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Differential response to water stress in two tropical common bean cultivars

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ABSTRACT

The common bean (*Phaseolus vulgaris* L.) yield comes from regions with some level of water deficiency. Maintaining crop yield under drought stress is thus one of the biggest challenges. In order to improve our understanding of the responses involved of common bean to drought stress, we studied photosynthesis, antioxidant system, photorespiration estimated by glycolate oxidase activity, and proline content in leaves of two common bean cultivars (Expedito and Macotaço) subjected to water stress induced by polyethylene glycol 6000. The stress imposed affected the two common bean cultivars by decreasing net photosynthesis rate and increasing glycolate oxidase (GO) activity due to stomatal closure. Increased flux of electrons through the photosystem II led to reactive oxygen species (ROS) production, as observed by increased hydrogen peroxide (H_2O_2) content and it enhanced the activity of the enzymatic antioxidant system such as superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT), and guayacol peroxidase (GPOD) in Macotaço. Both cultivars here studied showed similar effects on photosynthesis parameters, however, Expedito had a better response on antioxidant system and proline content, which may represent an advantage over Macotaço.

Key words: chlorophyll *a* fluorescence; gas exchange; oxidative stress; *Phaseolus vulgaris* L.; physiological drought

Resposta diferencial ao déficit hídrico em dois cultivares de feijão comum

RESUMO

A maior parte da produção de feijão comum (*Phaseolus vulgaris* L.) provém de regiões com algum nível de deficiência hídrica, sendo o rendimento de culturas sob tais condições um grande desafio. A fim de melhorar a nossa compreensão sobre as respostas envolvidas do feijoeiro ao estresse hídrico foi avaliado a fotossíntese, sistema antioxidante, fotorrespiração estimada pela atividade da glicolato oxidase e o teor de prolina em folhas de duas cultivares de feijão (Expedito e Macotaço) submetidos ao estresse hídrico induzido por polietileno glicol 6000. O estresse imposto afetou as duas cultivares de feijão pela diminuição da taxa de fotossíntese líquida e aumento da atividade da glicolato oxidase (GO), devido ao fechamento dos estômatos. O aumento do fluxo de elétrons através do fotossistema II induziu a produção de espécies reativas de oxigênio (ROS), como observado pelo aumento de peróxido de hidrogénio (H_2O_2) e aumentou da atividade do sistema antioxidante enzimático, tais como superóxido dismutase (SOD), ascorbato peroxidase (APX), catalase (CAT) e guaiacol peroxidase (GPOD) em Macotaço. Ambas as cultivares apresentaram efeitos semelhantes na fotossíntese, no entanto, Expedito teve uma melhor resposta do sistema antioxidante e teor de prolina, o que pode representar uma vantagem sobre Macotaço.

Palavras-chave: fluorescência da clorofila *a*; trocas gasosas; estresse oxidativo; *Phaseolus vulgaris* L.; seca fisiológica

Introduction

Common bean (*Phaseolus vulgaris* L.) is one of the most important food legume (Rosales et al., 2012) from the Fabaceae family that is cultivated worldwide for human consumption. For instance, almost half of global output bean productivity is from the semi-arid regions in Latin America (Broughton et al., 2003). Water deficit represents the major constraint leading to loss of yield in common bean by ranging about 50% due to the limitation of growth in at least one stage of the plant cycle (Singh et al., 2014).

Drought responses have been widely studied however, the effects of drought are expected to increase due to global climatic changes (Christensen et al., 2007), and it will probably make water shortage and even greater limitation to plant productivity across an increasing amount of land (Chaves et al., 2009). In this sense, an improved understanding of common bean cultivars is of importance for sustainable agriculture, contributing for increasing the productivity of this food legume.

The response of plants to water stress in general depends on the development stage, the length and severity of the stress applied (Torres et al., 2006). Even under mild water stress a rapid closure of stomata to avoid further loss of water through transpiration is observed (Farooq et al., 2009). The reduction in stomatal and leaf mesophyll conductance can restrict the diffusion of CO_2 from the air into the carboxylation sites (Sade et al., 2014). This way, the ability to maintain the functionality of the photosynthetic machinery under water stress is of major importance in drought tolerance.

Despite of the fact that photosystem II (PSII) is drought resistant (Yordanov et al., 2003) under conditions of water stress photosynthetic electron transport through PSII is impaired (Zlatev, 2013). Changes in the apparatus photosynthetic can be monitored by chlorophyll (Chl) a fluorescence which has been widely used to study impacts caused by water deficiency on photosynthetic metabolism specially when coupled with other non-invasive measurement, such as gas exchange analysis (Baker, 2008).

The stomatal closure upon water stress not only decreases CO_2 influx and reduces de carboxylation (Farooq et al., 2009), but also enhances redox imbalance of the photosystems. Thus, more electrons are directing to form reactive oxygen species (ROS) (Farooq et al., 2009), and increase the energy dissipated as heat or its reemission as non-photochemical quenching of fluorescence (Baker, 2008). Deleterious effects of ROS are reported under water stress (Blokhina & Fagerstedt, 2010), as oxidative damage to lipids (lipid peroxidation) and consequently membrane injuries, protein degradation and enzyme inactivation (Demidchik, 2015). In order to cope with oxidative damage plants induce an efficient antioxidative defence system, composed of both enzymatic and non-enzymatic components (Rosales et al., 2012; Demidchik, 2015). The modulation in the activities of antioxidant enzymes may be one of the important factors in tolerance of various plants to environmental stresses including drought (Rachoski et al., 2015).

The restriction of CO_2 at the assimilation site in chloroplasts (Yordanov et al., 2003) also induces photorespiration as a mechanism to facilitate energy dissipation and preventing photoinhibition. Glycolate oxidase (GO) is a key enzyme in photorespiration that catalyses the glycolate oxidation producing glyoxylate and H_2O_2 (Rosales et al., 2012). Besides that, plants accumulate solutes such as proline that is supposed to play a role in the osmotic adjustment in response to drought (Claussen, 2005).

Polyethylene glycol 6000 (PEG 6000) compounds have been used to simulate water stress effect in plants (Murillo-Amador et al., 2002). Therefore, to better understanding differences in physiological and biochemical mechanisms that underlie drought responses at early stage, we studied the Chl *a* fluorescence transients and gas exchange, antioxidant system, photorespiration estimated by glycolate oxidase enzyme, and proline content in leaves of two Brazilian common bean cultivars subjected to water stress induced by PEG 6000.

Material and Methods

Plant material, growth conditions and treatments

This study was carried out with two common bean (*Phaseolus vulgaris* L.) cultivars (Macotaço and BRS Expedito). The seeds were sown in sand and with 10-days-old (V1 stage; Schoonhoven & Pastor-Corrales 1987) the seedlings were transferred to a hydroponic system containing nutrient solution as described by Hoagland & Arnon (1950). The growing conditions in the greenhouse were as follow: light intensity of 400 - 500 µmol m^{-2} s^{-1} on the top of the plants, provided by natural light and temperature variation between 18 - 25 ºC. Plants with uniform vegetative growth were selected for the study, and the experiment was initiated 12 days after transferring to a hydroponic system. The water stress treatment was performed by regulating the osmotic potential of the Hoagland's solution by supplementation with PEG 6000 (5 % w/v; - 0.05 MPa). This treatment was compared with the control under normal water conditions containing only Hoagland nutrient solution, which was replaced every four days, and the pH adjusted to 6.5 during the time-course of the experiment. The experimental unit consisted of one pot containing one plant, in a fully randomized design. The number of replicates per treatment is indicated as follow in the figures. The leaf harvest and non-destructive analysis were done with three days of water stress induced by PEG 6000 (fully expanded first trifoliate leaf stage -V3), when harvested the material was stored at -80 ºC until analysis. The data were analysed by analysis of variance (ANOVA) and F-test. When *F* was significant, the treatment means for each genotype were compared by the Tukey test ($p \leq 0.05$). Statistical analyses were performed using the SAS 8.0 statistical software program.

Chlorophyll *a* fluorescence transients

Chlorophyll *a* fluorescence transients of dark-adapted leaf of plants were measured using a Handy PEA (Plant Efficiency Analyzer, Hansatech, UK). Leaves were kept in dark for at least 30 min in specially provided clips that fit onto the discs. The fluorescence was induced by one saturating flash with 3 000 µmol photons m^{-2} s^{-1}. The polyphasic fluorescence rise, OJIP, was measured during the first second of illumination.

The quantification of the OJIP fluorescence transient is based on the polyphasic fast fluorescence rise from the lowest intensity F_0 (minimum fluorescence) to the highest intensity F_M (maximum fluorescence) (Strasser & Tsimilli-Michael, 2001). The fluorescence intensities determined at 50, 100 and 300 µs ($F_{50\mu s}$, $F_{100\mu s}$ and $F_{300\mu s}$, respectively), 2 and 30 ms (F_{2ms} - F_J and F_{30ms} -F_I) and at F_M (maximum fluorescence) were used to calculate the JIP-test parameters (Strasser & Strasser, 1995). The intensity measured at 50 µs was considered to be the initial fluorescence (F_0). The plotted fluorescence values were the averages of 10 measurements of each treatment.

Gas exchange analysis

Gas exchange was measured in the first fully expanded leaf using a portable infra-red CO_2 analyser (model LI-6400XT LI-COR, Inc., Lincoln, NE, USA). The measurements were taken between 10:00 and 11:00 a.m., with an in-chamber CO_2 concentration of 380 µmol mol^{-1} and a photon flow density of 1 250 µmol photons m^{-2} s^{-1}, using the light source LI-COR 6400-02 attached to the measuring chamber.

Enzymatic activity assays

For the measurement of enzyme activities, leaves (± 0.2 g) were ground using liquid N_2 in porcelain mortars, containing 5 % (w:v) polyvinylpolypyrrolidone (PVPP) and homogenized in 1.8 mL of 100 mM potassium phosphate buffer, pH 7.8, containing 0.1 mM ethylenediaminetetraacetic acid (EDTA) and 20 mM sodium ascorbate. The homogenate was centrifuged at 12 000 *g* for 20 min and the supernatant obtained was used as crude enzyme extract. All steps in the preparation of the enzyme extract were carried out at 4 °C, as described by Azevedo Neto et al. (2006).

Superoxide dismutase (SOD - EC 1.15.1.1) activity was assayed by monitoring the inhibition of the nitroblue-tetrazolium (NBT) coloration at 560 nm. Catalase (CAT - EC 1.11.1.6) activity was assayed, by monitoring the decline in absorbance at 240 nm. Ascorbate peroxidase (APX - EC 1.11.1.11) activity was assayed through ascorbate oxidation at 290 nm. Guayacol peroxidase (GPOD 1.11.1.7) activity was assayed by monitoring the tetraguayacol production at 470 nm. Glycolate oxidase (GO; EC 1.1.3.1) activity was assayed as described by Bai et al. (2014). Fresh leaf tissue (0.2 g) was ground in a chilled mortar with liquid nitrogen and then homogenised with 5 % PVPP and 50 mM Tris-HCl buffer (pH 7.8) containing 0.01% Triton X-100 and 5 mM dithiotreithol (DTT). The homogenate was centrifuged at 12.000 g for 20 min at 4 ºC. GO activity was assayed by following the formation of glyoxylate phenylhydrazone at 324 nm.

Hydrogen peroxide content and lipid peroxidation measurement

Leaves (0.2 g) were ground in 0.1 % (w:v) trichloroacetic acid (TCA). The homogenate was centrifuged (12 000 g, 4 ºC, 20 min) and the supernatant was used for the analyses as described by Velikova et al. (2000). Lipid peroxidation, was determined by using thiobarbituric acid (TBA), which determines malondialdehyde (MDA) as an end product of lipid peroxidation. The amount of MDA-TBA complex (red pigment) was calculated from the extinction coefficient (ε =155 x 10^3 M^{-1} cm^{-1}).

Proline content

For free-proline determination, leaves (0.2 g) were ground by using liquid N_2 in porcelain mortars and homogenized in 3 % (w/v) aqueous sulphosalycylic acid followed by centrifugation at 5 000 g for 15 min. The supernatant was used to estimate the proline concentration by using ninhydrin reagent according to the method of Bates et al. (1973).

Results

Chlorophyll *a* fluorescence transient analysis

The relative variable fluorescence [$V_t = (F_t - F_0) / (F_M - F_0)$], represented on a logarithmic time scale starting at 50 µs, is present in the Fig 1A. We observed reduction in relative variable fluorescence at J-step (2 ms) (V_J) in both cultivars of common bean under drought stress simulated by PEG 6000. However, the relative variable fluorescence at I-step (30 ms) (V_I) was not altered by drought stress. The responses of drought stress are evaluated on the O-I phase of the fluorescence transient OJIP by normalisation as the relative variable fluorescence between steps O and I [$V_{OI} = (F_t - F_0) / (F_I - F_0)$]. This curve can evaluate the sequences of events from trapping by the reaction centre (RC) of the PSII until the reduction of the plastoquinone. In both cultivars under drought stress were observed a low oxidation rate of the final electron acceptor on the acceptor side of PSII (Figure 1B).

To evaluate the L-band, fluorescence data were normalized between O (50 µs) and K (300 µs) steps, as $V_{OK} = (F_t - F_0) / (F_K - F_0)$, and plotted as difference kinetics $\Delta V_{OK} = V_{OK(stress)} - V_{OK(control)}$ in the time range of 50-300 µs revealing the L-band. The L-band has been suggested to be an indicator of the energetic connectivity (grouping) of the PSII units. A higher cooperative results in an efficient consumption of the excitation energy and a higher stability of the system (Yusuf et al., 2010).

Furthermore, fluorescence data were normalized between the steps O and J (2 ms), as $V_{OJ} = (F_t - F_0) / (F_J - F_0)$, and plotted with the difference kinetics ΔV_{OJ} between the samples relatively to their controls in the 0-300 µs range revealing the K-band. The positive K-band indicates an increased reduction rate of quinone (Q_A), the primary electron acceptor of PSII, from Q_A to Q_A^-, which could mean that the oxygen evolving complex (OEC) becomes leaky and offers access to non-water electron donors. A positive K-band (at about 300 µs) suggests that the OEC is either inactivated or there is an increase in the functional PSII antenna size (Yusuf et al., 2010). We did not observe the presence of K- or L-band (data not shown) in both cultivars upon water stress. The fluorescence data were normalized between the steps I (2ms) and P (t_{FM}), as [$V_{IP} = (F_t - F_I) / (F_M - F_I)$ and showed on linear scale between 30 and 300 ms showed an increase in the sequences of events of electron transfer from the reduced plastoquinone to the final electron acceptor of PSI (Figure 1C) upon water stress.

The biophysic parameters derived from the transient chlorophyll fluorescence OJIP and JIP-test (Strasser &

Strasser, 1995) are shown in the Figure 1D. All parameters were normalized to their respective control. All these parameters allow understanding the energy distribution in the photosynthetic apparatus.

Water stress decreased the relative variable fluorescence at J-step, thereby increased the electron transport flux (ET_0/RC) and the electron flux reducing end electron acceptor at the PSI acceptor side per RC (RE_0/RC). An increase in the quantum yield for electron transport (ϕ_{Eo}), quantum yield for reduction of end electron acceptor at the PSI acceptor side (ϕ_{Ro}) and the probability (at time 0) that a trapped exciton moves an electron into the electron transport chain beyond Q_A^- (ψ_{Eo}) was observed in both cultivars. On the other hand, no differences in comparison to the control was observed in the maximum yield for primary photochemistry (ϕ_{Po}) and the probability with which an electron from the intersystem electron carriers moves to reduce end electron acceptors at the PSI acceptor side (δ_{Ro}) as well as dissipation flux per RC (DI_0/RC). An increase was also observed in the performance index (PI_{ABS}) for energy conservation from exciton to the reduction of PSI end acceptors and total performance index (PI_{total}) for conservation from exciton to the reduction of PSI end acceptors (Figure 1D).

Gas exchange measurements

Net photosynthesis rate (*A*), intercellular CO_2 concentration (C_i), transpiration rate (*E*) and stomatal conductance (g_s) under water stress are shown in the Figure 2. Water stress mediated by PEG 6000 induced reduction of *A*, g_s and *E* in both cultivars in relation to the control treatment. Considerable reduction was observed in g_s and *E,* ranging about 37 and 25 % in Expedito, 54 and 35 % in Macotaço respectively, which have influenced the reduction in *A*, 21 and 30 %, respectively in Expedito and Macotaço. The C_i was slightly affected in relation to the control, with reductions about 5 % in Expedito and 8 % in Macotaço.

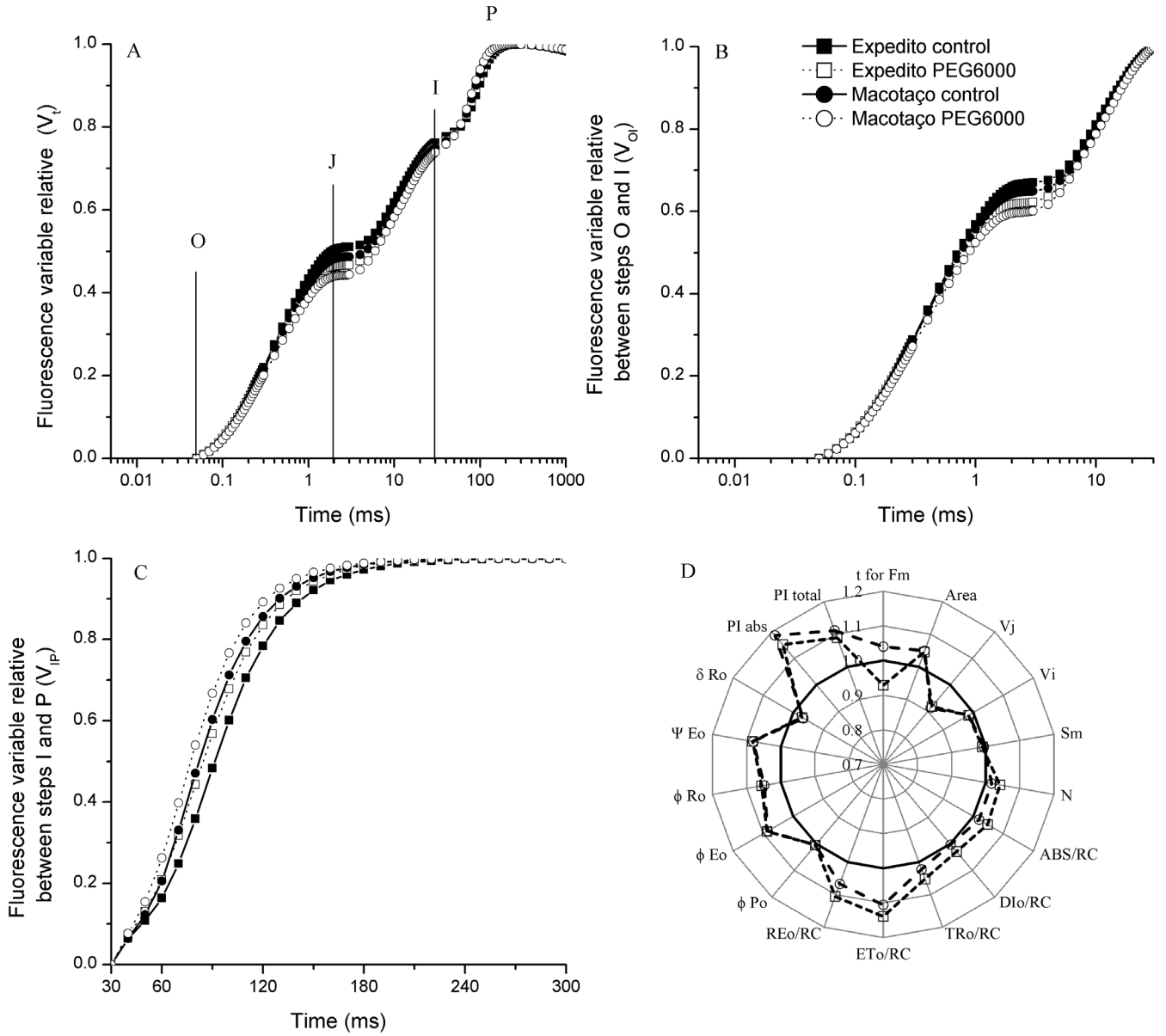

Figure 1. Chlorophyll *a* fluorescence transient in two common bean cultivars subjected to water stress by PEG 6000. A - Relative variable fluorescence [$V_t = (F_t - F_0) / (F_M - F_0)$]; B - Relative variable fluorescence between F_0 and F_I to $W_{OI} > 1$; C - Relative variable fluorescence between F_I and F_M [$V_{IP} = (F_t - F_I) / (F_M - F_I)$]; and D - Relative values of JIP-test parameters (n = 10).

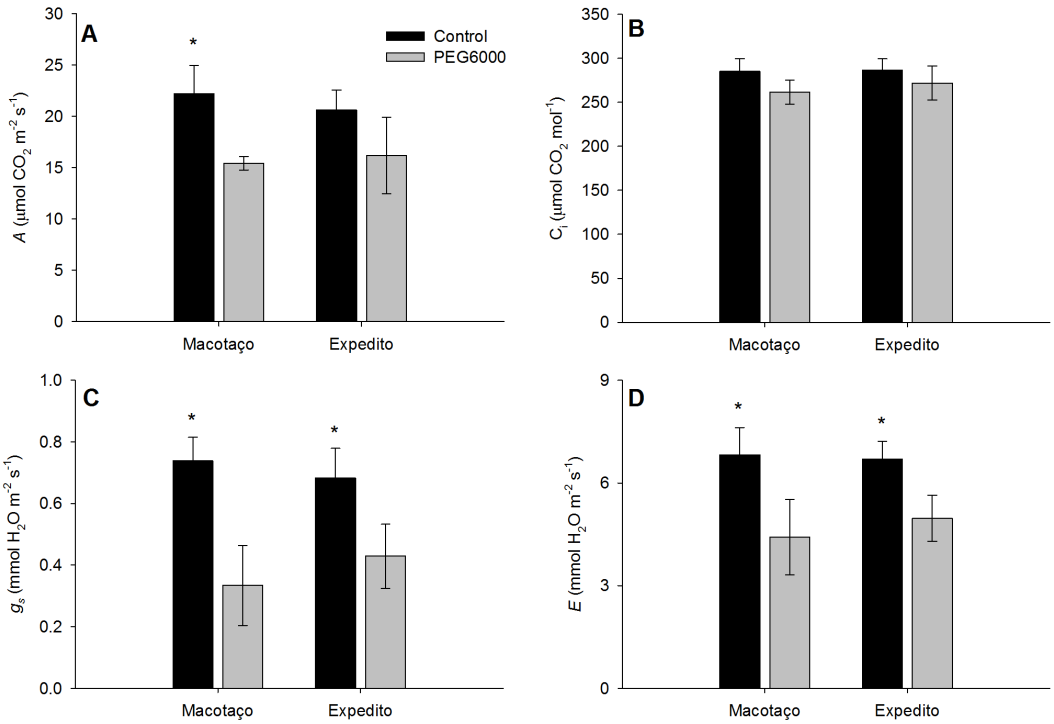

Figure 2. Gas exchange measurement in leaves of two common bean (Macotaço and Expedito) cultivars subjected to water stress by PEG 6000. A - net assimilation rate (A); B - intercellular CO_2 concentration (C_i); C - stomatal conductance (g_s); and D - transpiration rate (E). Asterisk symbol (*) indicates significant differences by Tukey's test ($p \leq 0.05$) between the treatments for each genotype. Values represent the mean ± SD (n = 5).

The reduction in gas exchange imposed by water stress was more pronounced in Macotaço than Expedito (Figure 2).

Antioxidant enzyme activity

The antioxidant enzyme activities of SOD, CAT, APX and GPOD are given in the Figure 3. Regarding SOD, APX and CAT, an increase of these enzymes were observed in Macotaço in response to water stress, whilst no changes occurred in Expedito (Figure 1A, B and C). This response was enzyme and genotype-specific and might be due to oxidative stress imposed by water stress. Strong activity induction of GPOD was observed in relation to the control, in both cultivars,

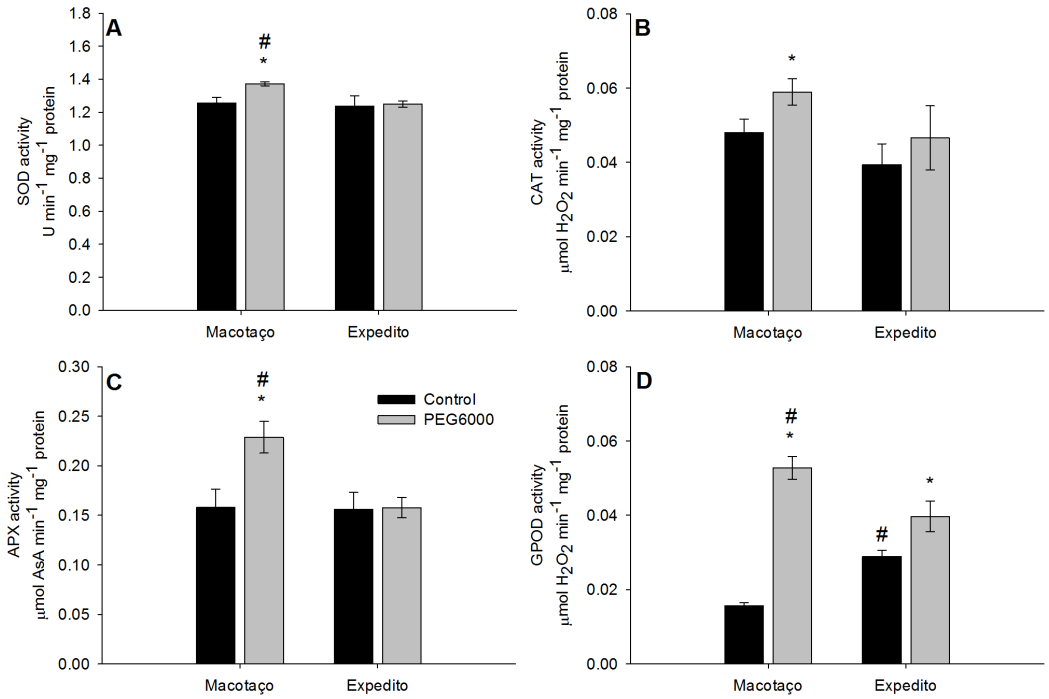

Figure 3. Antioxidative enzyme activity in leaves of two common bean (Macotaço and Expedito) cultivars subjected to water stress by PEG 6000. A - superoxide dismutase (SOD); B - catalase (CAT); C - ascorbate peroxidase (APX); and D - guayacol peroxidase (GPOD). Hash symbol (#) indicates significant differences by Tukey's test ($p \leq 0.05$) between genotypes for each treatment. Asterisk symbol (*) indicates significant differences by Tukey's test ($p \leq 0.05$) between the treatments for each genotype. Values represent the mean ± SD (n = 4).

however in Macotaço the increase was about 3.5-fold higher while 0.5-fold higher in Expedito (Figure 3D).

Glycolate oxidase activity

GO activity was assayed to explore its response associated to photorespiration upon water stress. Both cultivars, Macotaço and Expedito showed an increase (~50 %) in GO activity in comparison to their respective control upon water stress (Figure 4).

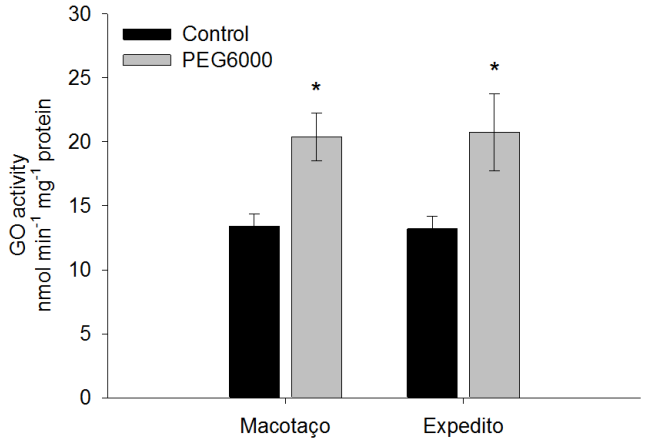

Figure 4. Glycolate oxidase (GO) activity in leaves of two common bean (Macotaço and Expedito) cultivars subjected to water stress by PEG6000. Asterisk symbol (*) indicates significant differences by Tukey's test ($p \leq 0.05$) between the treatments for each genotype. Values represent the mean ± SD (n = 4).

Hydrogen peroxide and lipid peroxidation

H_2O_2 and lipid peroxidation are consider indicators of oxidative stress (Blokhina & Fagerstedt 2010) and their concentration are shown in the Figure 5. An increase of the concentration of hydrogen peroxide was detected in both cultivars in response to water stress, thereby their content increased 55 % in Expedito while it was 2-fold higher in Macotaço (Figure 5A). Differences in lipid peroxidation were not observed in both cultivars, though an increase and decrease were detected in Macotaço and Expedito, respectively in comparison to the control (Figure 5B).

Proline content

Proline accumulation is supposed to participate in osmotic adjustment (Claussen 2005). An increase in proline content was observed in leaves of Macotaço, by accumulating about 3-fold higher in comparison to the control, whereas in Expedito no accumulation of this metabolite was detected (Figure 6).

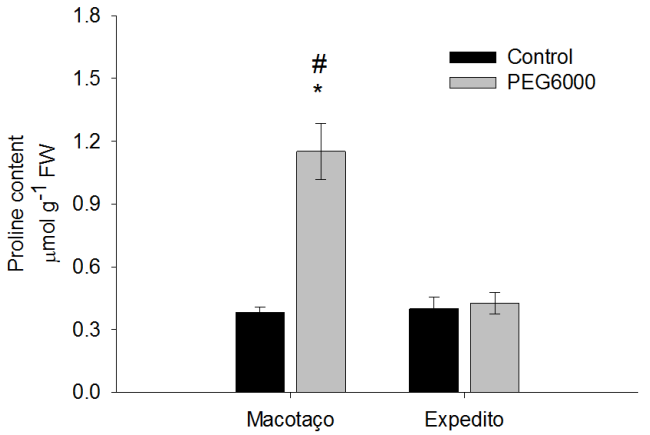

Figure 6. Proline content in leaves of two common bean (Macotaço and Expedito) cultivars subjected to water stress by PEG 6000. Hash symbol (#) indicates significant differences by Tukey's test ($p \leq 0.05$) between genotypes for each treatment. Asterisk symbol (*) indicates significant differences by Tukey's test ($p \leq 0.05$) between the treatments for each genotype. Values represent the mean ± SD (n = 4).

Discussion

Drought leads to a permanent depression of photosynthetic carbon assimilation (Chaves et al., 2009) as a result of stomatal and non-stomatal limitations (Zlatev, 2013), a constraint that has reflected considerable losses of yield in common bean over the last years. Therefore, water stress will become more frequent in some areas and is supposed to increase losses in productivity due to global climatic changes (Christensen et al., 2007). In addition, researchers are putting effort to better understanding the traits in an attempt to select cultivars better adapted to water deficit (Zlatev, 2013). Hence, this particular stress condition affects all development stages (Singh et al., 2014); this study was done by applying three days of water stress induced by PEG 6000 in a hydroponic system using common bean (fully expanded first trifoliate leaf stage) to characterize physiological and biochemical parameters at early stage of two Brazilian cultivars widely cultivated.

Three days of water restriction affected the two common bean cultivars displaying metabolic alterations as shown in the

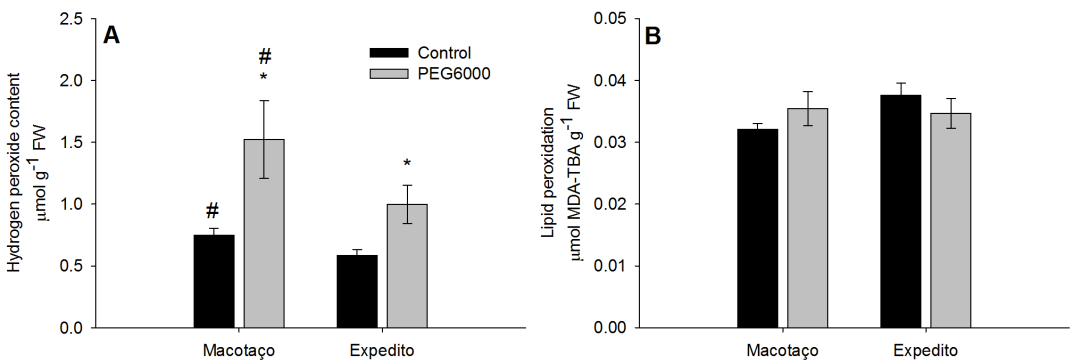

Figure 5. Hydrogen peroxide content (A) and lipid peroxidation (B) in leaves of two common bean (Macotaço and Expedito) cultivars subjected to water stress by PEG 6000. Hash symbol (#) indicates significant differences by Tukey's test ($p \leq 0.05$) between genotypes for each treatment. Asterisk symbol (*) indicates significant differences by Tukey's test ($p \leq 0.05$) between the treatments for each genotype. Values represent the mean ± SD (n = 4).

figures 1-6. The absorption of light energy exceeded (Figure 1) the capacity for CO_2 reduction due to stomatal closure decreasing net photosynthesis rate (Figure 2) and increasing photorespiration through glycolate oxidase activity (Figure 4). The reduction in relative variable fluorescence emission in both cultivars increased the flux of electrons through the PSII leading to an increase in PI_{ABS} and PI_{Total} and a slight increase in quantum yield (Figure 1). In addition, the increased electron flow through the PSII might have increased the electron scape to produce ROS, as observed by increased H_2O_2 production (Figure 5) enhancing the activity of the enzymatic antioxidant system (Figure 3) to counteract possible effects caused by ROS during the stress, once no damage to membrane was observed (lipid peroxidation). Both cultivars here studied showed similar effects and, as they are not characterized as sensitive and tolerant ones, Expedito had a better response to water stress in comparison to Macotaço considering the traits evaluated.

As the variable chlorophyll *a* fluorescence decreased in both cultivars upon water stress, an increase in photochemical reactions was observed due to low oxidation rate of the final electron acceptor on the acceptor side of PSII and increased flow of electron from plastoquinone reduced to the final electron acceptor of PSI as showed by the fluorescence data extracted from OJIP analysis of the PSII of dark-adapted leaves (Figure 1). Water deficit has more impact on PSI than PSII, especially on I-P phase that reflects the PSI performance. Thereby, no electron limitation was observed on J-step allowing the redox reaction of the Q_A (Xin et al., 2013).

Additionally, the period of water deficit imposed by PEG 6000 did not cause a blockage in the PSII reaction centres and the dissociation of the major antenna complexes or a shift in the redox-state equilibrium of PSII (Bukhov et al., 1990). As reported by González-Cruz & Pastenes (2012), the results suggest that any possible shift in the PSI redox state cannot originate from a reduction in the PSII electron acceptor side. Additionally, increases in PI_{total} over the control (Figure 1D) is an indicative of a positive tension on the system, which demonstrates a good performance of the photosynthetic machinery under stress conditions (Yusuf et al. 2010). On the other hand, increase in yield for reduction of the final electrons acceptor (Figure 1D), could be related to a cyclic electron transport around the PSI instead to be used for energy production once there was a decline in CO_2 assimilation (Figure 2A). Souza et al. (2004) reported that drought stress has relatively little effect on PSII, the parameter F_v/F_m decreases only under severe drought stress conditions.

The low stomatal conductance induced by water deficit has a direct adverse effect on photosynthesis (Chaves et al., 2009). This effect is evident in both cultivars with significant decline in net photosynthesis rate (A) upon drought induced by PEG 6000 (Figure 2A). It is noteworthy that photosynthesis was hampered at first due to stomatal limitation, once no significant alterations were observed in the functional activity of PSII, which are in agreement with other reports that underlie stomatal limitation as a primary event (Zlatev, 2013). Stomatal closure under drought is an avoidance response adopted by plants to save water and maintain the turgor.

Stomatal closure decreases the conductance (Figure 2C) restricting CO_2 availability at the assimilation site in chloroplasts (Yordanov et al., 2003) inducing photorespiration to facilitate energy dissipation and preventing photoinhibition. Glycolate oxidase catalyses the glycolate oxidation by producing glyoxylate and H_2O_2 (Rosales et al., 2012). Our results demonstrate that increases in GO activity may have contributed to H_2O_2 production in both cultivars (Figure 5A). On the other hand, increases in photorespiration contribute to improve plant performance as well as drought resistance by regulating photosynthesis through a feedback inhibition on Rubisco activity (Rosales et al., 2012)

In chloroplasts, limitation of CO_2 fixation coupled with over-reduction of the electron transport chains is the main cause of ROS production (Rachoski et al., 2015) or they are generated as a result of photorespiration via glycolate oxidase. In both cultivars were observed increase in hydrogen peroxide content, and it was higher in Macotaço than Expedito in comparison to their respective control (Figure 2). The production of H_2O_2 might be related to the increased flow of electron transport and leakage through the PSII (Figure 1). ROS are produced in different compartments of the plant cell, both under normal and stressful conditions (Grene, 2002).

On the other hand, no significant oxidative damage caused by ROS was observed in both cultivars (Figure 5B), where no differences in lipid peroxidation was detected in leaves under water stress in comparison to the control and it is in agreement with results of quantum yield efficiency (Figure 1D). In this sense, oxidative damage is normally related to linoleic acid peroxidation which is a major fatty acid component of thylakoid membranes in plants, and its degradation has been reported to affect the quantum efficiency of the PSII (González-Cruz & Pastenes, 2012) by also affecting the thylakoid proteins.

High ROS concentration is therefore a stress symptom, and plants maintain ROS concentration within a certain level by inducing enzymatic antioxidant system besides non-enzymatic one (Grene, 2002). The induction of the water stress enzymes is highly correlated with the severity of the stress and our results demonstrate high activity of the enzymes (SOD, CAT, APX and GPOD) responsive for scavenging ROS in Macotaço (Figure 3) upon water stress. In addition, higher activities of GPOD, CAT and APX in Macotaço are correlated with higher production of hydrogen peroxide in this cultivar in comparison to Expedito (Figure 5A). A reduced activity of the Calvin-Benson cycle under drought stress conditions could lead to an increase in the production of superoxide on the acceptor side of PSI, which supports the increased activity of SOD upon water stress (Figure 3A) and contribute for H_2O_2 production.

Interestingly that proline content increased only in Macotaço while no accumulation of this metabolite was detected in Expedito upon water stress (Figure 6). Studies have been suggested that proline is accumulated as a symptom of stress or a consequence of osmotic adjustment though information about its accumulation is scarce. In addition, proline accumulates in drought-susceptible common bean cultivars (Rosales et al., 2012)

Taken together, our results suggest that Expedito may present a metabolic advantage over Macotaço due to its better capacity of counteracting the effects of water stress in order to survive. Net photosynthesis rate is very sensitive to drought

due to stomatal limitation caused by reduction in both gas conductance and CO_2 influx at the carboxylation site, as already well reported, once the PSII is not impaired under water stress in both cultivars. The effects of water stress on photochemical efficiency and electron partitioning reveal that the excessive energy might be dissipated as heat once the chl a fluorescence did not increase under the conditions that the plants were subjected. The electron leakage and ROS formation were higher in Macotaço than in Expedito, with concomitant increase in the antioxidant enzymatic system. Photorespiration plays an important role in protecting the photosynthetic apparatus from photoinhibition. Hence, the operation of these photoprotective mechanisms during water stress allows the reduction of photodamage, therefore helping to preserve the integrity of the photosynthetic apparatus with higher performance in Expedito than in Macotaço. Furthermore, other analyses should be performed to support the tolerance of common bean to water deficit.

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