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Heat Tolerance in Blackberry Blossoms: Effects on Fruiting and Photosynthetic Performance

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Abstract

In 2018, at beginning of blooming stage of seven blackberry genotypes (cultivars BRS Xingu, BRS Cainguá, Tupy and Brazos and the selections Black 139, Black 198, and Black 254) plants were exposed to three temperature regimes for four days (control - plants kept constantly at 20 ± 1 ºC; plants held at 29 ± 1 ºC, during the day (light period) and 19 ± 1 °C, at night; and plants constantly held at 30 ± 1 °C) aiming to test heat tolerance of blackberry flowers. Pollen germination, average number of drupelets per fruit, photosynthetic electron transport rate and electron losses in the leaves were evaluated. Based on the results we concluded `BRS Cainguá´ released by Embrapa in 2019, was among the most tolerant to heat conditions whereas selection Black 254 was the most sensitive.

The cultivation of temperate fruit species in Brazil is concentrated in the South and Southeast regions, where it has an important economic and social role. Climatic conditions, that limit the expansion of these crops, have motivated research aimed to expand production to warmer regions, such as the São Francisco Valley (Lopes et al., 2010; Lopes et al., 2013). In addition, scientists all over the world consider climate change to possibly be the biggest problem influencing future fruit production, as it will affect maximum and minimum temperatures, rainfall, ocean temperatures, cloudiness, relative humidity, and so on. Extreme temperatures are expected to be significantly influenced by global warming, which could cause considerable agricultural losses. According to Salomé et al. (1999), for a new introduced species or cultivar to be successful, it must be adapted to new environmental conditions.

Global climate change is defined as the possible rise in the Earth's surface temperature caused by the rapid rise of greenhouse gas levels, in the atmosphere (Cerutti, 2006). This rise affects life in general, and consequently agriculture and particularly fruit species. The fruiting process is highly influenced by climatic conditions which, when adverse, contribute to erratic fruit production. The reproductive phase is known to be the most vulnerable period to thermal stress (Hedhly et al., 2009). Very low temperatures (McLaren et al., 1996) and supra-optimum temperatures (Burgos et al., 1991; Beppu et al., 1997; McKee and Richards, 1998) may negatively influence fruiting.

The vulnerability of the reproductive process to temperature is widespread in angiosperms (Hedhly et al., 2009), not only regarding frost sensitivity (common in some areas of the Southern region of Brazil, depending on the topography), but also with respect to supra-optimum temperatures.

Some studies showed that high temperatures significantly reduced fruiting and consequently, the yield of several fruit species (Kumakura and Siilsiildo, 1995; Beppu, et al., 1997; Higuchi et al.,998; Hedhly et al., 2007). With global warming, winter chill accumulation is declining, which directly affects completion of dormancy in temperate fruit species. Therefore breeding programs

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are developing cultivars with lower chilling requirements that are better adapted to these conditions (Perez-Gonzalez, 2000; Byrne, 2010). However, genotypes adapted to mild winter conditions must also be heat tolerant, as there is a tendency for mild winter regions to have higher spring and summer temperatures (Jennings et al., 1991).

Breeding programs identified the need for hibernal cold as a priority, however tolerance to heat stress has received less attention (Hedhly, 2003). Some studies showed that many species are sensitive to high temperatures, particularly in the reproductive phase (Park et al., 1998; Hedhly et al., 2003; 2004; 2005; Kozai et al., 2004). The subject was also investigated at Embrapa Temperate Climate (a Research Center in Southern Brazil), starting with field observations in 2005, followed by designed experiments (Couto and Raseira, 2004; Raseira et al., 2005; Couto, 2006; Couto et al., 2010; Zanandrea et al., 2009; Carpenedo, 2015; Carpenedo et al., 2015; 2017; 2018).

Although the efforts of plant breeders have resulted in considerable progress in recent decades, for most agronomic species of interest, annual genetic improvements are decreasing. Incorporating new tools and developing/validating methodologies may enhance commonly used phenotyping procedures. Hopefully this would increase the accuracy and refine phenotyping, to aid in selecting superior genotypes that will be more tolerant to different kinds of stresses.

In recent years, chlorophyll *a* fluorescence analysis has been extensively used to examine photosynthetic performance of plants subjected to biotic and abiotic stresses. This approach has been leveraged by a better understanding of the relationship between fluorescence parameters and photosynthetic electron transport *in vivo*. This knowledge, along with more user-friendly interfaces, allows plant scientists to use various aspects of photosynthesis to study the response to plant stress. In the present study, the fruiting process was evaluated, along with some chlorophyll fluorescence variables, to examine the heat stress perturbations on some photosynthesis parameters, using the imaging-PAM (Pulse-Amplitude-Modulation) analysis system. The objective of this study was to use photosynthetic parameters to identify heat-tolerant blackberry genotypes to be used in future hybridizations.

Material and Methods

Blackberry plants of cultivars BRS Xingu, BRS Cainguá, Tupy and Brazos and the selections Black 139, Black 198 and Black 254, obtained by root cuttings, were planted in pots with substrate and kept in a screen house, until used in this experiment. All genotypes with the exception of cv. Brazos were developed by the Embrapa Temperate Climate breeding program.

At the beginning of flowering and before treatment application, flower buds at the green tip stage or very early pink stage were marked.

The plants were kept under 14 h of daily light, provided by two mixed light sources (Fluorescent and White LED). These light sources provided 320 μmol m^{-2} s^{-1} of photosynthetic photon flux of photosynthetically active radiation (PAR) at 50 cm from dossel top. For four days plants were subjected to the following treatments: 1. Control: plants kept constantly at 20 ± 1 ºC; 2. Plants subjected to 29 ºC during the day (light period) and 19 ± 1 ºC, at night and 3. Plants exposed constantly to 30 ± 1 °C for four days and in very low light (about 30 μmol m^{-2} s^{-1} of PAR). Following treatment, all plants were returned to the screen house.

On the first day after treatment, pollen samples were collected for germination tests *in vitro.*Pollen grains were scattered on slides with a solidified germination medium (10g sugar and 1g of agar in 100 ml of distilled water), incubated for 3 hours at 23 ± 1 ºC, after which the percentage of pollen germinated was recorded. The pollen was considered germinated when the pollen tube length exceeded the pollen grain size. Four samples

of 100 pollen grains were counted for each treatment.

The immature fruits (just at beginning of turning red) developing from the previously marked flowers, were harvested and the number of drupelets per fruit, in 10 random fruits was counted.

The plants under control conditions and those exposed to 29 °C/19 °C were used for chlorophyll *a* fluorescence analysis. The plants were acclimated in the dark for 60 minutes before leaf samples were collected. Subsequently, completely expanded young leaves from the apex were collected and placed in a Petri dish containing a water film to preserve their turgidity during the analysis. These leaves were immediately analyzed, using a Maxi image fluorometer of Imaging-PAM and Imaging Win software (Heinz Walz GmbH, Effeltrich, Germany). The transient emission of chlorophyll *a* fluorescence was captured by a CCD camera (charge-coupled device) with a resolution of 640 x 480 pixels in a 25 mm diameter circular area taken three times along the leaf length, excluding the central leaf rib. The leaves were positioned 18.5 cm from the CCD camera.

Prior to the beginning of the fast light curve construction or curve of rapid light (CRLs), the leaves were exposed to weak modulated light (0.5 μmol photons m^{-2} s^{-1}, 100 μs, 1 Hz, a red light color) for the initial fluorescence determination (F_0), when all PSII reaction centers were "open", i.e. oxidized. An important feature of this measuring beam is that its intensity must be low enough so it does not drive significant PSII photochemistry. Subsequently, a 2400 μmol m^{-2} s^{-1} (10 Hz) saturating light pulse was applied for 0.8 s to ensure the emission of maximum fluorescence (F_m), i.e. when all PSII reaction centers are "closed" or reduced. From these initial determinations, the maximum photochemical efficiency was calculated by the ratio between variable fluorescence and maximum fluorescence, which is described as $F_v / F_m = [(F_m - F_0) / F_m)]$ (Osorio et al., 2014; Rolfe and Scholes, 2010; Scholes and Rolfe, 2009). After 10 s in darkness, the rapid light curve, programmed to gradually increase the actinic light at each step (0, 21, 56, 111, 186, 281, 336, 396, 461,531, 611, and 701), was initiated through the blue light provided by the source LEDs at regular intervals of 20 s. At the end of each step, a light saturation pulse was applied. During the construction of the CRLs, the ambient light was kept off to avoid interference from another light source than that provided by the fluorometer system. Such procedures were performed between 9:00AM and 11:00AM. The initial values of F_0 and F_m were used to calculate other parameters related to the photochemical and non-photochemical extinction coefficients using equations in the manual for the ImaginWin software version 2.46i, which were described by Van Kooten and Snel (1990), Maxwell and Johnson (2000) and Baker (2008) as follows: $qP = (F_{m'} - F_s) / (F_{m'} - F_{o'})$, $qN = 1 - (F_{m'} - F_{o'}) / (F_m - F_o)$, $NPQ = (F_m - F_{m'}) / F_{m'}$.

For calculation of the electron transport rate, the equation is (ETR = Yield x PAR x 0.5 x Absorptivity), where absorptivity describes the fraction of incident light that is actually absorbed. This equation application assumes an equal light distribution between PSII and PSI (Krall and Edwards, 1992; Rolf and Scholes, 2010).

Values of chlorophyll *a* fluorescence measurements were plotted using SigmaPlot software (13.0 version, Systat, USA).

Pollen viability and fruiting data were analyzed by SISVAR program using a factorial (3 treatments x 7 genotypes) arrangement in a completely randomized design with four replications of 100 hundred pollen grains for pollen viability and 10 replications of one single fruit per treatment for number of drupelets per fruit. For the analysis, percentages of pollen germination were transformed as arcsen$\sqrt{X}$ (where x was the percentage) The number of drupelets was transformed as $\sqrt{X}$ (x= number of drupelets per fruit) and then submitted to the normality test using a Shapiro-Wilk test and it was non-normally

Table 1. Percentage of pollen germination and number of drupelets per fruit obtained from flowers of seven blackberry genotypes, following four days of exposure to different daily temperature patterns.

Genotypes	Pollen germination (%)				Drupelets (number)			
	29 °C/19 °C	20 °C	30 °C	Average	29 °C/19 °C	20 °C	30 °C	Average
Tupy	9.50 cA[x]	12.50 cA	3.25 eB	8.42 d	63.60 aA	65.80 aA	42.87 aB	58.46 a
BRS Xingu	37.50 bA	43.75 aA	15.75 dB	32.33 b	55.80 abA	63.00 aA	49.35 aA	55.81 a
BRS Cainguá	39.75 bA	36.00 abA	34.25 cA	36.67 b	49.33 abA	58.43 aA	49.40 aA	52.13 ab
Black 198	58.25 aA	45.50 aB	51.75 bAB	51.83 a	60.80 aA	52.00 aA	19.10 bB	43.97 bc
Black 254	1.50 dC	39.75 abA	15.50 dB	18.92 c	37.00 bcB	69.60 aA	11.27 bC	31.54 d
Black 139	35.25 bAB	30.75 bB	43.00 bcA	36.33 b	44.30 abB	60.50 aA	10.22 bC	37.74 cd
Brazos	33.00 bB	36.75 abB	67.75 aA	45.83 a	21.67 cB	27.38 bB	55.08 aA	39.44 cd
Average	30.68 B	35.00 A	33.04 B		47.49 B	56.67 A	33.90 C	
CV (%)	9.52				20.83			

[x] Means followed by the same upper case letters in the rows and lower case in the columns do not significantly differ by Tukey ($p \leq 0.05$).

distributed thus there was no value in using the transformation, and the non-transformed data were used.

Results and Discussion

There was a significant interaction between genotypes and treatment for pollen germination as well as number of drupelets per fruit.

Compared to the control treatment, flowers of `Tupy´,`BRS Xingu´ and selection Black 254, developed after four days at 30 ºC, under low light conditions, had reduced pollen germination, whereas `Brazos´ and selection Black 139 had higher pollen germination than the control, but ´BRS Cainguá´ and Black 198 did not differ from the control plants (Table 1). Compared to control plants, pollen germination was generally increased or unaffected by exposure to 29 °C during the day and 19 °C at night, with 14 hours of daily light, with exception of selection Black 254. In a similar study, using five cultivars and three selections, exposing the plants for 48h to 32 ºC, Carpenedo et al. (2018) observed that the Black 198 selection was among the most heat tolerant genotypes at the beginning of flowering. In the same study, cv. Brazos had near 20% reduction in pollen viability, when exposed to 32 ºC, compared to flowers of plants maintained at 20 ºC; `Tupy´ had a larger reduction than the cited genotypes. Additional work is needed to determine the individual and combined effects of temperature and light on pollen germination.

Compared to the control plants, the number of drupelets per fruit was lower for all genotypes (except `Brazos´) exposed to 30 ºC but not significantly lower in fruits of BRS `Cainguá´ and `BRS Xingu´. All genotypes exposed to 29 °C/19 °C did not significantly differ from their controls, with the exception of selections Black 139 and Black 254. (Table 1).

When exposed to 30 ºC, the cv. Brazos, considered a heat tolerant cultivar (Nesbitt et al., 2013), had the highest pollen germination and the higher number of drupelets per fruit compared to the other genotypes, but did not differ from `BRS Xingu´ and `BRS Cainguá´. Additional research is needed to explain why pollen germination and drupelet number of `Brazos´ flowers were higher in flowers of treatment 3 (30 °C), where light was very low.

The Black 254 selection was the most heat sensitive, based on both pollen viability and number of drupelets.

'Tupy' had low pollen germination, but high numbers of drupelets for all treatments. This is something that has also occurred in most years when testing pollen viability of field plant flowers. We still do not understand this phenomena. Since it is not rare to have an interaction between genotype and the media for testing pollen viability, perhaps we should have tested different media for Tupy´pollen germination.

We tested only floricane blackberry cultivars which are considered the most heat tolerant species among the berry crops (Nesbitt et al., 2013), but primocane cultivars are less heat tolerant.

In summary, the parameters used (pollen viability and number of drupelets/fruit) were fairly adequate for identifying blackberry genotypes that were sensitive or not sensitive to heat stress.

When exposed to biotic and abiotic stresses, structural and functional changes in the thylakoid membranes in the chloroplast lead to changes in the characteristics of fluorescence signals. These can be detected and quantified noninvasively in leaves of plants exposed to stress (Baker, 2008). The rapid light curve construction (RLC) technique provides detailed information about electron transport saturation as well as the photosynthetic performance of a plant. In recent years plant breeders have used fluorescence analysis for phenotyping, to identify more stress-tolerant cultivars (Kalaji et al., 2016; Makonyaa, et al., 2019; Sharma et al., 2019). In our study, genotypes generally had the

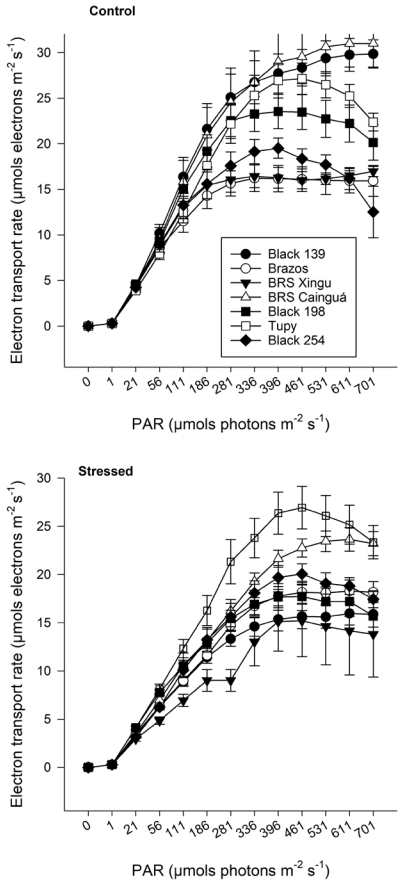

Fig. 1. ETR after exposure to progressive light during rapid light curve construction (n=3 biological replicates; bars indicate standard error values) in seven blackberry genotypes submitted to heat stress (temperature of 29 °C at day time and 19 °C night temperature) and under control conditions (temperature 20 °C/12 °C).

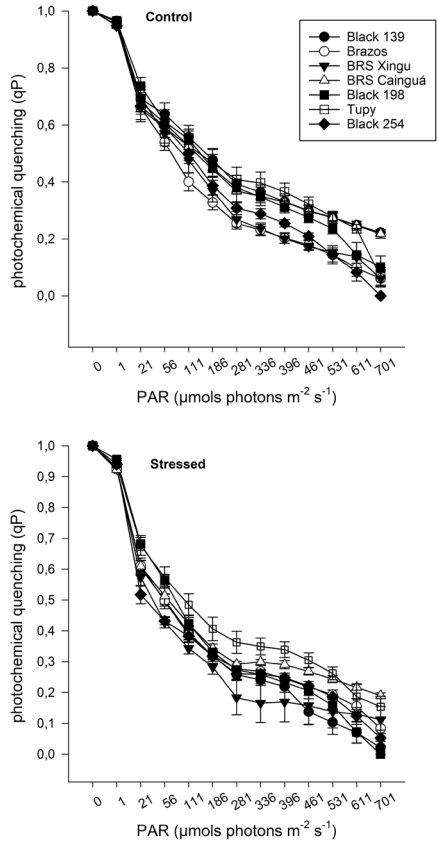

Fig. 2. qP course after exposure to progressive light during rapid light curve construction (n=3 biological replicates; bars indicate standard error values) in seven blackberry genotypes submitted to heat stress (temperature of 29 °C at day time and 19 °C night temperature) and under control conditions (temperature 20 °C/12 °C).

highest electron transport rate (ETR) at 461 µmol m^{-2}·s`. At this RLC stage in the control treatment, the cultivar BRS Cainguá had the highest electron transport and was 30% more efficient compared to the average of the others and 84.34% higher than the Black 254 genotype, which had the worst performance (Fig. 1). There was a similar trend when plants were stressed, but differences were less pronounced. `BRS Cainguá´ was 18.20% higher than the average of the other genotypes evaluated, and 34.60% higher than the worst genotype at that light intensity (`BRS Xingu´). The parameter qP gives an indication of proportion of PSII reaction centers that are open. Then, stress stimulus, such as supra-optimum temperatures can induce changes in qP leading to closure of reaction centers, as a result of saturation of photosynthesis by light. `BRS Cainguá´ had high qP values across RLC regardless of temperatures regimes (Fig. 2); additionally, `BRS Cainguá´ had low NPQ values (Fig. 3). As the excess excitation energy in the photosystem (PSII) antenna complex can be harmlessly dissipated as heat in response to some stress conditions, this is observable as a process named nonphotochemical quench-

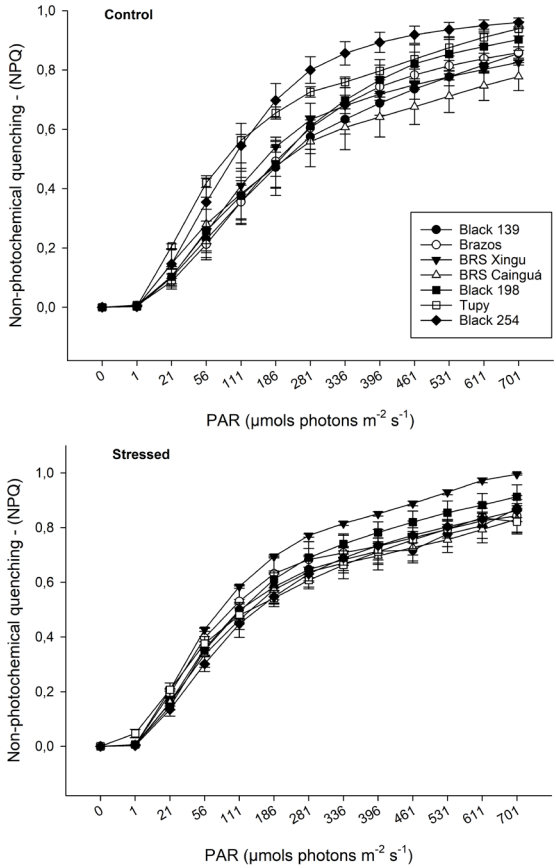

Fig. 3. NPQ after exposure to progressive light during rapid light curve construction (n=3 biological replicates; bars indicate standard error values) in seven blackberry genotypes submitted to heat stress (temperature of 29 °C at day time and 19 °C night temperature) and under control conditions (temperature 20 °C/12 °C).

ing of chlorophyll fluorescence (NPQ). Taken together, these data suggest a contrasting performance among evaluated genotypes. For example, `BRS Cainguá´ showed a great ETR and qP stability regardless of environmental conditions, and had lower NPQ activation. On the other hand, Black 254 was the most sensitive genotype considering all five variables measured in this study.

Regarding the non-photochemical behavior (NPQ) or non-photochemical quenching of the evaluated genotypes, `BRS Cainguá´ had the lowest values under the two environmental treatments. However, under stress, Black 254 had the highest values for NPQ (Fig.3). These responses suggest that this genotype invested in energy dissipation as heat in response to supra-optimum temperature exposure in detriment to photochemical quenching. NPQ is normally associated with the xanthophyll cycle, involved in photon energy dissipation, preventing/reducing photochemical pathway damage, before it results in the accumulation of intermediate reactive substances in the photosynthetic chain (Ralph et al., 2002). In this context,

the higher non-photochemical dissipation rate for Black 254 is possibly associated with non-photochemical dissipation-dependent energy removal suggesting a more sensitive response to supra-optimum temperatures; which is linked to the relaxation of the pH gradient along the thylakoid membrane, increasing the rate of energy conversion into heat. The contrasting chlorophyll *a* fluorescence performance shown by `BRS Cainguá´ and Black 254 genotypes and its relationship with fruiting process suggests great potential use of this approach to refine the phenotypic procedures for blackberry breeding for heat stress tolerance.

Using the nonparametric correlation test Spearman's Rank-Order there was not a significant correlation between the photosynthetic measuments (ETR and NPQ) with pollen germination or drupelets number. This might be because all the approaches used were only suitable to separate the most tolerant and the least tolerant genotypes, but did not differenciate all of them. Considering all the results, we conclude that as far as fruit set is concerned, `BRS Cainguá´ proved to be an interesting heat tolerant genotype unlike Black 254, which was one of the most sensitive. Heat tolerance of these genotypes during fruit ripening period still remains to be studied.

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