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Deficiency in phytochromobilin biosynthesis enhances heat-stress-induced impairments to the photosynthetic apparatus in tomato

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Abstract

Plants are continuously exposed to unfavorable environmental conditions, such as heat stress, which negatively affect plant growth and productivity. There is evidence that phytochromes are involved in plant response to different abiotic stresses. We investigated the possible phytochrome-dependent responses to heat stress in photomorphogenic tomato mutants *aurea* (*au*, phytochromobilin-deficient, PΦB) and *high-pigment 1* (*hpl*, hyperresponsive to phytochrome-mediated responses), as well as the wild-type Micro-Tom (MT). In comparison with MT, reductions in photosynthetic rate promoted by a high temperature were more pronounced in *au*, whereas less pronounced in *hpl*. All genotypes subjected to the heat stress exhibited adjustments in the capture and dissipation of energy, which were indicated by increases in the initial fluorescence and decreases in the maximum photochemical efficiency of photosystem II (PS II). The effective quantum yield of PS II and the apparent electron transport rate showed greatest alterations in the *au* mutant. In addition, heat-triggered anatomical changes occurred in all genotypes but were most conspicuous in the *au* mutant, followed by MT. Thus, phytochrome-dependent mechanisms played pivotal roles in the plant responses to the heat stress, and deficiency in phytochromobilin biosynthesis enhanced the heat-induced impairment of photosynthetic performance.

Additional key words: *aurea* mutant, chlorophyll *a* fluorescence, *high-pigment 1* mutant, phytochromes, thermotolerance, *Solanum lycopersicum*.

Introduction

In natural or agricultural systems, plants are often exposed to environmental stressors including low rainfall, oscillations of temperature, fluctuations in irradiance, high salinity, and excess of heavy metals, which are unfavorable to their development and growth (Rizhsky *et al.* 2002, Kreslavski *et al.* 2007, Soto *et al.* 2014). These

stressful conditions directly hinder a wide range of physiological processes and dramatically reduce crop yield (Doupis *et al.* 2013, Mutava *et al.* 2015). Considering the recent climate predictions regarding global warming, heat stress has been highlighted as a threat to agricultural production (Grover *et al.* 2013,

Submitted 11 February 2018, last revision 23 August 2018, accepted 18 September 2018.

Abbreviations: *au* - *aurea*; c_i/c_a - internal to external CO₂ concentration ratio; DDB1 - UV-damaged DNA-binding protein 1; E - transpiration rate; ETR - apparent electron transport rate; F_o - initial fluorescence; F_v/F_m - variable to maximum fluorescence ratio (maximum photochemical efficiency of photosystem II); F_v'/F_m' - effective quantum yield of photosystem II; g_s - stomatal conductance; *hpl* - *high-pigment 1*; MT - Micro-Tom; NPQ - non-photochemical quenching; P_N - net photosynthetic rate; PΦB - phytochromobilin; PS II - photosystem II; Rubisco - ribulose-1,5-bisphosphate carboxylase/oxygenase.

Acknowledgements: The authors would like to thank the financial support from the Coordenação de Aperfeiçoamento Pessoal de Nível Superior (CAPES), the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), the Financiadora de Estudos e Projetos (FINEP), the Fundação de Amparo à Pesquisa do Estado de Goiás (FAPEG), and the Instituto Federal Goiano, Campus Rio Verde. The authors are also grateful to Dr. Lázaro E. P. Peres (Escola Superior de Agricultura Luiz de Queiroz, ESALQ-USP) and Dr. Rogério F. Carvalho (Universidade Estadual Paulista Júlio de Mesquita Filho, UNESP) for providing the mutant tomato seeds.

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Mathur *et al.* 2014). Heat may cause oxidative stress from increased production of reactive oxygen species. Their excess can lead to protein denaturation, enzyme inactivation, and decreased photosynthesis (Ingraham *et al.* 1985, Kreslavski *et al.* 2007, Allakhverdiev *et al.* 2008, Lubovská *et al.* 2014, Sajid *et al.* 2018)).

Phytochromes are photoreceptors, which are composed of an apoprotein associated with a chromophore (phytochromobilin in higher plants). They are involved in the control of germination, plant elongation, flowering, shade tolerance (Possart *et al.* 2014), biosynthesis of enzymes in the photosynthetic apparatus (McCormac *et al.* 2001, Moon *et al.* 2008), and stomatal density (Boccalandro *et al.* 2009, Melo *et al.* 2011). These photoreceptors also play roles in cellular signaling plant defense against abiotic stresses, including high and low temperatures, water deficit, and salinity (Carvalho *et al.* 2011a, Alves *et al.* 2016). Phytochromes activate the expression of genes related to the biosynthesis of heat shock protein and control stomatal conductance, both of which may increase plant heat tolerance (Boccalandro *et al.* 2003, Zhou *et al.* 2014). Considering these functions, studying the role of phytochromes in plant responses at high temperatures is a promising strategy to improve the yield of agricultural crops exposed to harsh environmental conditions (Zhang *et al.* 2013).

Photomorphogenic mutants, such as the *aurea* (*au*) and *high-pigment 1* (*hpl*) tomato mutants, have been used

Material and methods

Plant growth and heat stress treatment: Tomato (*Solanum lycopersicum* L.) seeds from the MT wild-type cultivar and the *au* and *hpl* mutants were sown in trays containing *Bioplant*[®] substrate (Nova Ponte, Minas Gerais, Brazil). During the germination period, the seedlings were grown in a greenhouse under controlled conditions of temperature (24 - 27 °C), air humidity (60 - 80 %), photoperiod (13 h), and irradiance (550 ± 50 μmol m⁻² s⁻¹). After 17 d, the seedlings of a similar size and vigor were transplanted into 0.5-dm³ pots containing red dystroferic latosol (*Oxisol*) and sand at a 2:1 (v/v) ratio. A fertilizer mix was added to the substrate to supply crop requirements. Two weeks after transplanting, the plants were initially acclimated in a growth chamber (*BOD, LIMATEC LT 320TFP-I*, Cruz das Almas, Bahia, Brazil) with a mean temperature of 25 °C, a 75 % relative humidity, a 12-h photoperiod, and mean irradiance of 150 μmol m⁻² s⁻¹ for 5 days. These conditions reduced the possible occurrence of photo-oxidative damage induced by an excessive irradiance combined with heat stress, as proposed by Ferreira-Silva *et al.* (2011).

After the acclimation period, the plants were separated into two groups comprising four plants of each genotype. Group 01 was maintained under the same initial conditions in the growth chamber. Group 02 was maintained in another growth chamber and subjected to a

in studies of phytochrome-mediated physiological processes. The *au* mutant is deficient in phytochromobilin (PΦB) biosynthesis, which impairs the formation of functional phytochromes (Muramoto *et al.* 2005). Consequently, the *au* mutants present a distinct phenotype of elongated hypocotyls and yellow to light-green leaves due to a lower content of anthocyanins and chlorophylls (Terry and Kendrick 1996). The *hpl* presents a mutation in UV-damaged DNA-binding protein 1 (DDB1), one of the repressors of the phytochrome signaling pathway, which results in hyper-responsiveness to high irradiance. Measurably, the *hpl* mutants present dark-green leaves and fruits as well as a higher activity of light-dependent enzymes such as phenylalanine ammonia lyase and nitrate reductase (Goud *et al.* 1991, Goud and Sharma 1994, Peters *et al.* 1998, Liu *et al.* 2004).

These mutations were introgressed in a miniature tomato cultivar Micro-Tom (MT), which is used as a model in genetic and physiological studies due to its relatively short life cycle and the convenience of cultivation (Carvalho *et al.* 2011b). Elucidating the physiological effects related to phytochrome could lead to the development of plant cultivars or lineages with improved yield under stressful conditions. Therefore, the aim of this work was to assess the influence of phytochrome-dependent responses on the photosynthetic process of the photomorphogenic tomato mutants subjected to heat stress.

temperature of 42 ± 2 °C for 7 consecutive days by gradually increasing the temperature to 42 °C from 11:00 to 12:00 and decreasing to 25 °C from 17:00 to 19:00.

Leaf gas exchange, chlorophyll *a* fluorescence, and leaf water potential: Leaf gas exchange and chlorophyll *a* fluorescence were measured at 1, 3, 5, and 7 days after the heat treatment, and images of chlorophyll *a* fluorescence were obtained at the last evaluation time. The net photosynthetic rate [P_N, μmol(CO₂) m⁻² s⁻¹], transpiration rate [E, mmol(H₂O) m⁻² s⁻¹], stomatal conductance [g_s, mol(H₂O) m⁻² s⁻¹], and ratio of internal to external CO₂ (c_i/c_a) were determined in the fourth leaf from the base. Measurements were performed using a portable infrared gas analyzer (*LI-6400XTR, LI-COR*, Lincoln, Nebraska, USA) between 08:00 and 11:00 at an irradiance of 1 000 μmol m⁻² s⁻¹, a CO₂ concentration of 400 μmol mol⁻¹, a block temperature of 25 ± 1 °C, and a 75 % relative humidity.

The chlorophyll *a* fluorescence parameters were measured on the same leaves. For the assessments, leaves were dark-adapted to achieve complete opening the reaction centers (all electron carriers) with a minimum heat loss. Variable fluorescence (F_v) was a difference between maximum fluorescence (F_m) and initial fluorescence (F_o); maximum photochemical efficiency of

photosystem II (PS II) was calculated as F_v/F_m (Genty *et al.* 1989).

Variables from the slow phase of fluorescence induction were then obtained by applying actinic light to determine fluorescence of light-adapted leaves (F_o') before the saturating pulse. Maximum fluorescence of light-adapted leaves (F_m') was used to calculate effective quantum yield of PS II as $F_v'/F' = (F_m' - F_o')/F_m'$ (Genty *et al.* 1989). The F_v'/F_m' was also used to estimate apparent electron transport rate (ETR) as follows: $ETR = F_v'/F_m' \times \text{photosynthetic photon flux density} \times 0.5 \times \text{leaf absorptivity coefficient} [\mu\text{mol}(\text{electrons}) \text{m}^{-2} \text{s}^{-1}]$ (Kalaji *et al.* 2014). Non-photochemical quenching (NPQ) was calculated as $NPQ = (F_m - F_m')/F_m'$ (Bilger and Björkman 1990).

The F_o , F_v/F_m , F_v'/F_m' , ETR, and NPQ were also measured using an imaging fluorometer (*Imaging-PAM*, Heinz Walz, Effeltrich, Germany) to confirm the chlorophyll *a* fluorescence results obtained by the *LI-6400XTR*. The imaging fluorometer can be used to map the fluorescence parameters in a larger area than that obtained by the *LI-6400XTR*, thus enabling assessment of the alterations throughout the leaf. To verify whether the water status was affected by heat stress, the leaf water potential of the plants was assessed before dawn (between 04:00 and 06:00) on the first and last days of the heat treatment using the pressure bomb technique (Scholander *et al.* 1965).

Content of chlorophylls and carotenoids: The content of photosynthetic pigments was determined on the last day of the heat treatment according to the method proposed by Meneguelli-Souza *et al.* (2016). The values were calculated using equations proposed by Wellburn *et al.* (1997).

Results

Regardless of genotype, P_N decreased after 3 days at 42 °C compared to the control at 25 °C (a significant interaction $G \times T \times D$) (Fig. 1A,B,C; Table 1 Suppl.). The negative effect of the high temperature on P_N was reduced in the *hpl* mutant compared to the other genotypes. Usually, the mutant *au* showed reductions that were more pronounced in P_N when subjected to the heat stress compared to the *hpl* and MT genotypes during the evaluation period. The respective decreases compared to the control counterparts were 27, 18, and 50 % for genotypes MT, *hpl*, and *au* subjected to 42 °C for 7 d.

Changes in P_N were coupled with alterations in g_s and E in MT and the *hpl* mutant (a significant interaction $G \times T \times D$) (Fig. 1D,E,F; Table 1 Suppl.). Compared to the controls, g_s increased by 83 and 193 % in MT and the *hpl* mutant when subjected to 42 °C for 1 day, respectively. The E increased by 64 and 115 % under these conditions, respectively. Generally, the increases in g_s and E triggered by the heat stress decreased progressively and

Anatomical analyses: To characterize the possible anatomical changes, leaf samples of the first node were collected on the last day of the heat treatment and stored for 24 h in a Karnovsky fixative (Karnovsky 1965). The plant material was next dehydrated in a series of increasing ethanol concentrations and then pre-infiltrated and infiltrated in *Histo-resin* (Leica, Nussloch, Germany) according to the manufacturer's recommendations. For histochemical assays, samples were cross-sectioned at 5 μm in a rotary microtome (*model 1508R*, Biobase, Shandong, China) and stained with toluidine blue (0.05 %, m/v, in 0.1 M phosphate buffer, pH 6.8) (O'Brien *et al.* 1964). Observations were performed using an *Olympus* microscope (*model BX61*, Tokyo, Japan) with the bright field option.

Statistical analyses: Experiments were carried out in a completely randomized design with 4 replicates (a total of 24 experimental units). Data were subjected to an analysis of variance (ANOVA) and the Tukey test at a 5 % probability using the *SISVAR* software (v. 5.6). The following experimental designs were considered for ANOVA: the first was a $3 \times 2 \times 4$ factorial design consisting of 3 genotypes (MT, *hpl*, and *au*), 2 temperatures (25 and 42 °C), and 4 evaluation times (1, 3, 5, and 7 days of heat treatment) for the leaf gas exchange and chlorophyll *a* fluorescence. The second was a 3×2 factorial design for the content of photosynthetic pigments, anatomical changes, and image of chlorophyll *a* fluorescence (3 genotypes and 2 temperatures). The final design was a $3 \times 2 \times 2$ arrangement for the leaf water potential (3 genotypes, 2 temperatures), and 2 evaluation times (1 and 7 days of heat treatment).

were maintained in MT and the *hpl* mutant from 3 days of the heat treatment onwards (Fig. 1D,E,G,H). Surprisingly, in the *au* mutant, g_s and E were unchanged in response to the heat stress regardless of evaluation time (Fig. 1F,I).

Additionally, the heat stress increased c_i/c_a in MT and in the *hpl* mutant during the evaluation period (Fig. 1J,K). The c_i/c_a increased only in the *au* mutant when it was subjected to 42 °C for 7 days compared to the control (Fig. 1L). Therefore, the *hpl* mutant generally displayed a higher photosynthetic performance (higher values of P_N , g_s , and E) than the other two genotypes even under heat stress conditions (Fig. 1 and Table 1 Suppl.).

The values of F_v/F_m , F_v'/F_m' , ETR, and NPQ were usually lower in all the genotypes at 42 °C compared to their non-stressed counterparts (a significant interaction $G \times T \times D$) regardless of evaluation time (Fig. 2D-O and Table 1 Suppl.). Conversely, F_o was higher in the genotypes under the heat stress (Fig. 2A,B,C). Higher

decreases in the variables of chlorophyll *a* fluorescence were induced by the high temperature in the *au* mutant compared to the other genotypes (Fig. 2 and 3). On the third day of the high temperature treatment and onward, there were slightly lower reductions in the quantum yield of PS II in the *hpl* mutant compared to MT (represented

by F_v/F_m and F_v'/F_m'). Compared to the control counterparts, F_v/F_m showed significant reductions of 12, 8, and 27 % in MT, *hpl*, and *au* when treated for 7 d at 42 °C, respectively. The F_v'/F_m' showed significant reductions of 24, 15, and 53 % (Fig. 2 G-L).

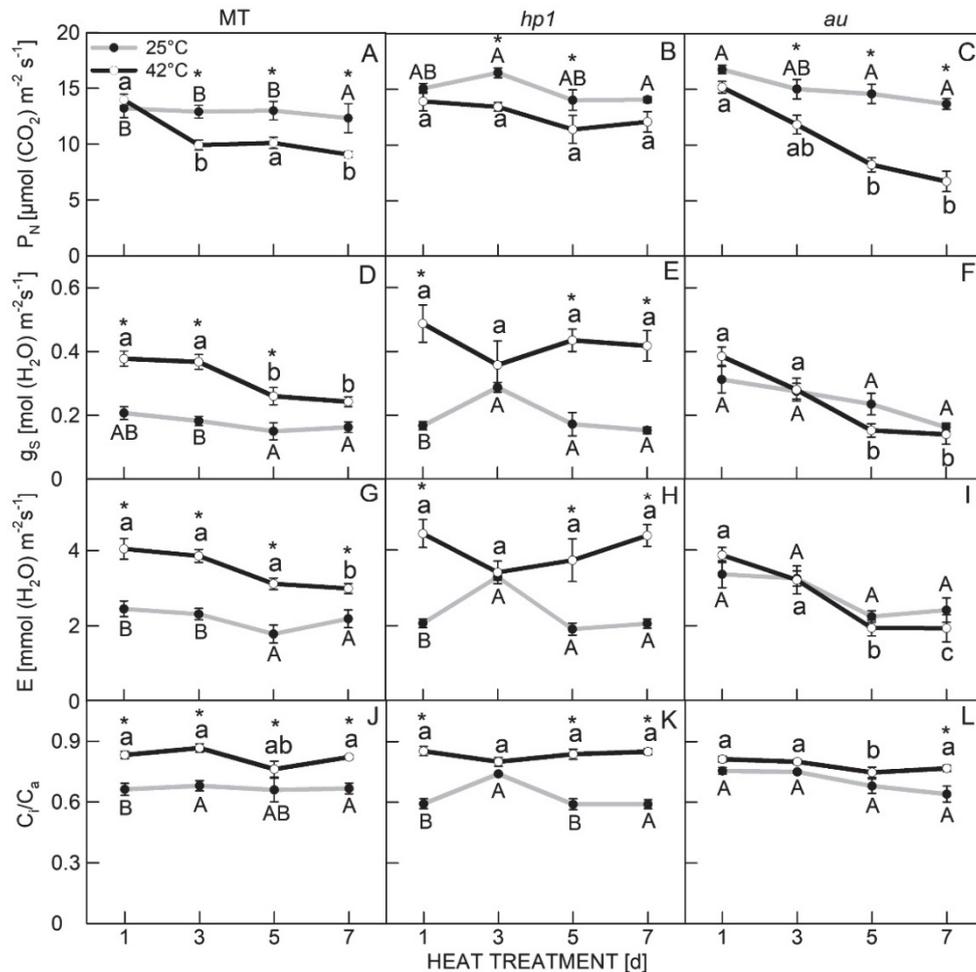


Fig. 1. Net photosynthetic rate (P_N ; A,B,C), stomatal conductance (g_s ; D,E,F), transpiration rate (E; G,H,I) and internal to external CO_2 ratio (c_i/c_a ; J,K,L) in tomato mutants *aurea* (*au*) and *high-pigment 1* (*hpl*), and in the wild type Micro-Tom (MT) subjected to 25 or 42 °C for 1, 3, 5, and 7 d. Means \pm SEs, $n = 4$. Within each day, there are no significant differences among genotypes in means that are followed by the same uppercase letter at 25 °C and lowercase letter for the treatment at 42 °C, and means that are not followed by an asterisk between temperatures based on the Tukey test at a 5 % probability.

Usually, the heat stress for 7 days increased F_0 by 29, 42, and 144 % in MT, *hpl*, and *au*, respectively, compared to their controls (Fig. 2A,B,C). The lowest impairments in the photosynthetic performance were observed in the *hpl* mutant, which is evidenced by the lowest decreases in F_v/F_m , F_v'/F_m' , and ETR (Fig. 3 B1-B6, C1-6, and D1-6). Irrespective of genotype, the high temperature decreased NPQ particularly in the *au* mutant (Fig. 3 E1-6). In non-stressful conditions, the variables of chlorophyll *a* fluorescence (F_0 , F_v/F_m , F_v'/F_m' , ETR, and NPQ) did not usually differ among the genotypes regardless of evaluation time, with the exception of the *au* mutant, which had a lower F_0 (Fig. 2).

The *hpl* mutant showed a lower water leaf potential than the *au* mutant and a similar water potential to MT irrespective of the temperature treatments and evaluation time (a non-significant interaction $G \times T \times D$), as shown in Fig. 4.

No significant relationship was observed between temperatures and genotypes for chloroplastic pigments (Table 1 Suppl.). However, the *au* mutant exhibited a lower content of chlorophyll *a*, chlorophyll *b*, and carotenoids compared to the *hpl* mutant and MT (Fig. 5A,C,E). Irrespective of genotype, there were a lower content of chlorophyll *a* and chlorophyll *b* and a lower chlorophyll *a/b* ratio in the plants under the heat

stress (Fig. 5B,D,F).

Irrespective of genotype, the heat stress changed the cellular structure of the mesophyll (Fig. 6). Heat-triggered anatomical changes at 42 °C were most

prominent in the *au* mutant followed by the MT genotype. These genotypes exhibited disorganization of the parenchyma cells, electrolyte leakage, and mesophyll retraction (Fig. 6B,F).

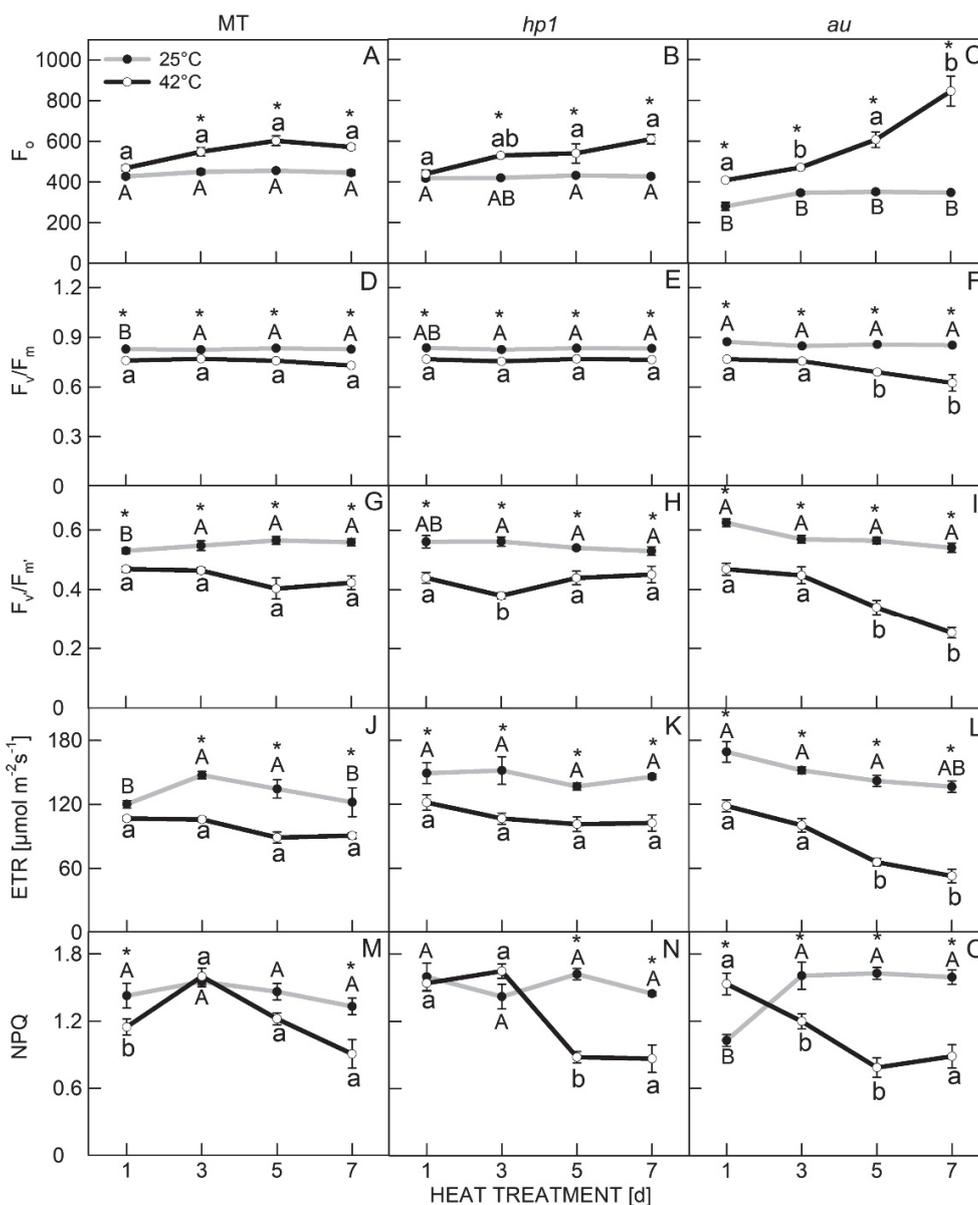


Fig. 2. An initial chlorophyll *a* fluorescence (F_0 , A,B,C), maximum photochemical efficiency of photosystem II (PS II) (F_v/F_m ; D,E,F), effective quantum yield of PS II (F_v/F_m' ; G,H,I), apparent electron transport rate (ETR; J,K,L), and non-photochemical quenching (NPQ; M,N,O) in the tomato mutants *aurea* (*au*) and *high-pigment 1* (*hp1*) and wild type Micro-Tom (MT) subjected to 25 or 42 °C for 1, 3, 5, and 7 days. Means \pm SEs, $n = 4$. Within each day, there are no significant differences between genotypes in means that are followed by the same uppercase letter at 25 °C and lowercase letter for the treatment at 42 °C, and means that are not followed by an asterisk between temperatures based on the Tukey test at a 5 % probability.

Discussion

This study investigated the role of phytochrome-dependent responses in plant tolerance to high temperature using tomato photomorphogenic mutants as

models. Such approach provided evidence that phytochrome plays a pivotal role in plant responses to heat stress, which was indicated by an enhanced

photosynthetic dysfunction observed in the *aurea* PΦB-deficient mutant. Although the heat stress negatively affected the leaf gas exchange in all of the genotypes, the impairments were less pronounced for the *hp1* mutant, which is hyperresponsive to irradiance. A similar physiological performance has been reported in other plant species subjected to high temperatures (Galmés *et al.* 2015).

The increased g_s and E observed in the *hp1* mutant in comparison to MT also contributed to the better photosynthetic performance of this mutant at the high temperature (Fig. 1E,H). Increased E is an efficient

mechanism for heat dissipation and maintaining adequate leaf temperatures and so mitigating damage induced by heat stress (Rizhsky *et al.* 2002, Lubovská *et al.* 2014). The effective heat dissipation in the *hp1* mutant may be related to its high stomatal density (Boccalandro *et al.* 2009, Melo *et al.* 2011). There is also evidence that phytochrome-dependent responses are positively related to both a high stomatal density and a high transpiration (Boccalandro *et al.* 2003, Schittenhelm *et al.* 2004).

The deficiency in PΦB biosynthesis in the *au* mutant affected the stomatal aperture (Fig. 1F). This indicates that functional phytochromes are important in the

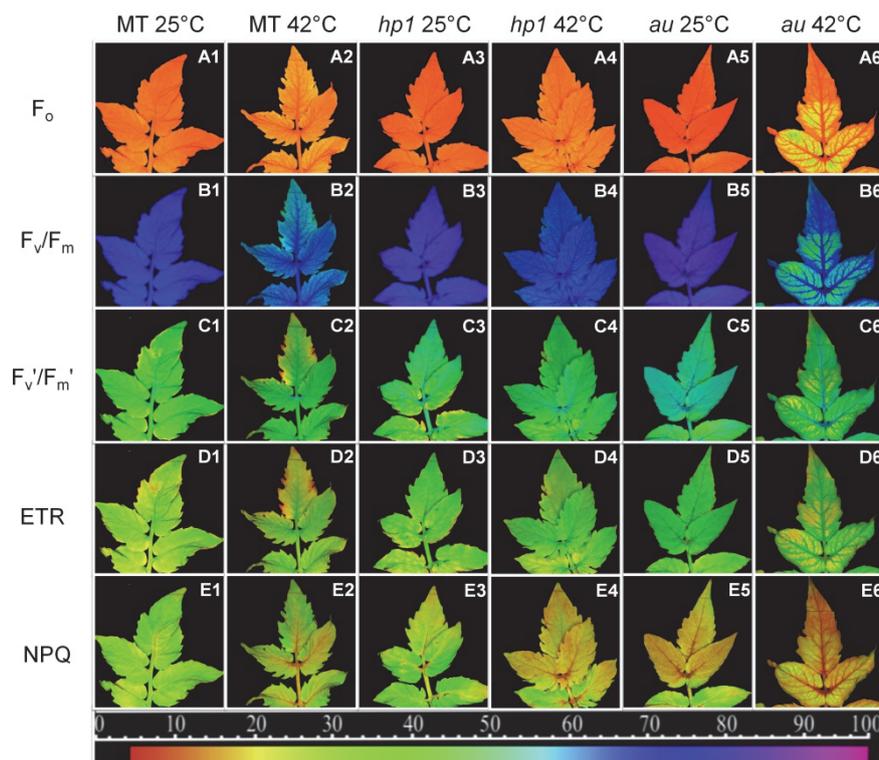


Fig. 3. Images of chlorophyll *a* fluorescence (F_0 ; A1-6), maximum photochemical efficiency of photosystem II (PS II) (F_v/F_m ; B1-6), effective quantum yield of PS II (F_v'/F_m' ; C1-6), apparent electron transport rate (ETR; D1-6), and non-photochemical quenching (NPQ; E1-6) in the tomato mutants *aurea* (*au*) and *high-pigment 1* (*hp1*) and wild type Micro-Tom (MT) subjected to 25 or 42 °C for 7 days.

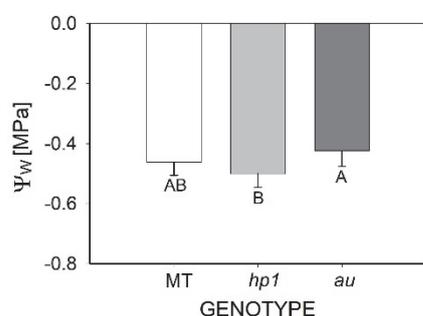


Fig. 4. Water leaf potential in the tomato mutants *aurea* (*au*) and *high-pigment 1* (*hp1*) and wild type Micro-Tom (MT) regardless the temperature and evaluation times. Means \pm SEs, $n = 16$. Means that are followed by the same letter are not significantly different (at a 5 % probability; Tukey test).

responses of plants to high temperatures *via* the regulation of stomatal opening. All the genotypes had a similar water status under the high temperature, which means that the changes observed in g_s were strictly related to the high temperature (Fig. 4). Melo *et al.* (2011) reported that the *au* mutant usually displays an unchanged stomatal density compared to MT, which corroborates the hypothesis that stomatal opening for heat dissipation is mediated by phytochromes (Wang *et al.* 2010, Chen *et al.* 2012). Thus, the lower gene expression of phytochromes stemming from the deficiency in PΦB synthase in the *au* mutant (Carvalho *et al.* 2011b, Bianchetti *et al.*, 2017) is linked to a higher thermo-sensitivity due to a decrease in heat dissipation.

In addition to the physiological damage, the heat stress caused a slight damage in the palisade and spongy

parenchyma cells in the *hp1* mutant in comparison to the other genotypes (Fig. 6D). The stability of cell structure is strongly related to plant thermotolerance and can be used to assess a putative heat tolerance (Latif and Mohamed 2016). Heat stress can damage membranes and

cause rupture in the cell walls of mesophyll tissue. This results in solute leakage and the collapse of parenchyma cells (Zhang *et al.* 2009), which was particularly evident in the *au* mutant.

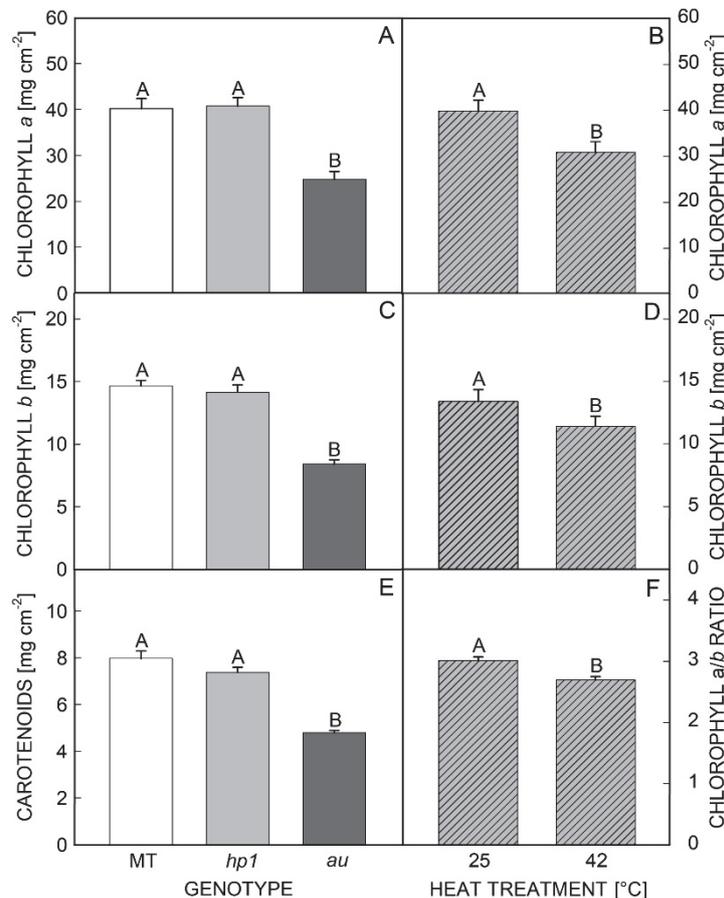


Fig. 5. Content of chlorophyll *a* (A), chlorophyll *b* (C), and carotenoids (E) in the tomato mutants *aurea* (*au*) and *high-pigment 1* (*hp1*) and wild type Micro-Tom (MT) regardless the temperature. The effect of temperatures of 25 or 42 °C for 7 days on the content of chlorophyll *a* (B), chlorophyll *b* (D), and chlorophyll *a/b* ratio (F) regardless the genotype. Means \pm SEs, $n = 8$ for genotypes and 12 for temperature. Means that are followed by the same uppercase letter are not significantly different based on the Tukey test at a 5 % probability.

The content of photosynthetic pigments is strongly influenced by phytochromes (Khudyakova *et al.* 2017). Thus, the lower content of photosynthetic pigments in the *au* mutant could also be related to its higher heat sensitivity because of a decreased energy dissipation through either photochemical or non-photochemical pathways (Fig. 5A,C,E) (Merzlyak and Solovchenko 2002). Both pathways are involved in the dissipation of excess of radiation (Merzlyak and Solovchenko 2002, Krüger *et al.* 2014). The reduction observed in the chlorophyll *a/b* ratio induced by the heat stress corroborates with findings that chlorophyll *a* is more heat sensitive than chlorophyll *b* (Murchie and Horton 1997).

The high c_i/c_a ratios observed in the MT and *au* mutant were probably due to a lower efficiency in CO₂ assimilation caused by the high temperature (Fig. 1J,L). This may have resulted from a reduction in the

carboxylation activity of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) due to an increased photorespiration (Mathur *et al.* 2014, Sharma *et al.* 2016). Another reason could be the denaturation of Rubisco activase due to heat stress (Grover *et al.* 2013). Moreover, high temperatures greatly compromise the expression of genes encoding for the synthesis of proteins involved in the Calvin cycle including Rubisco and Rubisco activase (Crafts-Brandner and Salvucci 2000, Salvucci and Crafts-Brandner 2004, Allakhverdiev *et al.* 2008). In contrast, the increased CO₂ content in the substomatal cavity recorded in the *hp1* mutant can be attributed to the higher g_s and heat dissipation rather than a lower efficiency of the Calvin cycle enzymes (Fig 1K).

The tomato plants subjected to the heat stress exhibited clear adjustments in the capture and dissipation of energy as indicated by the increases in F_0 and

decreases in F_v/F_m , F_v'/F_m' , and ETR, which were remarkably altered in the *au* mutant (Fig. 2). The changes in fluorescence parameters are an efficient tool for distinguishing heat stress tolerant genotypes (Allakhverdiev *et al.* 2008). A loss of thylakoid membrane integrity is most likely responsible for a loss in physiological performance in genotypes at high temperature indicated by concurrent reductions in leaf gas exchange and chlorophyll *a* fluorescence (Allakhverdiev *et al.* 2008, Teskey *et al.* 2014, Gerganova *et al.* 2016). Normal functioning PS II and the integrity of thylakoid

membranes play a key role in the adaptation of plants to stress (Kreslavski *et al.* 2007). In our study, the disruption in the PS II light absorption complexes evidenced by decreases in F_v/F_m accounted for the reduction in the effective capacity of electron transport in PS II, which was confirmed by reductions of ETR and F_v'/F_m' and hence impaired P_N in all genotypes. However, these responses were less evident in the *hpl* mutant and wild type suggesting the involvement of functional phytochromes in the plant tolerance to heat stress.

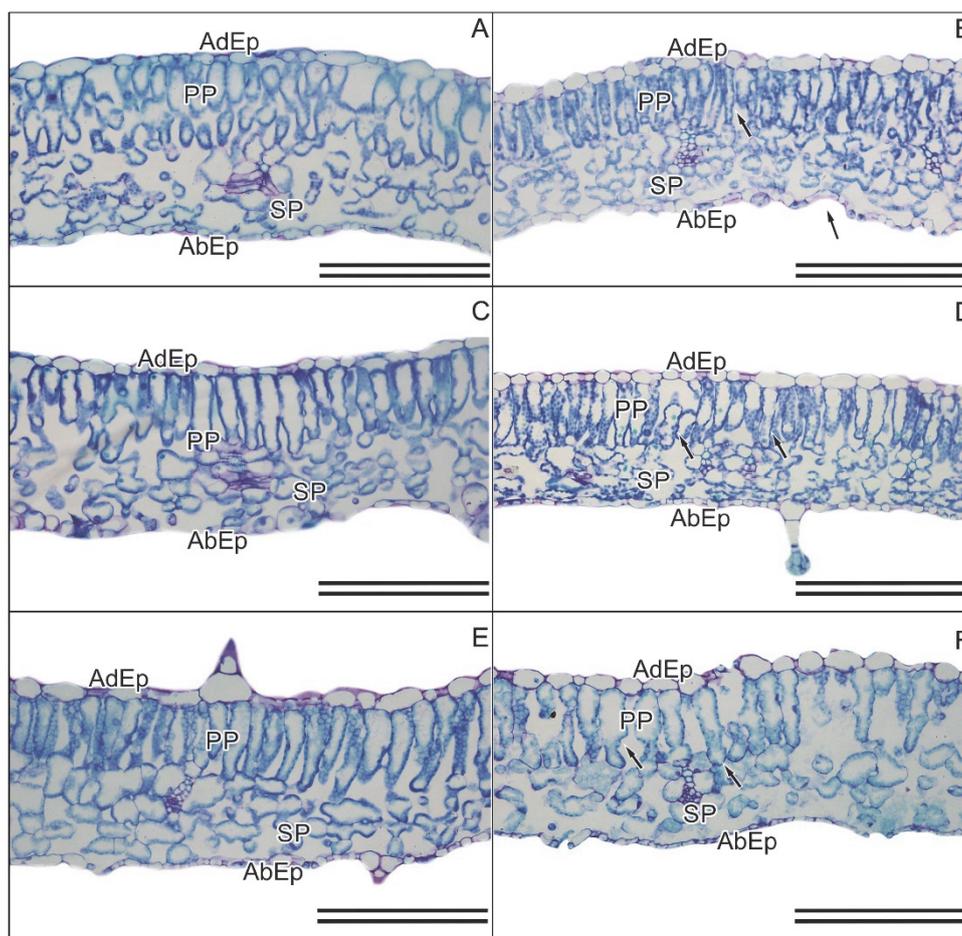


Fig. 6. Anatomical changes in response to heat stress in tomato leaves: the wild type cultivar Micro-Tom (MT; *A,B*) and mutants *high-pigment 1* (*hpl*; *C,D*), and *aurea* (*au*; *E,F*) exposed to 25 °C (*A,C,E*) and 42 °C (*B,D,F*) for 7 days. (AdEp - adaxial epidermis, AbEp - abaxial epidermis, PP - palisade parenchyma, SP - spongy parenchyma. Arrows indicate the cell collapse and mesophyll retraction. Scale bars: 200 μ m.

Increasing NPQ is linked to the photoprotective capacity of plants (Müller *et al.* 2001). Thus, impairments induced by high temperature require another protective mechanism against an excessive radiation to prevent photooxidative damage. In this regard, the increases in F_0 in all the genotypes indicate that an excessive light energy was mostly dissipated by Chl fluorescence in the PS II light-harvesting antennae complex. The higher increases in F_0 in the *au* mutant highlight the role of phytochromes in plant tolerance to the heat stress since

deficiency in the content of functional photoreceptor enhances the necessity of energy dissipation. This process carries a low energy to the photochemical stage of photosynthesis and causes losses in the *au* mutant.

Overall, our results suggest that a deficiency PΦB biosynthesis and the resulting decrease in the content of functional phytochromes enhanced the impairments in the photosynthetic performance of plants subjected to the heat stress, as shown by the *au* mutant responses at 42 °C. We also verified that the hyperresponsive phytochrome-

mediated responses of the *hpl* mutant corresponded to a higher efficiency in heat dissipation mechanisms. Consequently, *hpl* presented a stable carbon assimilation even at high temperatures. The ability to dissipate heat *via* transpiration associated with the photosynthetic

performance was a determining factor for a greater tolerance of the tomato plants to the heat stress. However, in-depth studies should be carried out to further elucidate the genetic and molecular mechanisms.

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