Low temperature impact on photosynthetic parameters of coffee genotypes

Fábio Luiz Partelli[1], Henrique Duarte Vieira[2], Alexandre Pio Viana[2], Paula Batista-Santos[3], Ana Paula Rodrigues[4], António Eduardo Leitão[3] and José Cochicho Ramalho[3]

[1]Universidade Federal de Goiás, Escola de Agronomia e Engenharia de Alimentos, Campus II Samambaia, Caixa Postal 131, CEP 74001-970 Goiânia, GO, Brazil. E-mail: partelli@yahoo.com.br [2]Universidade Estadual do Norte Fluminense Darcy Ribeiro, Centro de Ciências e Tecnologias Agropecuárias, Avenida Alberto Lamego, nº 2.000, Parque Califórnia, CEP 28013-602 Campos dos Goytacazes, RJ, Brazil. E-mail: henrique@uenf.br, pirapora@uenf.br [3]Instituto de Investigação Científica Tropical, Centro de Ecofisiologia, Bioquímica e Biotecnologia Vegetal, Avenida República, Quinta do Marquês, 2784-505 Oeiras, Portugal. E-mail: peichler@iict.pt, cochichor@iict.pt [4]Instituto Superior de Agronomia, Tapada da Ajuda, 1349-017 Lisboa, Portugal. E-mail: anadr@isa.unl.pt

Abstract – The objective of this work was to evaluate photoprotective mechanisms related to low positive temperatures in *Coffea canephora* (Conilon clones 02 and 153) and *C. arabica* ('Catucaí' IPR 102) genotypes, involved in cold temperature tolerance. To accomplish this, one-year-old plants were successively submitted to: temperature decrease of 0.5ºC day^{-1}, from 25/20ºC to 13/8ºC; a three-day chilling cycle at 13/4ºC; and a recovery period of 14 days (25/20ºC). During the experiment, leaf gas exchange, chlorophyll *a* fluorescence and leaf photosynthetic pigment content were evaluated. Total activity of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) and ribulose-5-phosphate kinase (Ru5PK) were quantified to measure the activity of photosynthesis key enzymes. All genotypes showed low temperature sensitivity, but displayed diverse cold impact and recovery capabilities regarding the photosynthetic-related parameters studied. Catucaí IPR 102 cultivar showed better ability to cope with cold stress than the Conilon clones, especially Conilon 02, and had full recovery of leaf gas exchange, fluorescence parameters, enzymatic activity, and higher contents of the photoprotective pigments zeaxanthin and lutein.

Index terms: *Coffea*, carotenoids, photoprotection, photosynthesis, rubisco.

Impacto de baixas temperaturas em parâmetros fotossintéticos de genótipos de cafeeiro

Resumo – O objetivo deste trabalho foi avaliar mecanismos de fotoproteção relacionados a temperaturas baixas positivas em genótipos de *Coffea canephora* (clones Conilon 02 e 153) e *C. arabica* ('Catucaí' IPR 102), envolvidos na tolerância a baixas temperaturas. Para tal, plantas com um ano de idade foram expostas sucessivamente a: decréscimo da temperatura (0,5ºC dia^{-1}), de 25/20ºC até 13/8ºC; um ciclo de três dias a 13/4ºC; e a 14 dias de recuperação (25/20ºC). Durante o experimento, foram avaliadas as trocas gasosas, a fluorescência da clorofila *a* e os teores de pigmentos fotossintéticos foliares. Foram quantificadas a atividade total da ribulose-1,5-bisfosfato carboxilase/oxigenase (Rubisco) e da ribulose-5-fosfato quinase (Ru5PK), para medir a atividade de enzimas-chave da fotossíntese. Todos os genótipos mostraram sensibilidade a baixas temperaturas, mas tolerância e capacidade de recuperação diferentes no que respeita aos diversos parâmetros fotossintéticos estudados. A cultivar Catucaí IPR 102 apresenta maior capacidade de suportar o estresse do frio que os clones de Conilon, em particular o Conilon 02, com completa recuperação dos parâmetros de trocas gasosas foliares, de fluorescência e das atividades enzimáticas, e teores mais elevados dos pigmentos fotoprotetores zeaxantina e luteína.

Termos para indexação: *Coffea*, carotenoides, fotoproteção, fotossíntese, rubisco.

Introduction

The genus *Coffea* has approximately 100 species, with commercial relevance for *C. arabica* and *C. canephora* (Davis et al., 2006). Brazil is the world's largest coffee producer and exporter. Coffee is a major source of income, employment and development in the producing and processing regions.

Low temperatures interfere with the photosynthetic process in several ways. They lower stomatal conductance, photochemical efficiency of the photosystem (PS) II, thylakoid electron transport rate, enzyme activity and carbon metabolism, as well as the photosynthetic pigment complex systems (Suzuki et al., 2008) and membrane lipids (Campos et al., 2003).

Coffee is particularly sensitive to cold, especially *C. arabica*, *C. canephora* and *C. dewevrei* (DaMatta et al., 1997; Ramalho et al., 2003), which are responsible for over 99% of the world's coffee production. Previous works showed that photosynthesis is strongly reduced below 18ºC (Ramalho et al., 2003), while temperatures around 4ºC dramatically depress photosynthetic performance and yield (DaMatta et al., 1997; Silva et al., 2004). However, a gradual exposure to low positive temperatures highlighted the possibility of photosynthetic cold acclimation in some coffee genotypes (Ramalho et al., 2003), which was related to membrane stability (Campos et al., 2003). Oxidative stress often occurs when plants remain under adverse environmental conditions (drought, high irradiance, extreme temperatures, and nutritional stresses) due to changes in the light energy capture balance and in its use (Demmig-Adams et al., 1995; Ramalho et al., 2003). In fact, when the energy trapped by the photosynthetic pigments exceeds consumption requirements for carbon assimilation, increased production of highly reactive molecules of chlorophyll (^{3}Chl) and oxygen ($^1O_2$,$^{\cdot}O_2^-$, H_2O_2 and OH) may occur, leading to damages in the photosystems, in enzymes, in membrane lipids and in DNA (Suzuki et al., 2008). Therefore, the xanthophyll cycle is an important photoprotective mechanism when excess of light energy occurs, since it performs thermal energy dissipation, preventing overproduction of highly reactive molecules of chlorophyll and oxygen (Ma et al., 2003; Ramalho et al., 2003; Cai et al., 2007).

Understanding the physiological and biochemical response mechanisms to low temperatures in *Coffea* can contribute to selecting tolerant genotypes and improving crop management. Therefore, the objective of this work was to contribute to the characterization of response mechanisms that might permit *C. canephora* and *C. arabica* plants to cope with low temperatures.

Material and Methods

The experiment was done at the Centro de Ecofisiologia, Bioquímica e Biotecnologia Vegetal, Instituto de Investigação Científica Tropical, in Oeiras, Portugal. One-year-old plants of *Coffea canephora* cv. Conilon, clones 02 (early ripening) and 153 (late ripening), widely cultivated genotypes, and *C. arabica* cv. Catucaí IPR 102, a newly bred genotype with potential cold tolerance, were used. Plants were grown in 3-L pots containing soil:sand (4:1) substrate and organic matter plus chemical nutrients, in a greenhouse with controlled environment to prevent night temperatures lower than 14ºC during the winter. They were then transferred to walk-in growth chambers (10000 EHHF, Aralab, Portugal) with 750–900 μmol m^{-2} s^{-1} of irradiance, external air CO_2 concentration of 380 μL L^{-1}, 70% relative humidity, 12-h photoperiod and 25/20ºC (day/night) temperatures for 15 days to allow plant stabilization in these conditions set as control. Afterwards, the plants were exposed to a gradual reduction in temperature of 0.5ºC day^{-1}, from 25/20ºC to 13/8ºC, for 24 days, to allow the expression of potential responses to low temperatures. After that, plants were exposed to a three-day chilling at 4ºC at night and in the first four hours of the next day, and to 13ºC during daytime (three days at 13/4ºC), followed by a recovery period of 14 days in which the temperature was raised to 20/15ºC in the first day and to 25/20ºC for the following 13 days. All determinations were performed in similar recently mature leaves of the upper part of the plants.

Photosynthetic net CO_2 assimilation (A) and stomatal water vapor (g_s) conductance rates, as well as internal CO_2 concentrations (C_i) were measured in ten leaves, using a portable IRGA open system (CIRAS 1, PP Systems, England). Measurements were carried out on five plants per genotype at 9, 11, 13, 15 and 17 h. The photosynthesis and the fluorescence data collected during the light period represent the average of those five points, integrating the whole day period.

Chlorophyll (Chl) *a* fluorescence and gas exchange were evaluated on the same leaves at the same times and also in the dark at the end of the night period using a PAM-2000 system (H. Walz, Effeltrich, Germany). Minimum (F_o) and maximal (F_m) fluorescence, as well as the photochemical efficiency of photosystem II (F_v/F_m) were determined in the dark. The photochemical (q_P) and nonphotochemical (q_{NP}) quenchings (Kooten & Snell, 1990), the estimation of the quantum yield of photosynthetic noncyclic electron transport (ϕ_e) (Genty et al., 1989) and the photosystem II (PSII) energy conversion efficiency ($F_{v'}/F_{m'}$) (Krüpa et al., 1993) were obtained under steady-state photosynthetic conditions (irradiance of 550 μmol m^{-2} s^{-1} and saturating flashes of 6,000 μmol m^{-2} s^{-1}) and calculated as described by Ramalho et al. (2002). Both measurements were taken from five plants per genotype (two measurements per

leaf) at night and during the day, and were done on the same leaves.

Photosynthetic pigments were analyzed in eight samples (with four foliar discs of 0.5 cm^2 each) randomly collected from recently mature leaves from four different plants, at the end of the dark period and after two hours of illumination. Samples were immediately frozen in liquid nitrogen at -80ºC until analysis. The homogenization of leaf tissues and subsequent reversed-phase HPLC separation, identification and quantification of individual carotenoids was based on Ramalho et al. (1997). The de-epoxidation state [DEPS = (0.5A + Z)/(V + A + Z)], involving the xanthophyll cycle and the antheraxanthin (A), violaxanthin (V) and zeaxanthin (Z) components, was calculated as in Schindler et al. (1994). Chlorophyll content was evaluated spectrophotometrically, according to Lichtenthaler (1987).

Four samples (with four foliar discs of 0.5 cm^2 each) were taken from the same leaves used for pigment analyses after two hours of illumination to measure the activity of photosynthesis key enzymes. Homogenization and evaluation of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) and ribulose-5-phosphate kinase (Ru5PK) total activity were done as described by Maroco et al. (1999).

Data were subjected to analyses of variance, at 5% of probability, in a factorial arrangement with two factors (genotype and temperature, including the recovery period) for pigment and for enzyme activity, and three factors (genotype and temperature, including the recovery and daytime periods) for gas exchange and fluorescence . Treatment means were compared by Tukey's test at 5% of probability.

Results and Discussion

The several daily determinations of leaf gas exchange and fluorescence parameters did not show significant differences. Accordingly, diurnal points were presented as a global daytime average for these parameters.

Stomatal closure is frequently pointed out as one of the first limitations for photosynthesis under low temperatures (Ramalho et al., 2003). However, despite the significant and positive correlation (r = 0.80) between A and g_s (Figure 1 A and B), the decrease in A was not caused by a lower C_i supply, since this parameter gradually increases when A decreases (Figure 1 C), as reflected in a significant and negative correlation (r = -0.86) between C_i and A. Conilon 02 presented higher C_i values during the entire experiment. The A values were strongly affected by the cold in the three genotypes. From 13/8ºC until the first day of the recovery period, A was dramatically affected, and showed negligible values in the three genotypes. However, after that, significant differences were detected amongst the genotypes, and showed recoveries of 100 (Catucaí IPR 102), 75 (Conilon 153) and 50% (Conilon 02) of their respective control values by the end of the experiment (Figure 1 A).

Concomitantly, g_s was enhanced in all genotypes, but with different recovery, with Catucaí IPR 102 with higher values than the control (25/20ºC) seven days after the end of the stress period, which is significantly higher than for the two other Conilon genotypes. C_i decreased during the recovery period, stabilizing around 200–250 $\mu L\ L^{-1}$, which could be enough to obtain maximum A values, particularly in Catucaí IPR 102, which completely recovered for A, g_s and C_i. Thus, low A values during exposure to low temperatures was not caused by CO_2 restriction at the carboxylation sites due to stomatal closure. Instead, they would be related to metabolic limitations caused by low temperature. Reductions in A and in growth rate under seasonal low temperatures (minimum of 8ºC) in field conditions (Silva et al., 2004), and A decreases in *C. arabica* cv. Red Catuaí and *C. canephora* cv. Conilon in the winter (19.4/13.9ºC) (DaMatta et al., 1997) were also reported. Red Catuaí showed strong A depression, followed by a g_s decrease of 75% and a C_i increase of 34%, while Conilon showed lower impact on A and insignificant changes in g_s and C_i, corroborating the data obtained in this experiment and pointing out to A limitations other than stomatal.

The moderately low temperature of 18/13ºC induced a significant decrease in Rubisco activity in Catucaí IPR 102 and Conilon 153 (Figure 2 A), which was not affected further at lower temperatures. These results agree with those reported for *C. canephora* cv. Apoatã and for *C. dewevrei* under similar conditions (Ramalho et al., 2003). Interestingly, Conilon 02 was significantly affected only after chilling; when Catucaí IPR 102 also showed lower values, but Conilon 153 had values close to the control (25/20ºC). The reduction in the activity of Rubisco with cold confirms the suggestion that A was not limited by C_i, and could be related to several factors. In fact, under

cold conditions, negative effects on Rubisco could result from the activity of highly reactive molecules that are commonly overproduced in cold sensitive genotypes, due to the lack of substrate, chemical energy and reducing power (Maroco et al., 1999), or to monosaccharide accumulation (Ramalho et al., 2003).

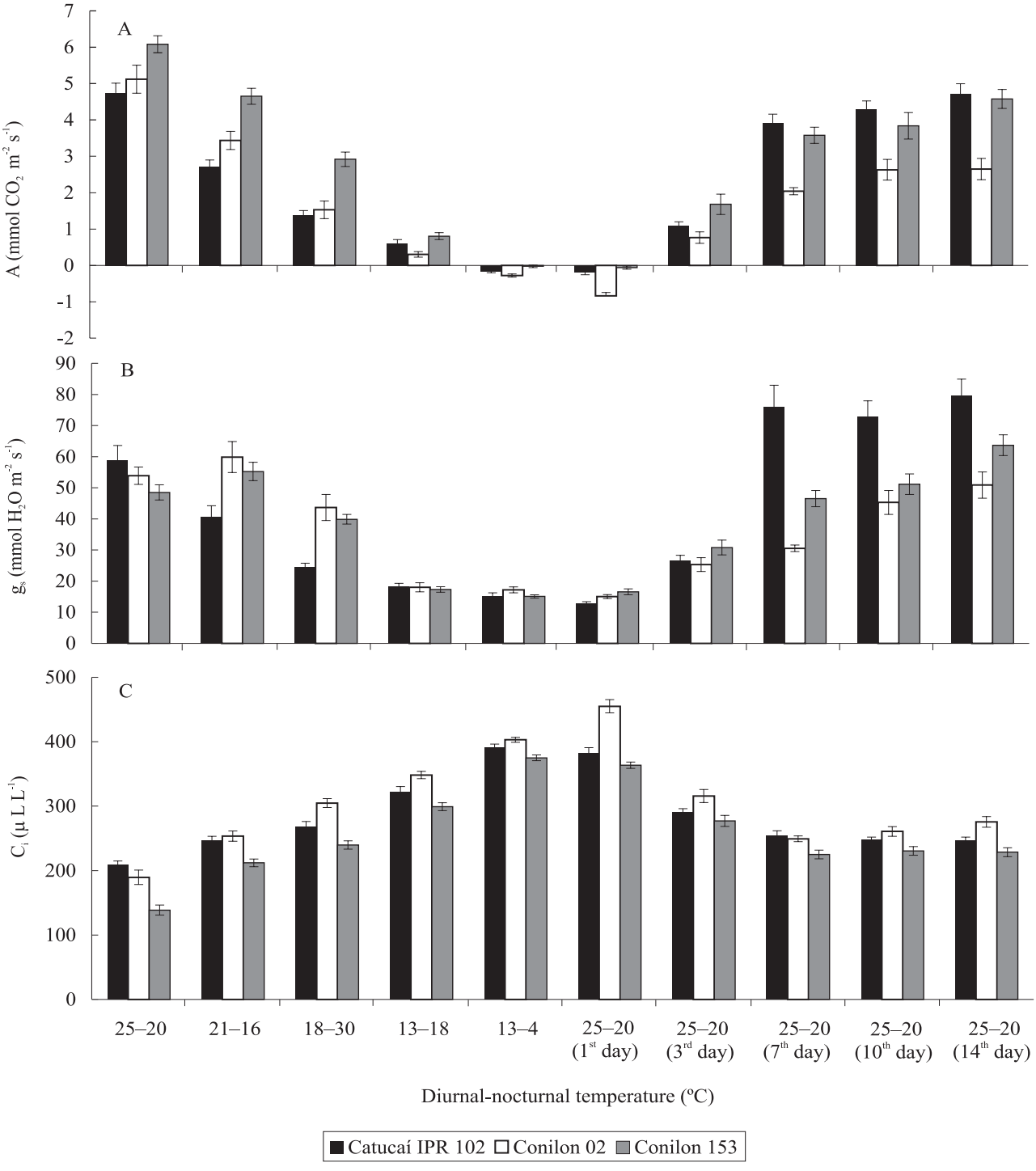

Figure 1. Changes in: A, net photosynthesis; B, stomatal conductance (gs) rates; and C, internal concentration of CO_2 (C_i) in *Coffea* sp. Catucaí IPR 102, Conilon 02 and Conilon 153 genotypes submitted to a gradual decrease of temperature (25–20ºC to 13–8ºC) for 24 days, three days at 13–4ºC, and to a recovery period of 14 days at 25–20ºC. Bars represent standard error (n = 25). Least significant difference ($p<0.05$) of 0.69 and 0.94 for genotypes and periods, respectively; coefficient of variation, 45.18%.

Furthermore, Ru5PK activity (Figure 2 B) decreased in Catucaí IPR 102 and Conilon 153, which may be caused by low availability of RuBP (Fredeen et al., 1990), thus strengthening the hypothesis that the observed strong A reduction with cold might include limited substrate availability for the Calvin cycle.

Conilon 02 showed lower effects on the Rubisco and Ru5PK activities than the other genotypes during gradual temperature reduction, and complete recovery was observed by the end of the experiment. This suggests that, at least in the recovery period, there are other restraints for the photosynthetic metabolism in Conilon 02, limiting A recovery to only 50% of its initial control value at 25/20ºC. Furthermore, Catucaí IPR 102 promptly and completely recovered enzyme activity after the end of stress, which agrees with complete A recovery; while Conilon 153 showed partial recovery of A (75%), Rubisco (73%) and Ru5PK (81%) activities by the end of the experiment.

Chlorophyll *a* fluorescence can give reliable information on chloroplast light energy capture and processing. With the gradual temperature decline, the maximum quantum efficiency of PSII (F_v/F_m) and the maximum fluorescence (F_m) decreased (Figure 3 A and B), as also observed in *Zea mays* under 5ºC (Ribas-Carbo et al., 2000). These values started to increase in the three genotypes during the third day of recovery, showing total recovery ten days after exposure to chilling. However, during the exposure to cold, the impact on F_v/F_m

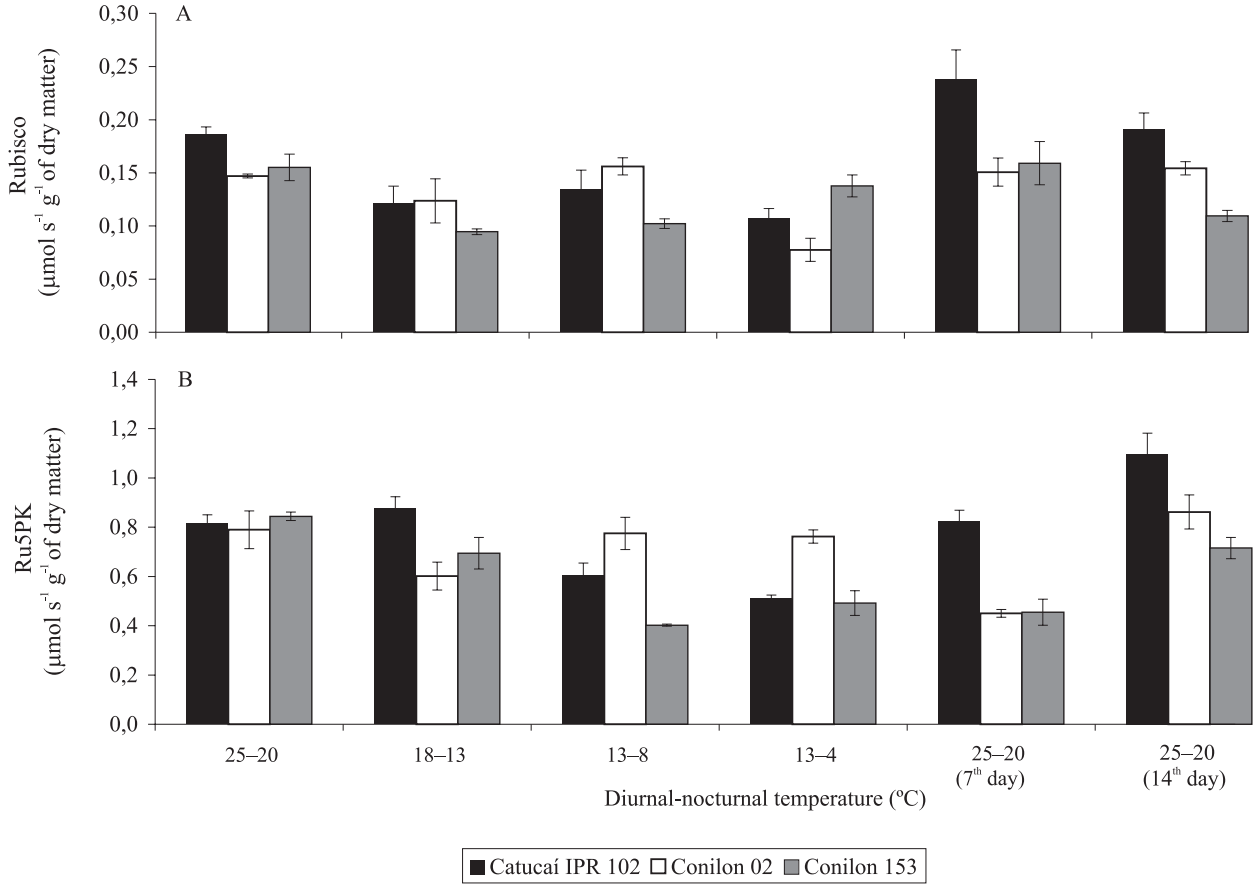

Figure 2. Changes in: A, Rubisco; and B, Ru5PK activities (µmol s^{-1} g^{-1} dry weight) in leaves collected after two hours of illumination in *Coffea* sp. Catucaí IPR 102, Conilon 02 and Conilon 153 genotypes submitted to: a gradual decrease of temperature (25–20ºC to 13–8ºC) for 24 days; three days at 13–4ºC; and to a recovery period of 0.046 and 0.057 for genotypes and periods, respectively; CV, 19.25%. Bars represent standard error (n = 4). For Rubisco, least significant difference (LSD, 5%) of 14 days at 25–20ºC. For Ru5PK, LSD of 0.173 and 0.212 for genotypes and periods, respectively; coefficient of variation (CV), 14.53%.

and $F_{v'}/F_{m'}$ (Figure 4 A), cannot be attributed solely to photochemical damages, since the diurnal and nocturnal zeaxanthin content increased with the cold conditions (Table 1). In fact, some xanthophylls are well-known photoprotective substances that act by thermal dissipation, thus avoiding energy overpressure of the photosynthetic apparatus (Demmig-Adams et al., 1995).

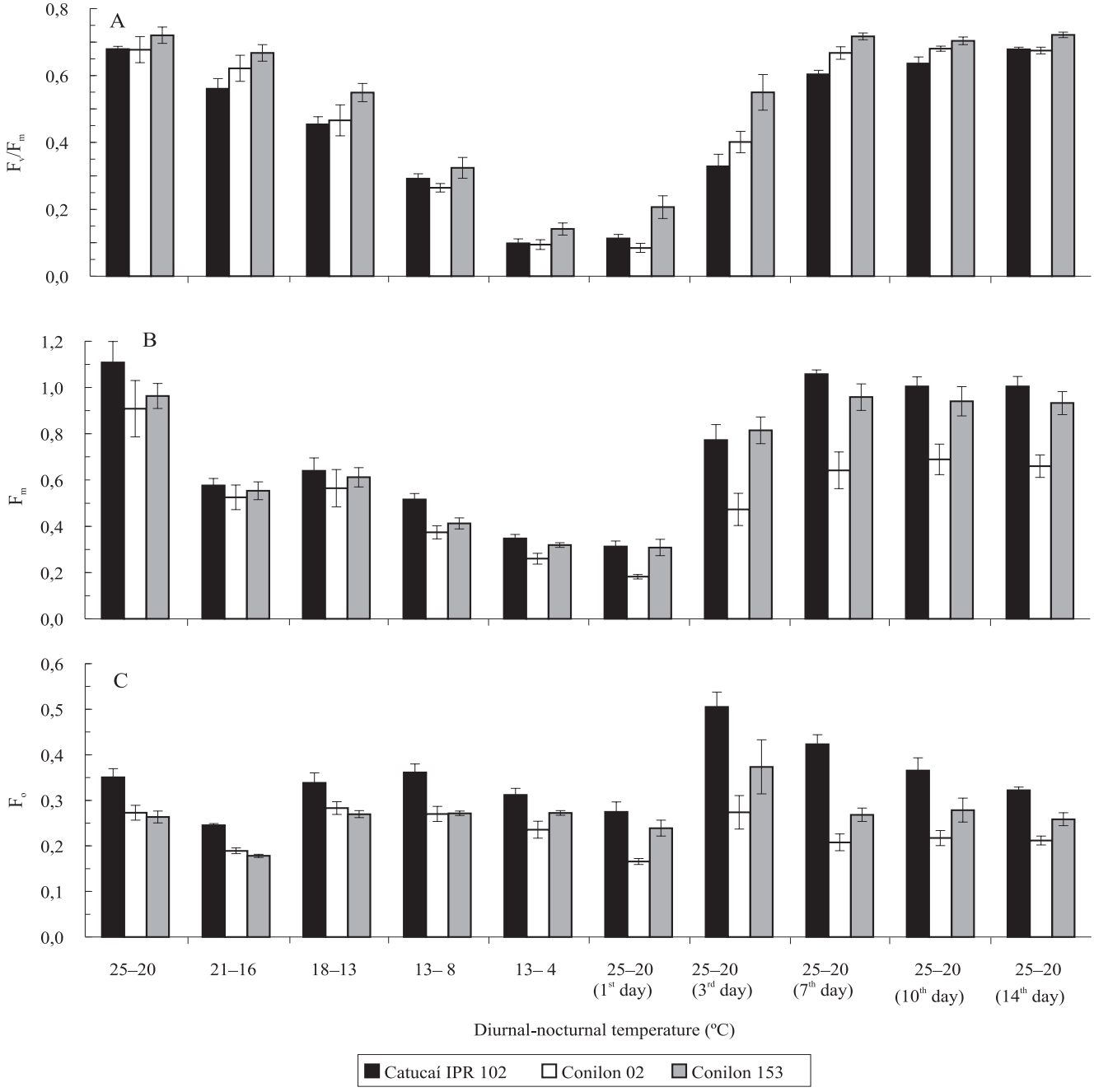

Figure 3. Changes in: A, maximum quantum efficiency of PSII (Fv/Fm), LSD of 0.083 and 0.113 for genotypes and periods, respectively, and CV of 11.60%; B, maximum (F_m), LSD of 0.185 and 0.251 for genotypes and periods, respectively, and CV of 19.03%; and C, minimum (F_o) fluorescence, LSD of 0.071 and 0.096 for genotypes and periods, respectively, and CV of 16.63%. Results from *Coffea* sp. Catucaí IPR 102, Conilon 02 and Conilon 153 genotypes submitted to: a gradual decrease of temperature (25–20ºC to 13–8ºC) for 24 days; three days at 13–4ºC; and a recovery period of 14 days at 25–20ºC. Bars represent standard error (n = 5). LSD, least significant difference; CV, coefficient of variation.

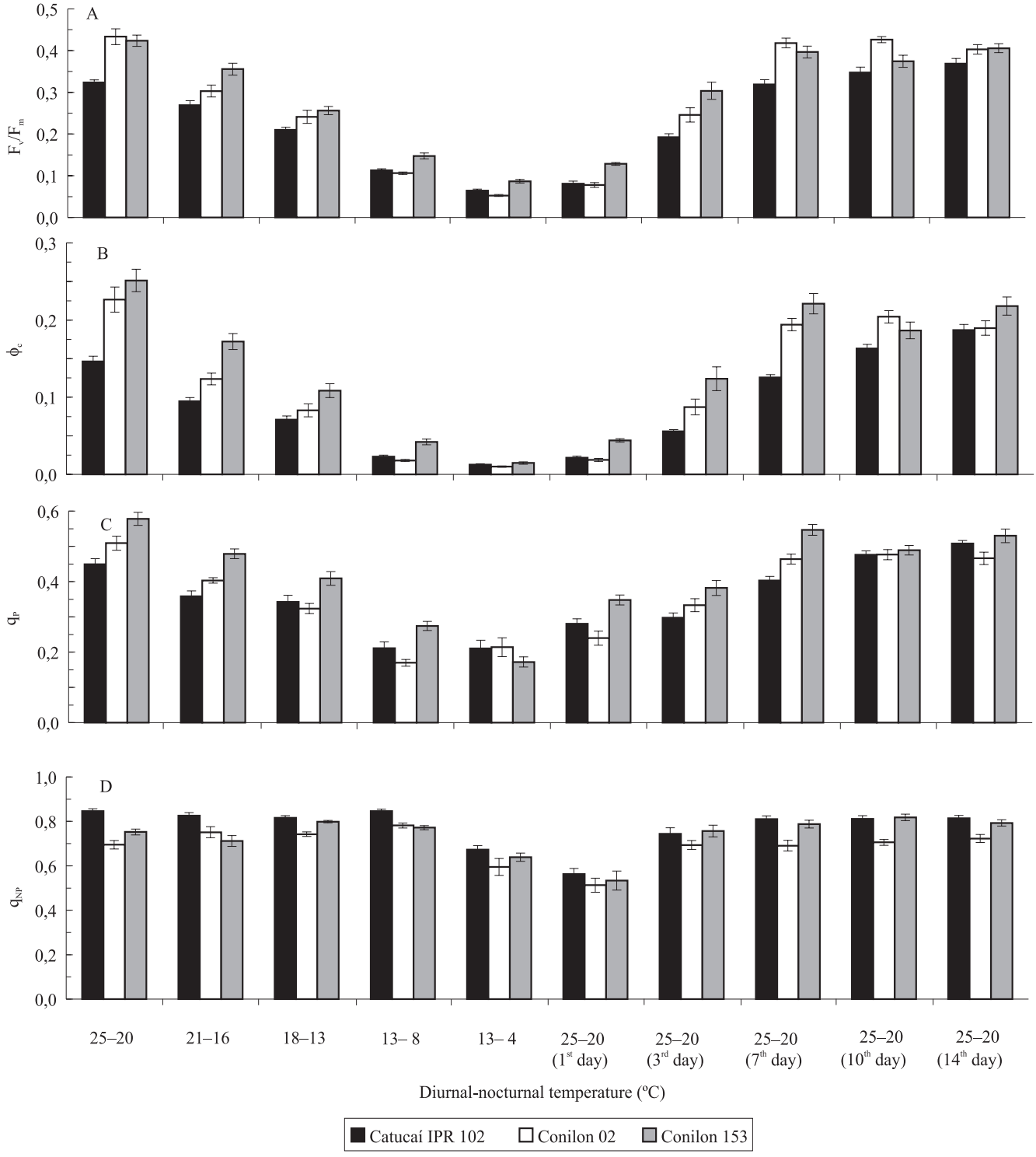

Figure 4. Changes in: A, photochemical efficiency of PSII (Fv'/Fm'), LSD of 0.037 and 0.050 for genotypes and periods, respectively, and CV of 21.27%; B, estimate of quantum efficiency of linear electron transport (ϕ_e), LSD of 0.027 and 0.036 for genotypes and periods, respectively, and CV of 35.64%; C, photochemical (q_P), LSD of 0.054 and 0.073 for genotypes and periods, respectively, and CV of 21.67%; and D, nonphotochemical (q_{NP}) quenchings, LSD of 0.067 and 0.091 for genotypes and periods, respectively, and CV of 13.87%. Results from *Coffea* sp. Catucaí IPR 102, Conilon 02 and Conilon 153 genotypes submitted to: a gradual decrease of temperature (25–20ºC to 13–8ºC) for 24 days; three days at 13–4ºC; and a recovery period of 14 days at 25–20ºC. Bars represent standard error (n = 25). LSD, least significant difference; CV, coefficient of variation.

Hence, the reduction of F_m, F_v/F_m and $F_{v'}/F_{m'}$ would contribute to the increase in nonphotochemical dissipation due to night retention and diurnal buildup of zeaxanthin (Table 1) (Demmig-Adams & Adams III, 1992; Ramalho et al., 2000, 2003). That decrease can also be linked to the presence of photochemical inactive reaction centers of the PSII that dissipate thermal energy. These defense mechanisms compete for energy with photochemical events, but reduce the photochemical efficiency of PSII (Krause, 1994). However, the maintenance of lower F_m in Conilon 02 and $F_{v'}/F_{m'}$ in all genotypes until the seventh day of recovery (Figure 4 A), when the zeaxanthin level was similar in Conilon clones or lower in Catucaí IPR 102 than in the controls (Table 1), suggests the presence of some not readily reversible deleterious effects despite the recovery of F_v/F_m.

In fact, Conilon 02 showed degradation of chlorophyll (Table 2) during the recovery period, leading to significantly lower chlorophyll contents in comparison with its own control and with the other genotypes, and agreeing with the lower F_m value. Since

Table 1. Changes in leaf carotenoid contents (μg g^{-1} dry matter) after two hours of illumination and in the night period (only zeaxanthin and DEPS) in the coffee genotypes Catucaí IPR 102 (Cat.), Conilon 02 and Conilon 153 exposed to a gradual decrease of temperature (25/20ºC to 13/8ºC) for 24 days, to three days at 13/4ºC and to a recovery period of 14 days at 25/20ºC[(1)].

Genotypes	25/20ºC	18/13ºC	13/8ºC	13/4ºC	25/20ºC (7th day)	25/20ºC (14th day)
			Alpha-carotene			
Cat. IPR 102	59.6Ba	34.2Bb	13.5Bc	8.2Ac	11.6Bc	26.8Bb
Conilon 02	88.2Aa	61.9Ab	22.5Ac	5.5Ad	10.4Bd	14.0Ccd
Conilon 153	48.3Ca	35.9Bb	22.1Ac	11.8Ad	36.5Ab	40.9Aab
CV			23.18%			
			Beta–carotene			
Cat. IPR 102	157.0Ba	110.4Bb	73.9Bb	68.4Bd	86.4Bcd	103.3Bbc
Conilon 02	165.2Ba	123.0ABb	70.3Bcd	65.6Bd	78.2Bcd	89.1Bc
Conilon 153	201.4Aa	138.4Ab	134.2Ab	100.9Ac	141.5Ab	133.6Ab
CV			14.09%			
			Alpha/beta-carotene			
Cat. IPR 102	0.38Ba	0.31Bab	0.18Bcd	0.12Ad	0.13Bd	0.26Abc
Conilon 02	0.54Aa	0.53Aa	0.32Ab	0.08Ac	0.13Bc	0.16Bc
Conilon 153	0.24Aab	0.26ba	0.16Bbc	0.11Ac	0.26Aa	0.31Aa
CV			22.51%			
			Lutein			
Cat. IPR 102	718.4Aa	558.1Ab	444.5Acd	486.6Abcd	517.3Abc	409.2Ad
Conilon 02	473.8Ba	447.0Bab	358.2Bbc	375.9Bbc	294.7Ccd	234.0Bd
Conilon 153	516.1Ba	421.7Bab	509.9Aa	413.8ABb	398.5Bb	339.5Ab
CV			15.00%			
			Zeaxanthin (diurnal period)			
Cat. IPR 102	235.5Ab	268.6Aab	238.3Bb	284.5Aa	109.3Ad	163.0Ac
Conilon 02	93.4Cd	151.0Cbc	189.9Cb	236.9Ba	80.2Ad	117.2Bcd
Conilon 153	180.3Bc	223.3Bbc	301.3Aa	255.9ABb	111.0Ad	183.5Ac
CV			15.86%			
			DEPS (diurnal period)			
Cat. IPR 102	0.71Ab	0.85Aa	0.90Aa	0.91Aa	0.56Ac	0.73Ab
Conilon 02	0.50Bd	0.78Bb	0.90Aa	0.92Aa	0.61Ac	0.78Ab
Conilon 153	0.69Ac	0.88Aa	0.92Aa	0.93Aa	0.60Ad	0.77Ab
CV			6.29%			
			Zeaxanthin (nocturnal period)			
Cat. IPR 102	65.1Bd	111.8Ac	148.5Ab	193.3Aa	35.7Ae	37.1ABe
Conilon 02	46.8Cd	81.9Bc	137.8Ab	177.1Ba	38.8Ad	28.2Bd
Conilon 153	89.8Ac	116.2Ab	105.9Bbc	170.8Ba	32.9Ad	45.4Ad
CV			14.84%			
			DEPS (nocturnal period)			
Cat. IPR 102	0.44Bd	0.62Bc	0.74ABb	0.80Ba	0.33Be	0.32Be
Conilon 02	0.44Bd	0.62Bc	0.76Ab	0.85Aa	0.45Ad	0.40Ad
Conilon 153	0.49Ac	0.67Ab	0.70Bb	0.87Aa	0.31Be	0.40Ad
CV			7.21%			

[(1)]Mean values (n = 8) followed by equal letters, capital in the columns and lowercase in the rows, do not differ by Tukey's test at 5% of probability. CV, coefficient of variation; DEPS, de-epoxidation status involving zeaxanthin cycle components.

F_v/F_m values were close to those of the control on the seventh day after chilling exposure and onwards, it can be assumed that the existing reaction centers are functional, but presumably in lower quantities. On the other hand, at the beginning of the recovery period (third day), F_o increased in all genotypes, especially Catucaí IPR 102 and Conilon 153 (Figure 3 C). As referred by Ramalho et al. (2002), such rise in F_o might be related to problems in light capture by the antenna pigments and to the transfer of excitation energy to the reaction center, that could be linked to inhibition of electron transfer between quinones Q_A and Q_B, suggesting damage of the D1 protein (Dias & Marenco, 2006). The observed recovery of this photoinactivation state is coherent with the gradual return of F_o to the control values in Catucaí IPR 102 and Conilon 153 after the third day of recovery (Figure 3 C), which is concomitant with the recovery of the A rate (Figure 1 A).

At 21/16ºC, ϕ_e was strongly affected in the three genotypes, and showed minimal values (around 4–6% of the control) after chilling exposure (Figure 4 B), suggesting severe impact on electron transport, as reported in droughted coffee plants (DaMatta et al., 1997). Suzuki et al. (2008) maintained that this lower quantum-yield efficiency of linear electron transport, together with reduced activity in PSII (given by $F_{v'}/F_{m'}$), associated with the reduction in temperature, affected the decrease in the redox capability of quinones. Very low ϕ_e values were still observed on the first day of recovery, justifying the negative values of A observed at this temperature, but complete recovery was observed on the seventh day.

The q_P (Figure 4 C) reflects the reduced state of the first stable electron acceptor in PSII, with Q_A providing an estimate of light energy driven to reduce $NADP^+$. With the temperature drop, q_P was strongly reduced, with maximal impacts of 55% (Catucaí IPR 102), 60% (Conilon 02) and 70% (Conilon 153) after chilling exposure. However, some recovery had already occurred one day after the stress period ended, reaching values similar to those of the controls by the seventh day. The impact on q_P was less severe than on A and ϕ_e, which also shows faster recovery, suggesting the presence of alternative electron drains, cyclic electron

Table 2. Changes in chlorophyll leaf content and total carotenoids (mg g^{-1} dry matter), as well as in their ratios, after two hours of illumination, in three genotypes of *Coffea* sp., Catucaí IPR 102 (Cat.), Conilon 02 and Conilon 153, exposed to a gradual decrease of temperature (25/20ºC to 13/8ºC) during 24 days, three days at 13/4ºC and during the recovery period of 14 days at 25/20ºC[(1)].

Genotype	25/20ºC	18/13ºC	13/8ºC	13/4ºC	25/20ºC (7th day)	25/20ºC (14th day)
			Chlorophyll *a*			
Cat. IPR 102	3.40Ba	3.33Aa	2.40Bb	2.38Ab	2.31Bb	2.53Ab
Conilon 02	3.70Ba	3.39Aa	2.37Bb	2.02Abc	1.47Cc	1.49Bc
Conilon 153	4.45Aa	3.36Ab	3.37Ab	2.40Ac	2.96Abc	2.81Abc
CV			17.85%			
			Chlorophyll *b*			
Cat. IPR 102	1.34ABa	1.37Aa	0.96ABb	0.98Ab	0.91Ab	0.90Ab
Conilon 02	1.21Ba	1.16Aa	0.84Bb	0.73Bb	0.44Bc	0.39Bc
Conilon 153	1.49Aa	1.14Ab	1.16Ab	0.88ABb	0.97Ab	0.96Ab
CV			19.86%			
			Chlorophyll *a*+*b*			
Cat. IPR 102	4.74Ba	4.70Aa	3.37Bb	3.36Ab	3.22Ab	3.43Ab
Conilon 02	4.91Ba	4.55Aa	3.21Bb	2.75Abc	1.91Bc	1.88Bc
Conilon 153	5.94Aa	4.50Ab	4.53Ab	3.27Ac	3.93Abc	3.77Abc
CV			18.18%			
			Total carotenoids			
Cat. IPR 102	1.42ABab	1.44Aa	1.14Bc	1.28Aabc	1.19Abc	1.18Abc
Conilon 02	1.29Ba	1.24Aa	1.12Ba	1.19Aa	0.81Bb	0.77Bb
Conilon 153	1.55Aa	1.24Abc	1.37Aab	1.14Abc	1.09Ac	1.02Ac
CV			14.28%			
			Chlorophyll (*a*+*b*)/Total carotenoids			
Cat. IPR 102	3.35Ba	3.26Bab	2.94Bbc	2.61Ac	2.68Bc	2.90Bbc
Conilon 02	3.79Aa	3.63Aa	2.87Bb	2.27Bc	2.35Cc	2.43Cc
Conilon 153	3.84Aa	3.62Aab	3.32Ab	2.83Ac	3.58Aab	3.68Aab
CV			8.17%			

[(1)]Mean values (n = 8) followed by equal letters, capital in the columns and lowercase in rows, do not differ by Tukey's test at 5% of probability. CV, coefficient of variation.

transport around PSI, and photorespiration (Ribeiro et al., 2004).

The q_{NP} (Figure 4 D) reflects heat dissipation processes, namely those related to the increase of trans-thylakoid proton electrochemical potential difference (ΔpH) (Maxwell & Johnson, 2000). This parameter maintained high values during most of the experiment, denoting high nonphotochemical energy dissipation related to the *PsbS* protein, which is closely associated with the PSII reaction center. After protonation, *PsbS* triggers conformational changes in the thylakoid membrane which are necessary for q_E (the main component of q_{NP}, known as "high-energy quenching"), promoting a direct interaction between chlorophyll and zeaxanthin (Müller et al., 2001; Ma et al., 2003). However, q_{NP} decreased after chilling exposure and in the first day of recovery, when ϕ_e was strongly suppressed, which might have prevented ΔpH buildup and decreased effectiveness of this mechanism. Nevertheless, it must be taken into account that q_{NP} compares changes between light and dark-adapted status. Since F_m values probably decreased due to night retention of zeaxanthin (Table 1), q_{NP} values will only quantify further increases in thermal dissipation ability in light conditions, but not in the total sustained thermal dissipation (Maxwell & Johnson, 2000; Ramalho et al., 2003), which leads to an "erroneously" lower q_{NP} value.

The analysis of the photosynthetic pigments will give insights on the impact of stress on the photosynthetic apparatus. Amongst them, carotenoids are especially important, since they contribute to the stability of light-harvesting antenna complexes, the dissipation of excess excitation energy and the reactive oxygen species scavenging (Demmig-Adams et al., 1995). With low-temperature exposure, chlorophylls *a* and *b* were severely reduced in all genotypes (Table 2). This loss was further increased in Conilon 02 during recovery, when the chlorophyll (*a* + *b*) rate dropped to 38% of the control. The strongest impact was on chlorophyll *a*.

Total carotenoids also diminuished, with Conilon 02 showing the strongest reduction, particularly during the recovery period (Table 2). Nevertheless, carotenoids were less affected than chlorophylls, leading to a decrease in ratio (total chlorophyll/total carotenoids) in all genotypes, as observed in maize (Holá et al., 2007). This ratio decrease resulting from a stronger reduction of energy-capture pigments could be considered a response to low capture of excessive light energy under stress conditions. However, by the end of the experiment, despite the clear decrease in this ratio (to 60% of initial value) in Conilon 02, the massive pigment loss (38% of total chlorophylls and 60% of total carotenoids, reflected in a yellowish-green color of the leaves), suggests failures of photosynthetic mechanisms rather than a positive response to cold exposure. This could be linked to photobleaching phenomena, thus contributing to the poorest *A* recovery fourteen days after chilling exposure.

Values of zeaxanthin and the de-epoxidation status (DEPS) involving the zeaxanthin cycle components increased in all genotypes with the exposure to low temperatures during the day (Table 1), in agreement with results reported for other coffee genotypes (Ramalho et al., 2003). DEPS and zeaxanthin values were significantly higher in Catucaí IPR 102 and in Conilon 153 in comparison to Conilon 02 to 18/13ºC and 13/8ºC respectively (Table 1). This shows a higher thermal dissipation capability in those genotypes that could prevent the production of highly reactive molecules (^{3}Chl, ^{1}Chl, $^1O_2$, H_2O_2). Photoprotective pigments also avoid the over-reduction of the thylakoid electron transport chain and the over-acidification of the thylakoid lumen, which are known to trigger photoinduced PSII damages (Müller et al., 2001). Furthermore, zeaxanthin (together with lutein and beta-carotene) may also scavenge some of those highly reactive molecules, which are usually overproduced under stress conditions (Niyogi, 1999). After chilling, Catucaí IPR 102 still showed higher zeaxanthin content than Conilon 02, but upon recovery at 25/20ºC zeaxanthin levels tended towards the control values in Conilon clones, or to lower values in Catucaí IPR 102. Those findings confirm the role of the xanthophyll cycle as an important flexible mechanism that regulates PSII activity and avoids an energy overload in the photosynthetic apparatus in *Coffea* sp., as found with low temperature (Ramalho et al., 2003), water deficit (Cai et al., 2007) and high irradiance (Ramalho et al., 2000).

Concerning the xanthophyll cycle, what happens during the night is noteworthy. In the controls, zeaxanthin and DEPS of dark collected samples showed values clearly below those obtained under light (Table 1). Those values gradually increased with cold

exposure, showing maximum values after chilling, but still below the diurnal ones. As stated above, such retention will decrease the photochemical efficiency of PSII (F_v/F_m), and was suggested as coming from the sensitivity of zeaxanthin epoxidase to cold and from the partial maintenance of the ΔpH during the night, since ATPase does not perform H^+ transport across the thylakoid membrane under cold exposure (Gilmore, 1997).

Lutein also has an important photoprotective role in the photosystems and in the stability of the energy-capturing complexes (Pogson et al., 1998; Niyogi, 1999). Xanthophyll strongly decreased in all genotypes after chilling, with Conilon 153 being affected later than the other two genotypes. Nevertheless, values were consistently higher in Catucaí IPR 102 throughout the entire experiment when compared to Conilon 02, and Conilon 153 usually assumed intermediate values (Table 1), which confirms earlier results comparing *C. arabica* cv. Icatu with *C. canephora* cv. Apoatã (Ramalho et al., 2003).

Alpha and beta-carotene values significantly decreased with low temperatures (13/4ºC) (Table 1), reaching 86 and 56% in Catucaí IPR 102, 76 and 50% in Conilon 153 and 94 and 60% in Conilon 02 respectively. This could lead to impairments at the PS level, since beta-carotene is essential for its aggregation and photoprotection (Pogson et al., 1998) through ^{3}Chl and $^1O_2$ inactivation, releasing energy as heat (Niyogi, 1999). The (alpha/beta) carotene ratio also decreased with low temperatures, but tended to rise to the initial values in Conilon 153 upon recovery (Table 1). This reduction, interpreted as a leaf response to cold, was reported from coffee plants exposed to cold (Ramalho et al., 2003) and high irradiation (Ramalho et al., 2000). However, these reports assumed that the reduction results from a beta-carotene rise that increases photoprotection, as in *C. arabica* cv. Icatu (Ramalho et al., 2003), differently from what was observed in this work, which showed beta-carotene decreases of 50% or higher at 13/4ºC and a partial recovery thereafter.

Conclusions

1. All coffee genotypes show sensitivity to low temperature at the stomatal, biochemical and biophysical levels.

2. When photosynthetic parameters are considered (gas exchange and fluorescence), Catucaí IPR 102 presents the best performance after exposure to chill.

3. No significant differences were observed with the enzymes involved in the photosynthetic pathway (Rubisco and Ru5PK), among genotypes exposed to chill, although all genotypes show consistent decline in enzyme activity.

4. Catucaí IPR 102 shows higher contents of the zeaxanthin and lutein pigments than clone 02, and frequently higher than clone 153 as well.

5. Catucaí IPR 102 presents improved recoverying ability than the Conilon clones.

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