limited by biochemical reactions. In addition, Tezara *et al*. (1999) concluded that low ATP content, caused by a reduction in ATP synthase, was responsible for decreases in P_N.

The genotype A320 showed the earliest sensitivity

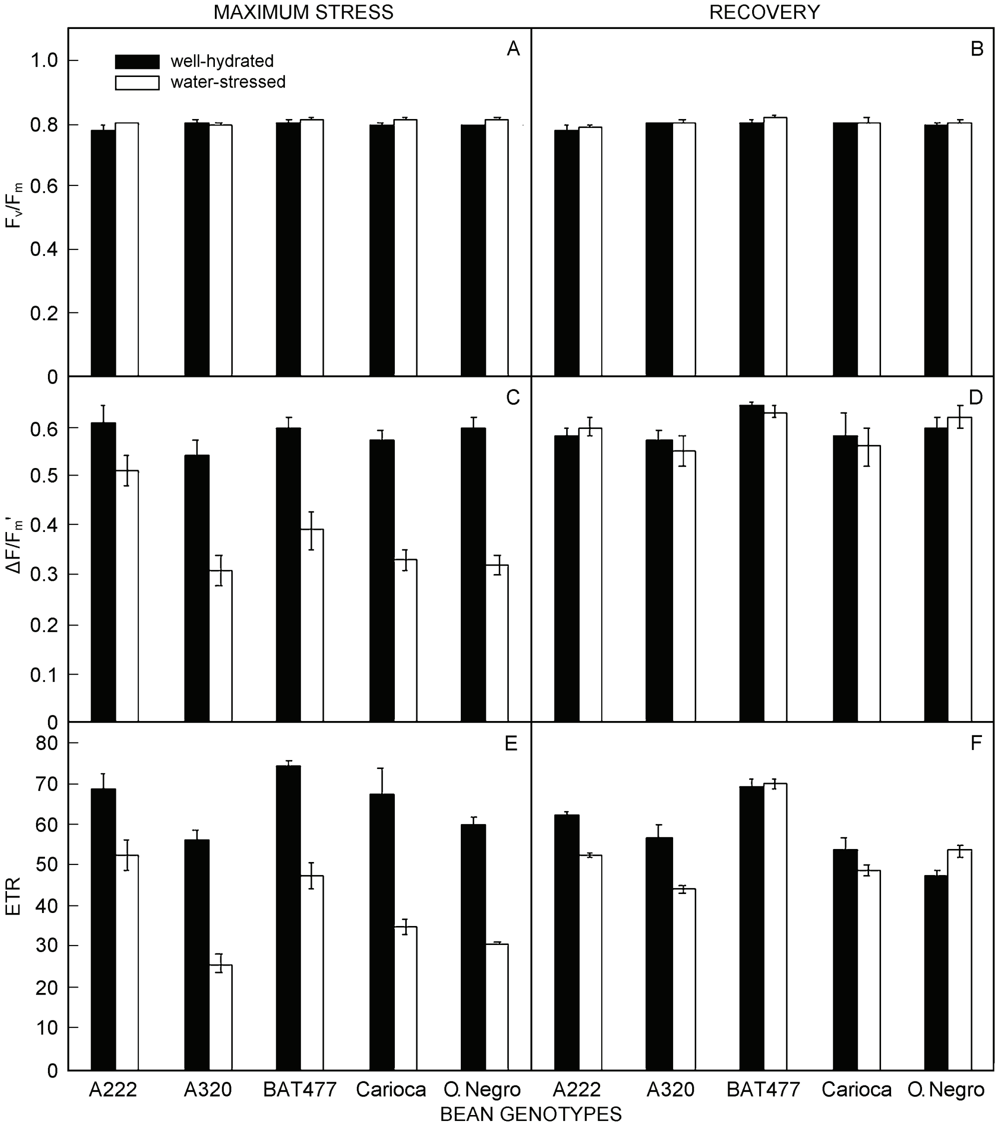

Fig. 2. Maximum quantum efficiency of photosystem 2 photochemistry (F_v/F_m - *A,B*), effective quantum efficiency of photosystem 2 ($\Delta F/F_m'$ - *C,D*), apparent electron transport rate (ETR - *E,F*), in genotypes A222, A320, BAT477, Carioca, Ouro Negro at 10 d of water withholding and 2 d after rehydration. Means ± SE, *n* = 4.

to water deficiency, reducing g_s on the 2nd day of water withholding and presenting the highest Ψ_w at the maximum water deficit (Fig. 1*C*). Curiously, A320 did not show full recovery of P_N after rehydration (Fig. 1*F*). Since P_N in plants subjected to water deficit were different as compared to the control plants even after the recovery of Ψ_w and g_s (Fig. 1*B*,*E*), it can be argued that A320 has greater sensitivity to a mild water deficit in relation to its photosynthetic machinery when compared to the other genotypes.

The first effect of a reduction in g_s is an increase in IWUE, *i.e.* P_N/g_s (Osmond *et al.* 1980), and the fast stomatal control in A320 led to an increase in IWUE from the onset of water deficit treatment. On the other hand, Ouro Negro showed the lowest IWUE at the maximum water deficit (Fig. 1*G*), which was caused by a large reduction in P_N (Fig. 1*E*). This genotype also showed the lowest Ψ_w at the maximum water deficit (Fig. 1*A*). Besides stomatal conductance, other factors can increase the water loss by leaves, reducing Ψ_w. Among them, we can consider the cuticular transpiration (influenced by the thickness of the wax epicuticular layer), the leaf area and the plant hydraulic conductivity (Kramer and Boyer 1995). Moreover, the hormonal balance performs an important role in the gas exchange under water deficit (Pospíšilová *et al.* 2000). Decreased content of cytokinins (due reduction in biosynthesis or enhanced degradation) and accumulation of abscisic acid in water-stressed plants lead to strongly increased abscisic acid/cytokinins ratio. In this moment the stomata closed.

Considering ETR as an overall measurement of

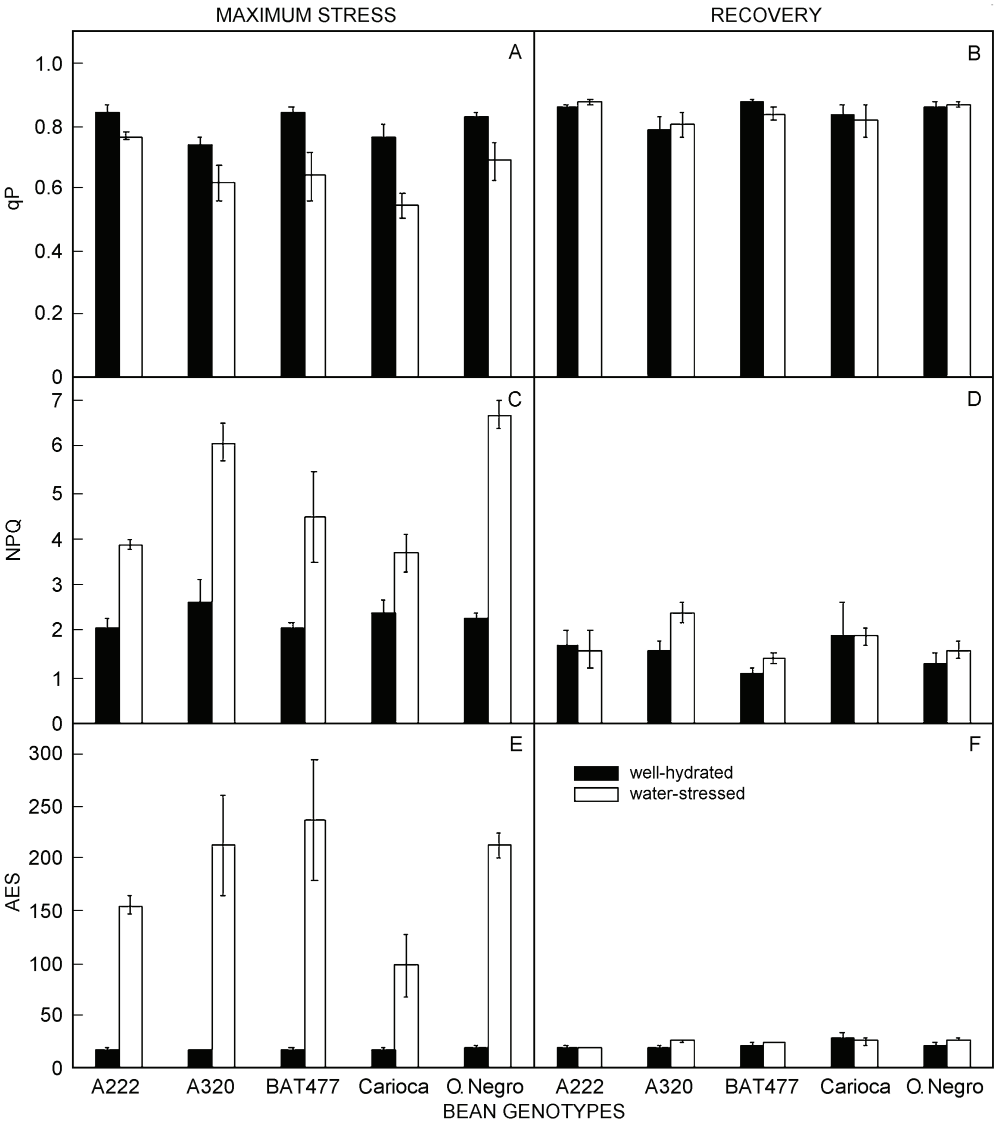

Fig. 3. Photochemical (qP - *A*,*B*), nonphotochemical quenching (NPQ - *C*,*D*) and alternative electron sink (AES - *E*,*F*) in genotypes A222, A320, BAT477, Carioca and Ouro Negro at 10 d of water withholding and 2 d after rehydration. Means ± SE, $n = 4$.

photochemical activity, our data show that photochemical activity was reduced at the maximum water deficit (Fig. 2*E*). Since all the genotypes showed a reduction in P_N from the 6th day of water withholding, it is suggested that low g_s was the main limitation to photosynthesis at the beginning of water deficit (until the 6th day). From this moment until the maximum water deficit, both stomatal and non-stomatal factors reduced the photosynthesis. At the maximum water deficit (Fig. 1*C*,*E*), the causes of photosynthesis reduction can not be distinguished, being consequence of stomatal closure and impairments in biochemical (g_s relatively constant and P_N continued to decrease) and photochemical reactions. However, the CO_2 assimilation of A320 did not show full recovery after two days of rehydration, which is in agreement with its diminished ETR values (Figs. 1*F*, 2*E*). Probably, the greater stomatal control of A320, which is favorable for maintaining a higher Ψ_w, caused a higher photoinhibitory effect due to a sharp reduction in c_i. On the other hand, A222 showed a full recovery in P_N values (Fig. 1*F*). Liu *et al.* (2006) discussed the drought stress intensity that PS 2 can tolerate, in progressive water deficit. Their results suggested that mild water deficit did not damage to photochemical apparatus, but reduced the activity.

In our paper, the chlorophyll fluorescence measurements indicated that moderate water deficit did not promote photoinhibition, as both water-stressed and well-watered plants presented F_v/F_m higher than 0.725 at the maximum water deficit (Fig. 2*A*). Souza *et al.* (2004) argued that despite reductions in photochemistry in cowpea plants during water deficit, the overall photosynthetic process was not limited by the supply of photochemical products. Although a reduction in ETR under drought stress (Fig. 2*E*) may suggest low tolerance to water deficit, an increase in NPQ (Fig. 3*C*) may be an important adaptation to deal with excessive light energy when plants have low P_N. Foyer *et al.* (1994) argued that the increase in AES activity (Fig. 3*E*) is related to the capacity of protective processes, such as the antioxidant system, the photorespiration and the reduction of molecular oxygen (Mehler reaction). In addition, reductions in ETR may also be caused by photochemical down-regulation under environmental constraints (Yordanov *et al.* 2000). Marques da Silva (2007) evaluated the photochemical reactions of three shrubs species under field conditions during the summer and autumn in the Mediterranean, with more limitation to photosynthetic machinery during the summer than autumn, possibly due to the influence of high temperatures and water shortage.

Our results showed that a decrease in g_s led to reductions in P_N in bean leaves even under mild drought stress. The genotypes A222 and A320 maintained a high Ψ_w. In addition, the excessive light energy due to reduced photosynthetic carbon metabolism under mild drought stress may be dissipated as heat through non-photochemical quenching or used by alternative electron sinks.

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