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RESEARCH PAPERS

Photochemical Heat-Shock Response in Common Bean Leaves as Affected by Previous Water Deficit[1]

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Abstract— The heat sensitivity of photochemical processes was evaluated in the common bean (*Phaseolus vulgaris*) cultivars A222, A320, and Carioca grown under well-watered conditions during the entire plant cycle (control treatment) or subjected to a temporal moderate water deficit at the preflowering stage (PWD). The responses of chlorophyll fluorescence to temperature were evaluated in leaf discs excised from control and PWD plants seven days after the complete recovery of plant shoot hydration. Heat treatment was done in the dark (5 min) at the ambient CO_2 concentration. Chlorophyll fluorescence was assessed under both dark and light conditions at 25, 35, and 45°C. In the dark, a decline of the potential quantum efficiency of photosystem II (PSII) and an increase in minimum chlorophyll fluorescence were observed in all genotypes at 45°C, but these responses were affected by PWD. In the light, the apparent electron transport rate and the effective quantum efficiency of PSII were reduced by heat stress (45°C), but no change due to PWD was demonstrated. Interestingly, only the A222 cultivar subjected to PWD showed a significant increase in nonphotochemical fluorescence quenching at 45°C. The common bean cultivars had different photochemical sensitivities to heat stress altered by a previous water deficit period. Increased thermal tolerance due to PWD was genotype-dependent and associated with an increase in potential quantum efficiency of PSII at high temperature. Under such conditions, the genotype responsive to PWD treatment enhanced its protective capacity against excessive light energy via increased nonphotochemical quenching.

Key words: Phaseolus vulgaris - chlorophyll fluorescence - photoprotection - photosynthesis - temperature - water deficit

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INTRODUCTION

Temperature is an environmental factor that normally imposes some physiological limitations on plant growth and development in tropical and subtropical regions. High temperatures (> 38°C) can be found at midday and during summer, if daily and annual cycles are considered. In such situations, the leaf temperature exceeds 36°C [1] due to excessive heat loading resulting from high available energy and inefficiencies of physiological and/or morphological mechanisms related to energy dissipation or heating avoidance.

Among high temperature effects on photochemistry, we may cite those related to the photochemical reactions themselves and those associated with the structural organization of thylakoid membranes [2–4]. Some consequences of heat stress upon the photochemical apparatus are as follows: inhibition of the water-splitting system, reduction in light energy trapping by PSII, impairments in electron flow between PSII and PSI [5, 6], suppression of nonphotochemical fluorescence quenching and membrane energization [7], migration of PSII reaction centers or LHCII to nonappressed regions in thylakoids [2, 3, 8], destabilization of lipid–protein interactions in membranes, and production of reactive oxygen species and oxidative damage [3].

Abbreviations: ETR—apparent electron transport rate; F_M—maximum chlorophyll fluorescence yield under dark conditions; F'_M—maximum chlorophyll fluorescence yield under light conditions; F_0—minimum chlorophyll fluorescence yield under dark conditions; F_S—steady-state chlorophyll fluorescence yield under light conditions; F_V—variable chlorophyll fluorescence under dark conditions; F_V/F_M—potential quantum efficiency of PSII; LHCII—light-harvesting chlorophyll *a*/*b* protein complexes of PSII; NPQ—nonphotochemical quenching of chlorophyll fluorescence; Q—photosynthetic photon flux density; PS—photosystem; PWD—previous moderate water deficit; Q_A—primary plastoquinone acceptor of PSII; Q_B—secondary plastoquinone acceptor of PSII; ΔF—variable chlorophyll fluorescence under light conditions; $\Delta F/F'_M$—effective quantum efficiency of PSII.

[1] The text was submitted by the authors in English.

In heat-stressed common bean plants, some researchers have observed a decline in the photosynthesis rate [2, 9, 10], an increase in nonphotochemical fluorescence quenching accompanied by a reduction in the potential quantum efficiency of PSII, photochemical fluorescence quenching and electron transport rates [10, 11]. However, heat sensitivity is species-dependent among legume plants, *Vigna unguiculata* being more tolerant than *Phaseolus vulgaris* to a high-temperature stress [10, 11]. Even within the same species (*P. vulgaris*), there are significant differences in heat sensitivity when comparing Carioca (more susceptible) and Negro Huatesco (more tolerant) cultivars [10, 11]. Also, the Carioca genotype exhibited a greater difference between the photosynthesis rates under optimum and natural conditions, thus indicating that CO_2 assimilation is more affected by air temperature and relative humidity than in Ouro Negro and Guarumbe genotypes [1]. In fact, heat tolerance mechanisms in common beans are under genetic control [12].

Thermal stability of chloroplast components affects the heat sensitivity of photosynthesis and plays an important role in maintaining a reasonable/superior photosynthetic capacity at high temperature [13]. In nature, water deficit is another common environmental constraint that reduces bean photosynthesis [14, 15]. Like in the case of heat tolerance, common bean cultivars also demonstrate different sensitivity to water deficit, Ouro Negro and A320 genotypes being less negatively affected when compared to the Carioca genotype [14]. On the other hand, water deficit may also increase plant tolerance to subsequent stresses as a result of enhanced PSII stability and tolerance at high temperature [5, 16]. In addition to high tolerance to heat stress, such physiological response to previous stresses may be important for the breeding of bean genotypes tolerant to warm environments.

In this study, we tested the hypothesis that the common bean (*Phaseolus vulgaris*) genotypes A222, Carioca, and A320 exhibit different responses to heat stress, and that heat tolerance is increased by a previous moderate water deficit (PWD). As physiological responses to high temperatures, changes in chlorophyll *a* fluorescence were assessed under dark conditions and during photosynthesis induction in leaf discs.

MATERIALS AND METHODS

Plant material and growth conditions. The experiments were conducted with three bean (*Phaseolus vulgaris* L.) cultivars: A320, Carioca, and A222. Cvs. A222 and A320 are tolerant to anthracnosis disease and known as maintaining the high shoot water status under drought conditions, and Carioca is a cultivar widely cultivated by growers [17].

Plants were sown and grown in plastic pots (10 l) containing a soilless mixture (Plantmax, Eucatex Inc., Brazil) fertilized with 0.18 g N, 0.15 g K_2O, 0.32 g P_2O_5, and 3 g of dolomitic lime. Micronutrients were also supplied in 300 ml of a nutrient solution: 210 μM $CuSO_4 \times 5H_2O$, 100 μM $ZnSO_4 \times 7H_2O$, 16 μM H_3BO_3, 240 μM $FeSO_4 \times 7H_2O$, and 1 μM $(NH_4)_6Mo_7O_{24} \times 4H_2O$ [18]. Twenty-five days after seedling emergence, an additional fertilization was done with 0.18 g N in each pot [14, 15]. Plants were grown under greenhouse conditions, where air temperature varied between 18 and 42°C, the minimum relative humidity was around 30%, and the maximum photosynthetic photon flux density was 1800 μmol/(m^2 s). Pots were irrigated daily until the PWD treatment.

Previous moderate water deficit. When plants were 34-day-old, half of them were exposed to water deficit for 10 days. This time was sufficient for the predawn leaf water potential to reach –0.50 ± 0.01 in cv. A222, –0.35 ± 0.03 in cv. A320, and –0.67 ± 0.09 MPa in cv. Carioca, which characterizes a moderate water deficit in common beans plants [14, 15, 19]. Measurements ($n = 3$) of predawn leaf water potential were performed with a pressure chamber (Soilmoisture, United States) in the fourth trifoliate leaf, a mature but nonsenescent leaf.

After 10 days of water deficit, plants were rehydrated and later maintained under this condition. Recovery of initial water status was observed in all cultivars after 2 days of rehydration, with predawn leaf water potential returning to –0.27 ± 0.02 in cv. A222, –0.27 ± 0.01 in cv. A320, and –0.28 ± 0.02 MPa in cv. Carioca. Therefore, two plant groups were defined: control, the plants well-irrigated during the entire plant cycle, and PWD, the plants experienced a moderate water deficit during the preflowering stage.

Heat treatment of leaf discs. Immediately after excision from leaves (similar to those used for water potential measurements), leaf discs (10 cm^2) were enclosed into an LD2/3 leaf chamber (Hansatech, United Kingdom) with a wet felt disc to maintain a constant leaf water status during chlorophyll fluorescence recording.

Leaf disc temperature was controlled by using an MA-127 water bath (Marconi, Brazil) and monitoring with an AWG 24 copper–constantan thermocouple (Omega Eng., United States) attached to the abaxial surface of the leaf disc. The temperature in the water bath was set to maintain leaf disc temperature at 25, 35, and 45°C. Different leaf discs were taken for each temperature treatment to avoid any short-term acclimation to increasing temperature.

Prior to heat treatment, bean plants were maintained under low light conditions [< 10 μmol/(m^2 s)] to prevent leaf photoinhibition. Then, leaf discs were excised and exposed to each temperature (25, 35, and 45°C) for 5 min in the dark. Heat treatment in darkness permitted to avoid light-induced PSII destabilization [7]. After 5 min at each temperature, chlorophyll fluorescence of both control and PWD samples was initially measured in the dark and then evaluated during photosynthesis

induction at a photosynthetic photon flux density of 200 μmol/(m^2 s). This irradiance was provided with an external light source (model LS3, Hansatech, United Kingdom).

Chlorophyll fluorescence measurements. The responses of chlorophyll fluorescence to increasing temperature were evaluated seven days after the complete recovery of plant water status, when plants were 53-day-old. Chlorophyll fluorescence was measured with a modulated FMS1 fluorometer (Hansatech, United Kingdom) attached to the leaf chamber used in heat treatment. Maximum (F_M) and minimum (F_0) chlorophyll fluorescence yield were measured in dark-adapted leaves, whereas steady-state (F_S) and maximum (F'_M) fluorescence yield were sampled in a light-adapted state [20]. Then, variable fluorescence yield was assessed in both dark ($F_V = F_M - F_0$) and light ($\Delta F = F'_M - F_S$) states. The maximum fluorescence yields in dark- and light-adapted leaves were attained during a 2.5-s saturation pulse (18 mmol/(m^2 s)).

The following chlorophyll fluorescence parameters were calculated: potential (F_V/F_M) and effective ($\Delta F/F'_M$) quantum efficiency of PSII, nonphotochemical fluorescence quenching (NPQ = $(F_M - F'_M)/F'_M$), and apparent electron transport rate (ETR = $\Delta F/F'_M \times Q \times 0.84 \times 0.5$), where Q is the photosynthetic photon flux density (200 μmol/(m^2 s)), 0.84 is the fraction of absorbed light, and 0.5 is the fraction of excitation energy distributed to PSII [21, 22]. In addition, stability and tolerance of the photochemical apparatus were evaluated by relative changes in F_0 and F_V measured at 25 and 45°C, i.e., stability of the light-harvesting complex LHCII at high temperature was evaluated by $F_0(25°C)/F_0(45°C)$ and the tolerance of the photochemical activity and O_2-evolving system was characterized by the changes in $F_V(25°C)/F_V(45°C)$ [8].

In control and PWD samples subjected to 25, 35,or 45°C, F_S, F'_M, and related variables $\Delta F/F'_M$, ETR, and NPQ were evaluated during photosynthesis induction at the intervals of 20 s during 6 min at a light intensity of 200 μmol/(m^2 s). Chlorophyll fluorescence was measured at ambient CO_2 partial pressure (~38 Pa).

Data analysis. The experiment was arranged in random block design, with two factors; namely previous condition (control or PWD plants) and leaf temperature (25, 35 or 45°C). Data were treated according to ANOVA procedure and mean values ($n = 3$) were compared by the Tukey test ($P < 0.05$ and $P < 0.01$) when appropriate. The data are represented by the means and their standard errors.

RESULTS AND DISCUSSION

Chlorophyll Fluorescence in Dark-Adapted Leaf Discs

A previous water deficit significantly affected the response of chlorophyll fluorescence to increasing temperature ($P < 0.01$), the effect being genotype-dependent (Fig. 1). Leaf discs showed increases in the minimum chlorophyll fluorescence yield (F_0) as a consequence of increasing temperature, with the highest F_0 values observed at 45°C (Figs. 1a–1c). When comparing the temperature shift from 35 to 45°C in control tissues, F_0 values increased more than by 4.2 times in A222 and Carioca genotypes and almost by 3.4 times in A320 (Figs. 1a–1c). Leaf discs of A222 and Carioca genotypes exposed to PWD exhibited F_0 3.2–3.3-fold rise between 35 and 45°C (Figs. 1a, 1b), while this increase was by 4.6 times in A320 (Fig. 1c).

Nevertheless, bean genotypes showed different patterns of the temperature response of F_0 especially after PWD treatment. An important genotypic difference was that control A222 samples exhibited a higher F_0 increase as compared to PWD plants ($P < 0.01$), which was not observed in Carioca and A320 genotypes (Figs. 1a–1c). In addition, these genotypic differences were noticed only when leaf tissues were subjected to 45°C (Figs. 1a–1c).

A sharp increase in F_0 reflects the development of heat injury in thylakoid membranes, which is accompanied by a reduction in the quantum yield of photosynthesis [13]. An increase in F_0 indicates both a partial dissociation of LHCII from PSII and a greater excitation energy loss during its transfer to reaction centers [8, 23]. In fact, the enhancement of F_0 emission was associated with the accumulation of Q_A^- and more active back electron transfer from Q_B to Q_A at high temperature [6, 24].

Increases in F_0 due to heat stress were also reported in the bean genotypes Carioca, Negro Huasteco, and Epace-10 [10, 11]. The Carioca genotype showed the highest increase in F_0 due to heat stress, revealing its higher sensitivity to this environmental constraint as compared to Negro Huatesco and Epace-10 [11]. Therefore, the bean genotypes exhibit different sensitivities to heat stress [10, 11, 25], the difference being affected by previous environmental conditions (Fig. 1).

The lower sensitivity to heat damage in the A222 genotype exposed to PWD was probably induced by more active interaction between PSII proteins and thylakoid membrane lipids due to the water deficit [16]. This hypothesis is reasonable since heat stress tends to increase the fluidity of thylakoid membrane lipids and destabilize the protein–lipid interaction, affecting both the organization and function of PSII [3].

The maximum fluorescence yield (F_M) was also affected by high temperature, showing significant decreases ($P < 0.05$) only in cvs. Carioca and A320 at 45°C (Figs. 1d–1f). Temperature and PWD did not affect ($P > 0.05$) F_M in cv. A222. In the Carioca and A320 genotypes, insignificant changes were observed between 25 and 35°C and between control and PWD plants (Figs. 1d–1f). However, a decreased F_M (32%) was noticed when the temperature elevated from 35 to 45°C in control Carioca and A320 leaf discs. Such

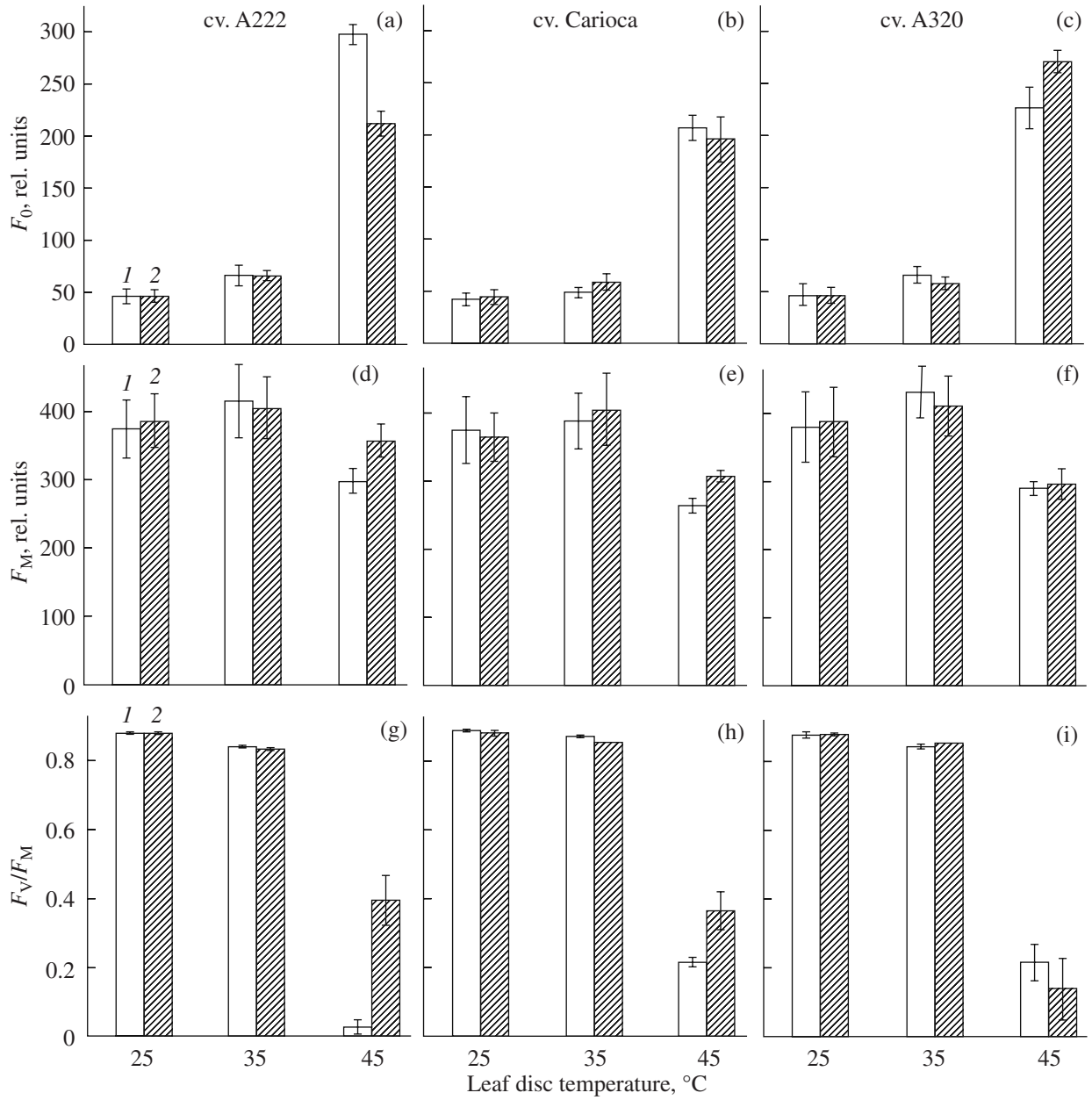

Fig. 1. Minimum (F_0 in a–c), maximum (F_M in d–f) and variable to maximum (F_V/F_M in g–i) chlorophyll fluorescence yield measured in leaf discs of common bean cultivars (A222 in a, d, g; Carioca in b, e, h; and A320 in c, f, i) at 25, 35, and 45°C.
(*1*) Potted plants were well-hydrated (control plants) or (*2*) exposed to PWD under greenhouse conditions. Leaf discs were dark-adapted prior to temperature treatment (5 min at each temperature in the dark).

decrease was less accentuated (~24–28%) in PWD plants (Figs. 1e, 1f). In fact, F_0 and F_M were suggested as potential indicators of heat-tolerance and damage in common bean plants [10, 11, 25], the tolerant varieties of which maintain these variables relatively more stable at high temperature than the sensitive ones.

A reduction in F_M was correlated with the inhibition of photosynthetic activity due to heat stress, mainly to heat damage of the PSII complex [4, 26]. Sharp decreases in F_M at high temperature suggest that this variable is an indicator of heat tolerance in the bean genotypes Carioca, Negro Huatesco, and Epace-10 [11]. However, this was not true for cv. A222, which maintained F_M values relatively constant ($P > 0.05$) between 25 and 45°C (Fig. 1e) and even showed a significant F_V/F_M decrease in control plants at 45°C (Fig. 1g).

Temperature-dependent changes in F_0 and F_M determined distinct patterns of potential quantum efficiency of PSII (F_V/F_M). In fact, there was a decreasing trend in F_V/F_M when the temperature increased from 25 to 45°C (Figs. 1g–1i), but as F_0 showed (Figs. 1a–1c), these changes occurred between 35 and 45°C ($P < 0.01$).

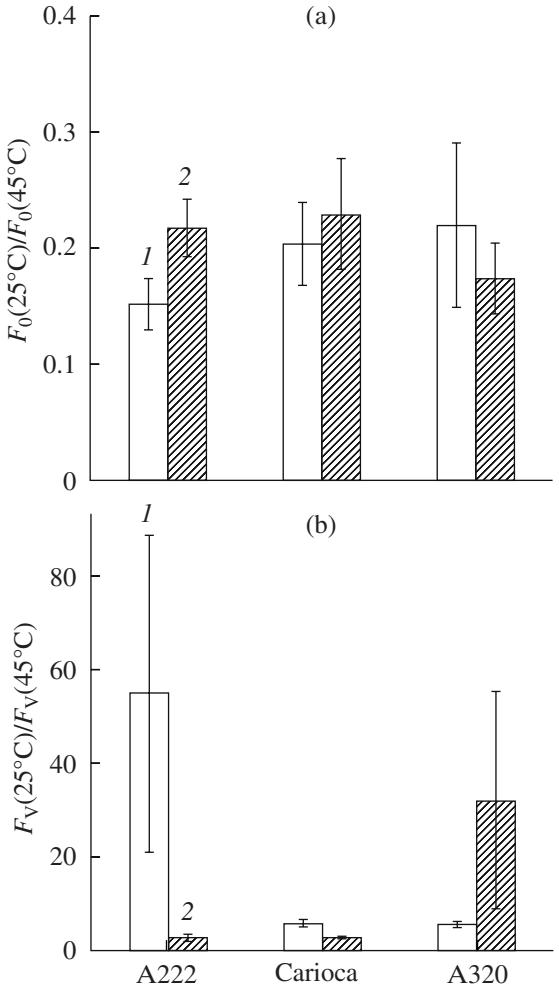

Fig. 2. Ratio between chlorophyll fluorescence yields (minimum, F_0; and variable, F_V) measured in leaf discs of common bean cultivars at 25 and 45°C.

Potted plants were (*1*) well-hydrated (control plants) or (*2*) exposed to PWD under greenhouse conditions. Leaf discs were dark-adapted prior to temperature treatment (5 min at each temperature in the dark).

A PWD treatment had a significant effect on the A222 and Carioca genotypes: PWD leaves showed higher F_V/F_M ratios than control ones ($P < 0.05$) at 45°C (Figs. 1g–1h). At this temperature, A222 leaves exposed to PWD exhibited F_V/F_M values 16 times higher than control plants ($P < 0.01$) while it was about 1.7 times in Carioca leaves. Insignificant difference in the temperature response of F_V/F_M was found between control and PWD plants of the A320 genotype. In control plants of Carioca and A320 genotypes, the F_V/F_M ratio was less affected by high temperature than in A222 plants (Figs. 1g–1i). On the other hand, when considering only PWD plants, A222 and Carioca genotypes showed the highest F_V/F_M values at 45°C. Therefore, reasonable photochemical activity was maintained at 45°C when cvs. A222 and Carioca were subjected to a PWD treatment.

Evaluation of temperature-dependent changes in minimum (F_0) and variable (F_V) fluorescence yield is an approach to assess the photochemical apparatus under heat stress [8, 27]. The ratio between F_0 measured at 25 and 45°C revealed insignificant differences among bean genotypes (Fig. 2a), suggesting a similar thermal stability of the LHCII [8]. However, dissimilar response to heat stress was observed in bean genotypes when relative changes in F_V at 25 and 45°C were evaluated (Fig. 2b). In addition, PWD treatment exerted a significant effect depending on bean genotype. The ratio $F_V(25°C)/F_V(45°C)$ gives information about the thermal tolerance of photochemistry and the O_2-evolving complex in PSII [8].

A222 control plants showed the highest $F_V(25°C)/F_V(45°C)$ ratio, which indicates reduced thermal tolerance of photochemical activity when compared to the other genotypes (Fig. 2b). The Carioca and A320 genotypes manifested a higher tolerance to heat stress when they did not experience a previous water deficit. Nevertheless, the A222 genotype responded significantly to PWD, showing a great increase in thermal tolerance as follows from reduced $F_V(25°C)/F_V(45°C)$. This phenomenon was also noticed in the Carioca genotype but was less intense as compared to A222 (Fig. 2b). In fact, the average reductions in the $F_V(25°C)/F_V(45°C)$ due to PWD were about 95 and 50% in A222 and Carioca, respectively. On the other hand, A320 plants subjected to PWD showed a 5.7-fold increase in this index when compared to control plants (Fig. 2b), thus indicating a reduction in thermal tolerance due to PWD. However, it is important to note that A320 leaves showed the highest water potential after ten days of water deficit (see "Previous Moderate Water Deficit" in the "Materials and Methods" section). This finding is probably related to the stomatal control of water loss and reduced leaf area in cv. A320, which maintains shoot hydration and low plant transpiration under water deficit conditions [17].

Relative changes in F_V due to elevated temperature are in accordance with F_V/F_M data, indicating that, in Carioca and A320 genotypes, the photochemical apparatus was more tolerant to heat stress and showed less sensitivity to thermoinhibition than A222 under control conditions, i.e., they were well-hydrated during the entire plant cycle (Figs. 1, 2). However, the most responsive to PWD bean genotype was cv. A222; it exhibited a significant increase in thermal tolerance and potential quantum efficiency of PSII at high temperature (Figs. 1, 2).

A further step is the evaluation of bean genotype capacities in the light, i.e., under conditions of constant energy supply to photosystems. Will photochemical differences observed in the dark-adapted state appear in light-adapted tissues?

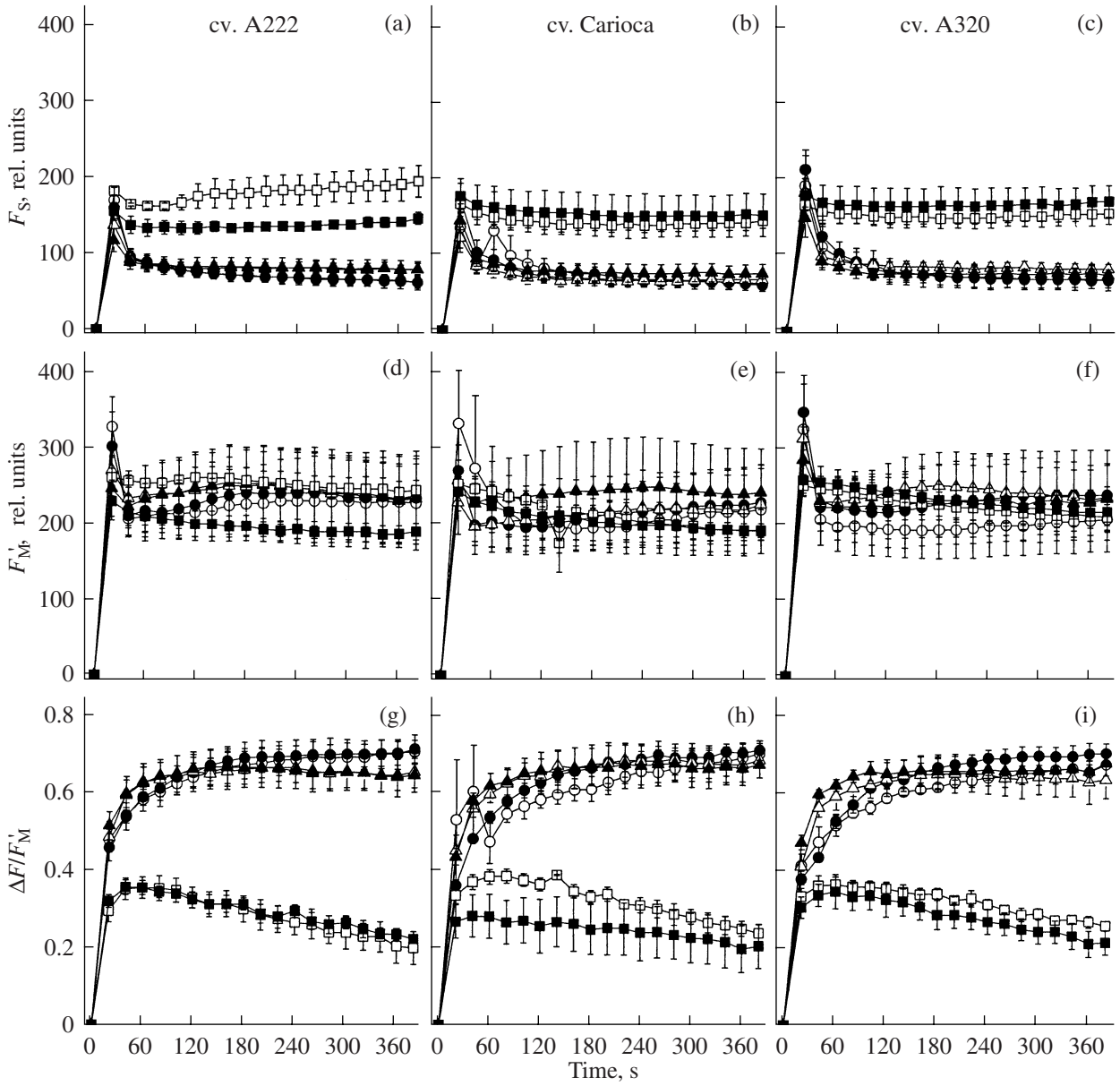

Fig. 3. Time-course of steady-state (F_S in a–c) and maximum (F'_m in d–f) and variable to maximum ($\Delta F/F'_m$ in g–i) chlorophyll fluorescence yield during photosynthesis induction in common bean cultivars (A222 in a, d, g; Carioca in b, e, h; and A320 in c, f, i) at 25°C (circles), 35°C (triangles), and 45°C (squares)

Potted plants were well hydrated (control plants, open symbols) or exposed to PWD (closed symbols) under greenhouse conditions. Leaf discs were illuminated during 6 min at photosynthetic photon flux density of 200 μmol/(m^2 s) after heating for 5 min in darkness. Measurements were taken at natural CO_2 concentration.

Chlorophyll Fluorescence during Photosynthesis Induction

The steady-state chlorophyll fluorescence yield (F_S) was affected by heat stress. In all bean genotypes, the highest F_S values were recorded at 45°C ($P < 0.01$) after 60 s of induction by low light intensity (Figs. 3a–3c). No significant changes in F_S dynamics during photosynthesis induction at 25 or 35°C were observed in the genotypes studied. Differences caused by PWD were noticed only at 45°C in the A222 plants (Fig. 3a); control leaves showed a higher F_S than PWD-treated leaves ($P < 0.01$). Maximum chlorophyll fluorescence yield in the light (F'_M) was not influenced by PWD ($P > 0.05$), and heat did not affect ($P > 0.05$) F'_M during photosynthesis induction (Figs. 3d–3f).

From F_S and F'_M changes (Figs. 3a–3f), we may conclude that the effective quantum efficiency of PSII

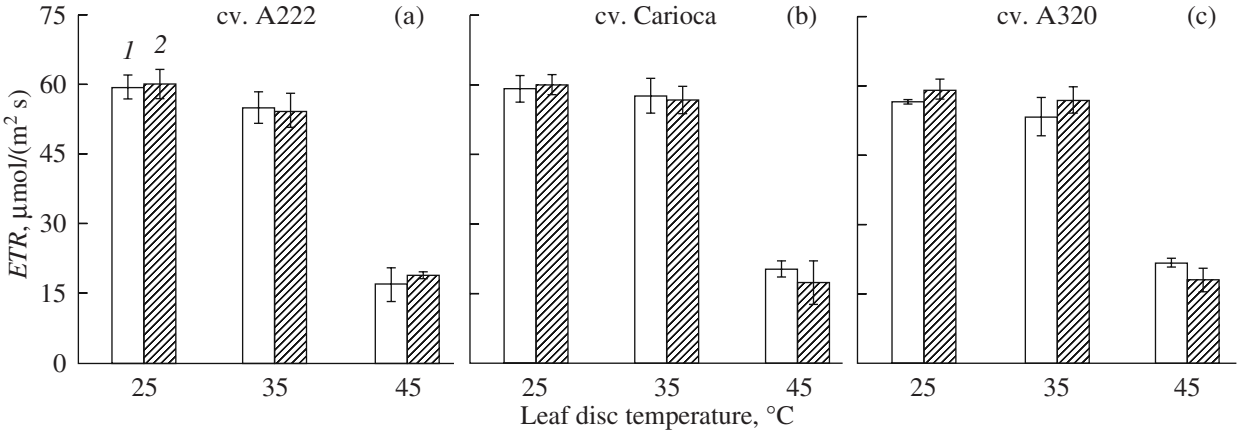

Fig. 4. Apparent electron transport rate (*ETR*) after photosynthesis induction in leaf discs of common bean cultivars. (a) cv. A222; (b) cv. Carioca; and (c) cv. A320. Experiment design like in Fig. 1.

($\Delta F/F'_M$) was affected only by F_S (Figs. 3g–3i). Regardless of bean genotype, leaves submitted to 45°C showed (i) lower $\Delta F/F'_M$ values ($P < 0.01$) than those at 25 or 35°C and (ii) a significant reduction in $\Delta F/F'_M$ ($P < 0.01$) during photosynthesis induction (Figs. 3g–3i). In general, the previous water deficit did not affect $\Delta F/F'_M$ during induction, and a peak in $\Delta F/F'_M$ was noticed after 180-s induction at 25 and 35°C, whereas $\Delta F/F'_M$ at 45°C showed some decrease from the beginning of illumination (Figs. 3g–3i). When compared to measurements taken at 25 and 35°C, $\Delta F/F'_M$ decreased by almost 70% after 360-s induction at 45°C.

As a consequence of changes in quantum efficiency, the apparent electron transport rate (ETR) was affected by heat stress (Fig. 4). All bean genotypes showed a significant reduction ($P < 0.01$) of ETR at 45°C, suggesting that the photochemical reactions were impaired by high temperature. At 45°C, ETR varied from 16.9 to 21.7 μmol/(m² s), indicating a 70% reduction when compared to the measurements taken at 25 or 35°C (Fig. 4). Again, PWD treatment did not cause any changes in ETR response to high temperature ($P > 0.05$). In C_3 plants, electrons from PSII are driven to both carboxylation (photosynthesis) and oxygenation (photorespiration) activities [22, 26]. Therefore, it is expected that between 35 and 45°C most ETR was allocated to photorespiration, a physiological process protecting the photochemical apparatus from excessive energy at high temperatures [22, 26].

As ETR may be considered an overall measure of photochemical activity between PSII and PSI [22], our data indicate that a previous water deficit did not increase the photochemical activity at high temperature. However, an important consequence of PWD may be found in defense mechanisms related to heat dissipation of excessive light energy at the PSII level. The nonphotochemical quenching of fluorescence (NPQ), a well-known dissipation route, can favor some insights into this hypothesis. NPQ represents any fluorescence decline that is not related to Q_A reoxidation [28, 29].

After the beginning of illumination, a sudden NPQ rise in all genotypes subjected to 25 or 35°C was observed (Fig. 5). In fact, an initial increase in NPQ was expected due to light-induced proton gradient [15, 28, 29]. Between 25 and 35°C, significant difference was noticed only in the Carioca genotype, which showed higher NPQ values at 35°C after 20 s of illumination (Fig. 5b). After 60 s, all control and PWD genotypes had NPQ varying between 0.7 and 0.9 in leaves exposed to both 25 and 35°C (Fig. 5). However, distinct patterns were noticed when the genotypes were compared at 45°C, the temperature at which the lowest NPQ values were observed ($P < 0.05$). Similar patterns of NPQ during photosynthesis induction at high temperature were also noticed in sunflower and maize plants [8]. Since energization of thylakoid membranes is related to NPQ and involved in ATP production [4, 21, 27], we suggest that the Calvin cycle operation was limited at 45°C (Fig. 5).

Fluorescence quenching by nonphotochemical processes is expected under heat stress and may be shown by a reduction in F_M (Figs. 1e, 1f) at a damaging temperature [28]. In this situation, a disconnection between chlorophyll molecules within antennae and/or between antennae and the reaction center can occur, which lowers F_M and rises F_0 by changing fluorescence lifetime and increasing the probability of an exciton to miss a functional reaction center [4, 23, 28, 29].

A PWD did not induce ($P > 0.05$) any changes in NPQ of the A320 leaves at 45°C (Fig. 5c), the NPQ values varied from 0.15 to 0.30 during photosynthesis induction. The absence of response is probably related to a higher plant hydration under maximum water deficit, when compared to other genotypes: the average values of leaf water potential were around –0.35 MPa in

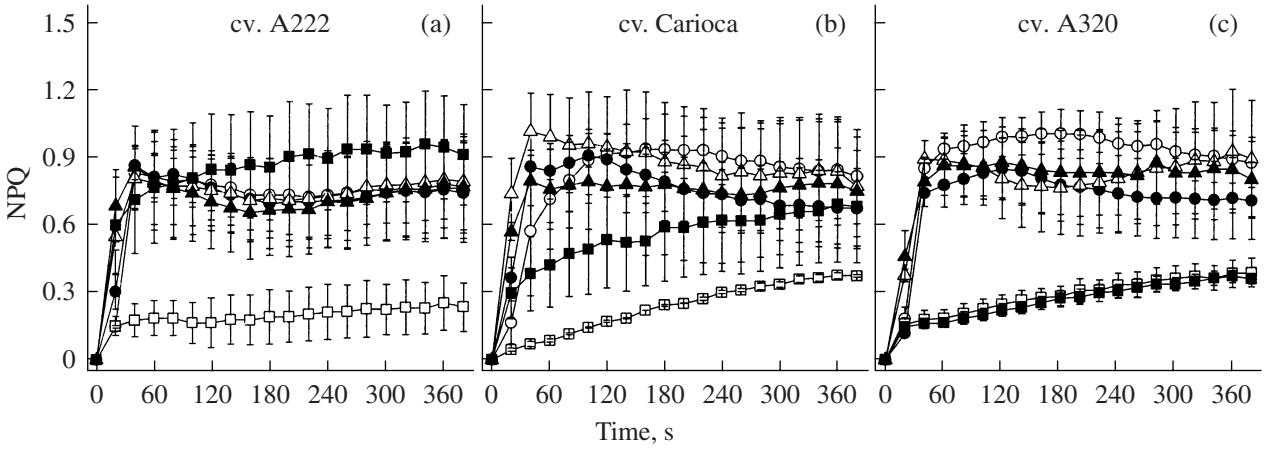

Fig. 5. Time course of nonphotochemical fluorescence quenching (NPQ) during photosynthesis induction of common bean cultivars (A222 in a; Carioca in b; and A320 in c) at 25°C (circles), 35°C (triangles), and 45°C (squares). Potted plants were well hydrated (control plants, open symbols) or exposed to PWD (closed symbols) under greenhouse conditions. Experiment design like in Fig. 3.

cv. A320, –0.50 MPa in cv. A222, and –0.67 MPa in cv. Carioca. On the other hand, a PWD improved NPQ in the A222 genotype at 45°C (Fig. 5a), that resulted in values similar to those observed at 25 and 35°C ($P < 0.01$). At the end of induction, A222 plants exposed to PWD had NPQ 3.9 times higher than control plants (Fig. 5a). Finally, the Carioca genotype showed an intermediate pattern of response to PWD and temperature when compared to cvs. A222 and A320. In relation to control plants, PWD treatment slightly increased the NPQ in Carioca plants at 45°C (Fig. 5b); however, this increase was insufficient to be statistically significant between control and PWD plants ($P > 0.05$).

Regarding excessive energy dissipation, NPQ is an important regulatory mechanism, controlling the quantum efficiency of PSII evaluated by $\Delta F/F'_M$ (Fig. 3h) and increasing leaf tolerance to photoinhibition suggested by high F_V/F_M (Figs. 1g, 1h) [8, 21, 28, 29]. Besides photoinhibitory quenching, NPQ is composed of the energy-dependent (ΔpH in thylakoid membranes) and state-transition (phosphorylation of LHCII) quenching coefficients [28, 29]. Transmembrane ΔpH may quench up to 90% of maximum variable fluorescence, being the principal fraction of NPQ and reflecting down-regulation of PSII under excessive light energy [28, 29]. In addition, a state-transition component is induced under heat treatment due to physical changes in thylakoid membranes, causing detachment of the LHCII from PSII complex [28].

Since A222 plants subjected to PWD treatment showed an increase in NPQ under high temperature stress, we may argue that a previous stressful event like water shortage enhanced this energy dissipative pathway (Fig. 5). Probably, PWD increased NPQ capacity at 45°C through changes in energy-dependent and state-transition quenching, which is related to structural modification of thylakoid membranes in the A222 genotype subjected to PWD treatment. This hypothesis should be tested in future work. In fact, NPQ is considered a protective and adaptive mechanism to cope with excessive light energy [28, 29], which commonly occurs at noon in tropical climates or is induced by photosynthetic impairment caused by environmental stresses.

To conclude, common bean genotypes have significant differences in photochemical sensitivity to heat stress and also in the ability to modify their photochemical apparatus due to a previous environmental constraint. Photochemical sensitivity of the common bean leaves to heat stress is altered by a previous transient water deficit; however, this adaptive change is genotype-dependent. Greater thermal tolerance was associated with an increase in the potential quantum efficiency of PSII (F_V/F_M) at high temperature. Under such conditions, the genotype responsive to the PWD treatment enhanced its protective capacity against excessive light energy by increasing nonphotochemical quenching.

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