

Can the critical temperature for photochemical damage in common bean plants be changed after a drought event?

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Abstract

Low water availability and high temperatures occur under field conditions and we hypothesize that the critical temperature for photochemical damage (T_c) in common bean (*Phaseolus vulgaris* L.) plants is increased by the occurrence of previous water deficit in a genotype-dependent manner. Five common bean cultivars A320, A222, Carioca, BAT477 and Ouro Negro were evaluated. Thirty days after seedlings emergence, one group of plants was exposed to water deficit for ten days and rehydrated and another one was maintained well hydrated during the experimental period. The minimum chlorophyll fluorescence (F_o) was monitored in leaf discs exposed to temperatures ranging from 25 to 45 °C and the T_c values estimated. The previous water deficit did not affect T_c , which varied between 38.8 and 43.8 °C when considering all cultivars and water regimes. Under well-watered conditions, BAT477 (41.9 °C) and Carioca (43.8 °C) presented higher T_c than Ouro Negro (38.8 °C). Our findings indicate a significant genotypic variation in thermal tolerance in *Phaseolus vulgaris*, an important crop trait to be considered in breeding programs.

Key words: chlorophyll fluorescence, hardening, heat stress, *Phaseolus vulgaris*, tolerance.

Low water availability and high temperatures are common stressful conditions under field conditions, causing significant reduction in photosynthesis and crop yield (Chaves et al., 2002; Prasad et al., 2011). The primary photochemistry is among the photosynthetic reactions affected by low water availability and extreme temperatures (Havaux, 1993; Yordanov et al., 1997). Under high temperature, photochemistry is affected through changes in physicochemical properties and functional organization of thylakoid membranes, with the photosystem II (PSII) being likely damaged (Havaux, 1992). The electron transport rate through PSII, an overall index of photochemical activity, is severely reduced when common bean leaves are exposed to 45 °C (Ribeiro et al., 2008). However, some authors have reported the resistance of PSII activity under water deficit (Santos et al., 2009; Schreiber & Bilger, 1987).

Both drought and high temperature may occur simultaneously or alone in nature, with common bean cultivars showing different acclimation to a changing environment (Ribeiro et al., 2004). In general, one may argue about an additive effect of drought and high temperature on plant physiology, increasing the limitation imposed to the photosynthesis (Grigorova et al., 2011; Prasad et al., 2011; Silva et al., 2010; Yordanov et al., 1997). However, the occurrence of a previous stress may improve plant performance during a next stressful event (Havaux, 1992, 1993). The capacity for improving photochemical performance during a second stressful event is genotype-dependent in *Phaseolus vulgaris* (Ribeiro et al., 2008) and it has been recognized in other plant species as well (Seemann et al., 1986). In fact, the

thermal stability of PSII was increased in leaves of tomato exposed to water deficit previously (Havaux, 1992).

As acclimatory responses to high temperature, membrane composition and then its properties may adjust to different growth temperature regimes and affect the thermal sensitivity of PSII (Schreiber & Berry, 1977; Smillie & Nott, 1979). One important and still current open question is how plants acquire tolerance to environmental stresses under natural conditions. In this paper we tested the hypothesis that the critical temperature for photochemical damage in common bean is increased by previous water deficit in a genotype-dependent manner. This would explain the lesser sensitivity of the potential quantum efficiency of PSII to high temperature reported previously in common bean plants subjected to water deficit (Ribeiro et al., 2008).

Five common bean (*Phaseolus vulgaris* L.) genotypes were evaluated: A320, A222, Carioca, BAT477 and Ouro Negro. A320 and A222 are tolerant to anthracnose and maintain high leaf water potential under water deficit (Pimentel et al., 1999a), whereas Carioca is widely cultivated in Brazil (Vicente et al., 2000). BAT477 is a drought tolerant genotype (Frahm et al., 2004; Pimentel et al., 1999b) and Ouro Negro is a black bean genotype commonly grown in Brazil (Pimentel et al., 1999b). Seeds were sown in plastic pots (10 L), using soilless mixture (Plantmax, Eucatex Inc., Brazil) fertilized with 0.18 g N, 0.15 g K₂O, 0.32 g P₂O₅, 3 g of dolomitic lime and with micronutrients in nutrient solution [210 μM CuSO₄·5H₂O; 100 μM ZnSO₄·7H₂O; 16 μM H₃BO₃; 240 μM FeSO₄·7H₂O; and 1 μM (NH₄)₆Mo₇O₂₄·4H₂O]. After 25-days of seedling emergence, an additional fertilization was done with 0.18 g N per pot. The plants were grown under greenhouse, where air temperature varied from 18 (night) to 42 °C (day), the air relative humidity reached 30% during the day and 100% at night and maximum photosynthetic photon flux density was 1800 μmol m⁻² s⁻¹ under a photoperiod of 13 h. The pots were irrigated daily until water deficit treatment.

Thirty days after seedlings emergence, one group of plants was exposed to water deficit for 10 days. There were significant differences between genotypes ($p < 0.05$) when considering the pre-dawn leaf water potential (Ψ_1) reached after 10 days of water withholding. As previously reported (Santos et al., 2009), A320 presented a reduction in Ψ_1 of 57% under water deficit, showing the highest Ψ_1 in such limiting conditions (-0.35 ± 0.01 MPa). A222 also presented a reduction in Ψ_1 and reached -0.50 ± 0.01 MPa under water deficit. The other three cultivars exhibited Ψ_1 values varying between -0.23 ± 0.01 MPa (Ouro Negro) and -0.32 ± 0.01 MPa (Carioca) under well-watered conditions and between -0.67 ± 0.03 MPa (Carioca) and -0.77 ± 0.01 MPa (Ouro Negro) under water deficit. According to the minimum Ψ_1 values cited above, common bean cultivars were subjected to a mild water deficit (Santos et al., 2009). Then, the plants were re-hydrated, presenting full recovery of Ψ_1 ,

and maintained in this condition. At this time, two plant groups were formed according to the previous conditions: well hydrated (reference, Ref) and water-stressed (PWD).

Leaf discs (10 cm²) were detached from plants of both groups and immediately enclosed into a LD2/3 leaf chamber (Hansatech, UK) with a wet felt disc to maintain the leaf water status. The leaf temperature (T) was controlled with a water-bath (MA-127 Marconi, Brazil) and monitored with an AWG 24 thermocouple attached to the abaxial leaf disc surface. Heating started when leaf disc temperature was around 25 °C and reached 45 °C, with a linear heating rate of 1.3 °C min⁻¹ under dark conditions. The accuracy for estimating the critical temperature for photochemical damage (T_C) was ± 0.1 °C. During the heating, the minimum chlorophyll fluorescence signal (F_0) was recorded in intervals of 1 s with a modulated fluorometer FMS1 (Hansatech, UK), under far-red background illumination to reoxidize the plastoquinone pool and avoid its reduction under high temperature (Schreiber & Bilger, 1987). F_0 was measured under steady-state conditions and F_0 vs. T curves were analyzed (Smillie & Nott, 1979). T_C was defined as the interception of linear regressions fitted to the fluorescence data collected before and after the curve inflection, as shown in figure 1.

As data did not present normal distribution, the T_C values of common bean genotypes in both water regimes were compared by the non-parametric Friedman test ($\alpha = 0.1$).

The F_0 vs. T curves reveal some interesting aspects of photochemistry in plants, with F_0 showing a large increase after a temperature threshold (Figure 1), herein defined as the critical temperature for photochemical damage. Such response reflects the separation of LHCII from PSII core and the inactivation of the PSII reaction centers

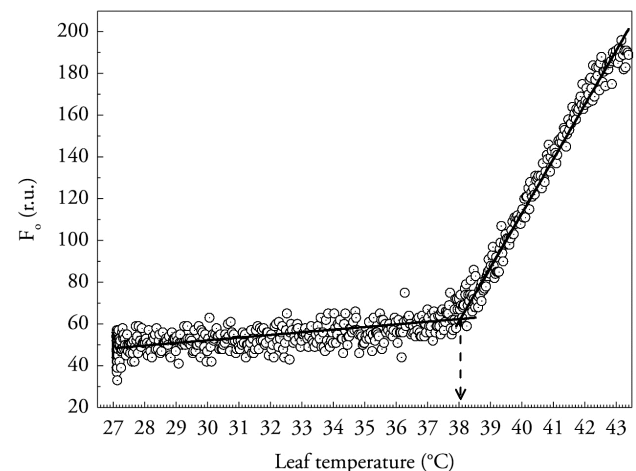


Figure 1. An example of F_0 vs. T curve in common bean cv. A222. Measurements were taken in intervals of 1 s, with an increasing temperature rate of 1.3 °C min⁻¹ in darkness. The critical temperature for photochemical damage is defined as the interception of regression lines fitted to fluorescence data, as shown.

(Yamane et al., 1997). High leaf temperature can change stability of PSII complexes and then affect the excitation transfer as a probable disconnection between the chlorophyll molecules within antenna and the reaction center occurs (Krause & Weis, 1984). According to Kouril et al. (2004), light-induced reduction of Q_A and the enhancement of back electron flow from Q_B to Q_A are the causes of increasing F_O when the temperature of barley leaves is between 40 and 50 °C. In general, F_O is related to the structural and functional conditions of electron transport chain in thylakoids, with changes in this fluorescence signal due to high temperature giving us information about the structural stability and fluidity of chloroplastial membranes (Kouril et al., 2004; Schreiber & Bilger, 1987).

We have found a significant variation in T_C among evaluated common bean cultivars; however, T_C was not affected by previous water deficit (Figure 2). BAT477 and Carioca cultivars presented higher T_C than Ouro Negro when growing under well-watered conditions. Such differences ranged from 3.1 to 5.0 °C when comparing Ouro Negro to BAT477 and Carioca, respectively. There was no significant difference among cultivars when they were previously subjected to water deficit (Figure 2), with T_C values varying between 39.5 and 41.1 °C. Although BAT477 and Carioca have presented reduction in absolute T_C value due to previous water deficit, such differences did not have statistical significance.

Our results indicate an interesting genotypic variation in thermal tolerance of common bean (*Phaseolus vulgaris*), as reported in dipterocarp species (Kitao et al., 2000). As T_C indicates the temperature in which the quantum yield of CO₂ fixation is damaged (Schreiber & Berry, 1977; Schreiber & Bilger, 1987), our data suggest differential sensitivity of

common bean photosynthesis to high temperature. In fact, Pastenes & Horton (1996) and Pimentel et al. (2013) have reported the differential photosynthetic response of bean cultivars to high temperature. Costa et al. (2003) have also found significant variation in common bean sensitivity to heat stress, with a large reduction in the apparent electron transport rate through PSII between 40 and 45 °C. These results are in accordance to the T_C values reported herein, which varied between 38.8 and 43.8 °C when all cultivars and conditions are taking into account (Figure 2). On the other hand, the T_C values found in common beans (Figure 2) are lower than the average T_C reported for tropical species by Smillie & Nott (1979), i.e., 46.0±0.6 °C. Such difference may be explained by the tropical species studied by Smillie & Nott (1979), which present differential leaf structure and metabolism and have plant cycle varying from perennial to annual.

The high thermal tolerance found in BAT477 and Carioca under well-watered conditions may be related to the presence of heat shock proteins (HSP) in chloroplasts, preventing the unfolding or misfolding of enzymes and structural components (Grigorova et al., 2011; Heckathorn et al., 1998). Besides this strategy, a stable conformational structure of thylakoid membranes due to a high proportion of unsaturated fatty acids as well as a close interaction between membrane components may be other ways to improve thermal tolerance (Chapman et al., 1983; Havaux, 1992; Raison et al., 1982). The biological bases of the tolerance to high temperature found in BAT477 and Carioca as well as the thermal sensitivity of Ouro Negro remain unsolved and should be further studied.

Theoretically, the occurrence of previous water deficit would improve the tolerance to high temperature by increasing the stability of PSII under heat stress (Havaux, 1992, 1993; Seemann et al., 1986). As the water deficit imposed was able to affect negatively leaf gas exchange and photochemistry of common bean cultivars (Santos et al., 2009), we may argue that the intensity and duration of water deficit would be enough to promote physiological changes in common bean plants. However, our data did not reveal any significant change in T_C between water regimes (Figure 2). This finding does not confirm our initial hypothesis about the improvement of thermal tolerance in common bean plants due to previous drought.

The data presented in this paper indicate a significant genotypic variation in thermal tolerance in *Phaseolus vulgaris*, an important crop trait that can be recognized by evaluating the chlorophyll fluorescence signal under high temperature. The correlation between T_C and crop yield under warmer conditions should be further investigated before suggesting this index as a selection criterion for high temperature tolerance in breeding programs. In fact, a significant increase in air temperature is suggested in the climate scenarios predicted by the International Panel for

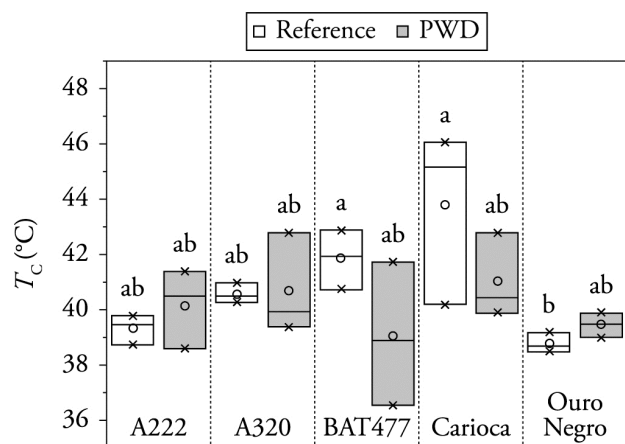


Figure 2. The critical temperature for photochemical damage in common bean genotypes grown under well-watered conditions (Reference) or subjected previously to water deficit (PWD). Different small letters mean statistical difference ($\alpha=0.1$) between genotypes by the Friedman test.

Climate Change (IPCC, 2014) and agriculture must be able to overcome such environmental changing without losses in crop yield. Among the agricultural strategies, the use of cultivars tolerant to high temperature is outstanding for promoting the agricultural sustainability and food security.

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