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Parameters associated with the resistance of coffee genotypes to low temperatures[1]

Parâmetros associados à resistência de genótipos de café a temperaturas negativas

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Highlights

Climate chamber are suitable for testing cold resistance in *Coffea.*
Cold resistance can be evaluated by exposing *Coffea* plants to -4°C and -5°C.
Physiological and biochemical tests indicate cold resistance differences in *Coffea.*
Coffea racemosa is resistant to cold.

Abstract

Physiological damage to coffee plants caused by cold stress can vary according to the intensity, exposure time, genotype, age, and nutritional status of the plants. The objective of this study was to evaluate the foliar, physiological, and biochemical damages resulting from the exposure of coffee seedlings to negative temperatures and, thus, determine the minimum lethal temperature for genotypes that could be used to study coffee plants with resistance to cold. Four progenies of *Coffea arabica* with introgression of *Coffea racemosa,* three progenies of *C. arabica* with introgression of *Coffea liberica,* and *C. racemosa* were evaluated, in addition to the cultivars *C. arabica* Mundo Novo IAC 376-4 and Catuaí Vermelho IAC 81, which were used as susceptible controls. The plants were subjected to temperatures of -2°C, -3°C, -4°C, and -5°C in a climatic growth chamber. The foliar and physiological damage of the seedlings was evaluated using qualitative (visual criterion) and quantitative methods (photosynthesis, ratio between the variable and maximum fluorescence of photosystem II [Fv/Fm], electrical conductivity of the imbibition solution of leaf disks, and protein content). The experimental design was completely randomized in a 5 × 10 factorial scheme, with five temperatures, 10 genotypes, and 4 replications. Data were subjected to

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analysis of variance, and means were compared using the Scott-Knott mean cluster test at 5% significance. Pearson's correlation was performed between the means of the genotypes. Visual damage was detected at -3°C, and when correlated with the other physiological parameters, resistance was observed only in *C. racemosa.* Temperatures between -4°C and -5°C were the most suitable for testing cold resistance in coffee progenies.
Key words: *Coffea racemosa.* Frost. Cold resistance. Visual assessment. Physiological analysis.

Resumo

Os danos fisiológicos em cafeeiros causados pelo estresse de frio podem variar de acordo com intensidade, tempo de exposição, genótipos, idade e status nutricional das plantas. O objetivo deste trabalho foi avaliar os danos foliares, fisiológicos e bioquímicos decorrentes da exposição das mudas de café a temperaturas negativas e, assim, determinar a temperatura mínima letal para genótipos que poderão ser úteis em futuras pesquisas de cafeeiros mais resistentes ao frio. Foram avaliadas quatro progênies de *Coffea arabica* com introgressão de *Coffea racemosa,* três progênies de *C. arabica* com introgressão de *Coffea liberica* e a espécie *C. racemosa,* além das cultivares de *C. arabica* Mundo Novo IAC 376-4 e Catuaí Vermelho IAC 81, as quais foram usadas como controles suscetíveis. As plantas foram submetidas às temperaturas de -2, -3, -4 e -5 ºC em câmara climática de crescimento. As avaliações dos danos foliares e fisiológicos das mudas foram feitas pelo critério visual (método qualitativo) e por métodos quantitativos: fotossíntese, razão entre a fluorescência variável e máxima do fotossistema II (Fv/Fm), condutividade elétrica de solução de embebição dos discos foliares e teor de proteína. O delineamento experimental foi inteiramente casualizado em esquema fatorial 5x10, sendo 5 níveis de temperaturas e 10 genótipos, com 4 repetições. Os dados foram submetidos à análise de variância e as médias comparadas pelo teste de agrupamento de médias Scott-Knott a 5% de significância. Realizou-se correlação de Pearson entre os dados para as médias dos genótipos. Os danos visuais foram detectados a partir de -3 °C e quando correlacionados com os demais parâmetros fisiológicos, observou-se resistência somente para a espécie *C. racemosa.* Temperaturas entre -4 e -5 °C foram as mais adequadas para testar resistência ao frio em progênies de café.
Palavras-chave: *Coffea racemosa.* Geada. Resistência ao frio. Avaliação visual. Análises fisiológicas.

Introduction

The climate in a large part of Brazil currently favors coffee cultivation. However, climate change has been heavily reducing productivity worldwide in areas suitable for coffee cultivation, especially of *Coffea arabica* L., as a result of severe droughts and high temperatures (Bunn et al., 2015; Craparo et al., 2015; Van der Vossen et al., 2015). Global warming resulting from anthropogenic activities has increased these factors (Intergovernmental Panel on Climate Change [IPCC], 2014), and cultivation of this crop at high latitudes and altitudes is considered an option. However, low temperatures can have deleterious effects on the development of the coffee plant, which can cease to grow or suffer from the frost.

Coffee is particularly sensitive to extreme temperatures. The optimal annual temperatures for growing *C. arabica* and *C. canephora,* for instance, are 18-23°C and 22-26°C, respectively (Matta & Ramalho, 2006). According to Ramalho et al. (2003), photosynthesis is strongly reduced below 18°C, and temperatures close to 4°C reduce performance and gas exchange. Lima and Silva (2008) compared methods for calculating degree-days and found that the minimum and maximum basal temperatures for culture are 12.9°C and 32.4°C, respectively.

Many plant species have mechanisms to survive in environments with prolonged cold periods. Cold tolerance or resistance can be genetic but it also depends on plant age (Saini et al., 2018).

Low temperatures affect the composition and structure of photosynthetic pigment complexes and the lipid matrix of cell membranes, particularly in chloroplasts, although to different degrees among Coffea progenies and species (Batista-Santos et al., 2011; Partelli et al., 2011; Ramalho et al., 2003). These changes may reflect deficiencies or damage; however, in some cases, they respond to the acclimatization of the plant to cold.

The acclimatization capacity of coffee progenies depends on the plant's ability to produce antioxidants as a form of defense against the formation of reactive oxygen species (ROS), which are often accumulated when less photochemical energy is used for photosynthesis e.g., under abiotic stress in low temperatures (Fortunato et al., 2010; Ramalho et al., 2003), water deficit (Lima et al., 2002), and high luminosity and N deficiency (Souza et al., 2015). This characteristic results in morphological and physiological variations of acclimatized *Coffea* spp.

The *C. arabica* cultivars in Brazil have low resistance to cold because they are originated from tropical regions of Africa. However, other *Coffea* species that are less commercially available are tolerant to biotic and abiotic stress and thus potential sources of genetic variability for breeding programs (Geromel et al., 2008). Nevertheless, testing resistance of plants to cold in a field experiment is complex because predicting the duration and intensity of the cold event is difficult, as is separating its effects from other elements (Rozzetto et al., 2017).

The objective of the present study was to evaluate foliar physical, physiological, and biochemical damages resulting from the exposure of coffee seedlings to negative temperatures and to verify whether these parameters can be used for selecting genotypes resistant to cold, especially to frost damage, which is common in production areas.

Materials and Methods

Ten coffee genotypes were evaluated: four *C. arabica* with *C. racemosa* genes (E0706 5-1-1, E0706 9-1-10, E0706 13-1-3, and E0706 27-2-2); three *C. arabica* with *C. liberica* genes (H147/1, IPR 100, and IPR 105); a progeny of *C. racemosa*; and the cultivars Catuaí Vermelho IAC 81 and Mundo Novo IAC 376-4, which are pure *C. arabica* and were used as susceptible controls (Table 1). The experiment was conducted at the Instituto de Desenvolvimento Rural do Paraná - IAPAR-EMATER (IDR-Paraná), Londrina, Paraná, Brazil (23°21'21"S, 51°09'46"W).

Table 1
***Coffea arabica* genotypes with *C. racemosa* and *C. liberica* genes used in this study.**

Genotypes	Description and Origin [1]
E0706 5-1-1	Progeny F_3 from 'Catuaí IAC 81' x ['Tupi' x (C1195-5-6-2 x 'Tupi')]
E0706 9-1-10	Progeny F_3 from 'Acaiá' x ['Tupi' x (C1195-5-6-2 x 'Tupi')]
E0706 13-1-3	Progeny F_3 from 'IPR 108' x ['Tupi' x (C1195-5-6-2 x 'Tupi')]
E0706 27-2-2	Progeny F_3 from 'IPR 104' x ['Tupi' x (C1195-5-6-2 x 'Tupi')]
H147/1	Progeny F_2 de 34/13 S353 4/5 x 110/5 S4 Agaro (differentiator of rust breeds carrying the genes S_H2, S_H3, S_H4 e S_H5) [2]
IPR 100	Cultivar derivative from 'Catuaí IAC 81' x ('Catuaí IAC 81' x IAC 1110-8) [2]
IPR 105	Cultivar derivative from 'Catuaí IAC 81' x ('Catuaí IAC 81' x IAC 1110-8) [2]
Coffea racemosa	A plant from the IDR-Paraná germplasm bank
Mundo Novo IAC 376-4	*C. arabica* (Sumatra x Bourbon Vermelho)
Catuaí Vermelho IAC 81	*C. arabica* (Caturra Amarelo x Mundo Novo)

[1] C1195-5-6-2 c.950 Ep209 = plant F_2 from crossing [(*C. arabica* x *C. racemosa* C1195) x *C. arabica*] x *C. arabica*, 'Tupi' = 'Tupi IAC 1669-33', 'Catuaí IAC 81' = 'Catuaí Vermelho IAC 81', 'Acaiá' = 'Acaiá 474/4'.
[2] *C. arabica* genotypes carrying *C. liberica* genes.

Seedlings were grown in a nursery under 50% shade for approximately 28 weeks. After this period, the seedlings with eight pairs of leaves were placed in a growth chamber (commercial reference S.S. Scientific) at the Environment Simulation Laboratory of IDR-Paraná. For each temperature evaluated, the plants went through a 24 h acclimatization period at 5°C, after which negative temperatures were applied.

The plants were placed in the test area of the chamber and subjected to negative temperatures of −2°C, −3°C, −4°C, and −5°C. The seedlings of the control treatment were kept in the nursery under 50% shade. The chamber was programmed to reproduce the natural conditions of a temperature drop to simulate a frost based on historical climatic data from the state of Paraná (Nitsche et al., 2019). The irradiance in the chamber simulated daylight for approximately 12 h. The seedlings were acclimatized for 24 h at a minimum temperature of 5°C and 60% relative humidity. Then, the temperature was gradually reduced until reaching the programmed minimum, starting at 13°C, followed by 2 h reaching a negative temperature at which it remained for 3 h 30 min, and then for at least 20 min at the test negative temperature. Photosynthetic irradiance was programmed to reach zero at 06h00, 123 µmol m^{-2} s^{-1} at 06h30, 429 µmol m^{-2} s^{-1} at 10h00, and 573 µmol m^{-2} s^{-1} at 12h00, at which it was maintained until 14h00; then, it decreased to zero around 18h00. After the test, the seedlings were transferred to a nursery in 50% shade.

Visual analysis of damage caused by negative temperatures was conducted 24 h and 15 days after exposure to low temperatures, using the following 1-5 scale: 1 = no damage; 2 = light damage (0.1-25% of leaf area damaged); 3 = moderate damage

(25.01-50% of leaf area damaged); 4 = severe damage (50.01-75% of leaf area damaged), and 5 = very severe damage (>75% of leaf area damaged) (Caramori & Maneti, 2002).

Damage to the photosynthetic apparatus was evaluated 24 h after exposure to low temperatures by measuring photosynthesis in the third pair of leaves with a portable Infra-red gas analyzer (IRGA) (commercial model LC pro-SD), using artificial light with photosynthetically active radiation incidence of 869 µmol m^{-2} s^{-1} on the leaf surface. The efficiency of photosystem II (PSII) was evaluated using the ratio of variable fluorescence (Fv) to maximum fluorescence (Fm), expressed as Fv/Fm, using a commercial model OS5p fluorometer. Initial fluorescence (F0) was obtained at low light saturation (<0.1 µmol m^{-2} s^{-1}), and maximum fluorescence was determined with a saturating photon pulse of 4.000 µmol m^{-2} s^{-1} for 0.3 s set to a frequency of 600 Hz. Both evaluations were performed 24 h after plants were subjected to negative temperatures on the third pair of developed leaves.

Damage to the membrane system was analyzed using an electrical conductivity test of the leaf disks. For this, two 1.2 cm^2 leaf disks were extracted from the plants 24 h after being subjected to negative temperatures. Then, two leaf disks of each repetition were immersed in disposable plastic cups with 30 mL of distilled water at 25°C for 24 h. The reading was then performed with a digital conductivity meter (commercial model Simpla PH140). The data are expressed in µmhos cm^{-1} (Manetti et al., 2018).

To determine the total protein content, leaf samples were taken from the four replicates of each genotype 24 h after exposure to low temperatures. The samples were stored in a paper bag in a freezer (-80°C) until the time of analysis. To obtain the extract, 0.250 g of leaf tissue was weighed from each sample and macerated in 5 mL of 50 mM potassium phosphate buffer (pH 7.0) and 4% polyvinylpyrrolidone (PVP) previously cooled to 4°C, and then used to avoid oxidation of the material. This was centrifuged for 10 min at 4°C and 785 rad s^{-1}, and the supernatant was transferred to 2 mL Eppendorf tubes and stored in a freezer (-14°C) until analysis. The concentration of soluble protein of the extracts was determined in triplicate using the calibration curve of the reagent with bovine serum albumin (BSA 0-15 µg $µL^{-1}$) as a standard, following Bradford (1976) and based on the color change of the Coomassie Brilliant Blue G-250 reagent when bound to protein. Total protein concentration was calculated by comparing the readings of the materials with those obtained from the standard curve and was expressed as mg of protein g^{-1} of fresh matter (FM).

The study was conducted in a completely randomized design in a 5 × 10 factorial scheme, with five temperatures (control, -2°C, -3°C, -4°C, and -5°C) and 10 genotypes, in four replications. The data were subjected to analysis of variance, and the means were compared using the Scott-Knott mean cluster test (Borges & Ferreira, 2003) at a 5% probability level. Pearson's simple correlation coefficients (r) were calculated between the cold stress treatments and the mean results of the genotypes, and the significance of the r values was determined using the t-test at 5% probability.

Results and Discussion

The visual analysis of damage showed no significant difference between the genotypes tested at the environmental temperature and those at -2°C when evaluated 24 h after exposure to negative temperatures (Table 2). At −3°C, the H147/1 progeny was more sensitive to cold than the other genotypes. By contrast, at -4°C, only *C. racemosa* presented less visual damage, similar to the samples at -5°C. All genotypes showed damage with a score of 4 (severe damage) at -5°C, except for *C. racemosa,* which presented a score of 3 (moderate damage).

Table 2
Visual analysis of damage observed in coffee seedlings 24 h and 15 days after coffee plants of different genotypes were subjected to negative temperatures of −2°C, −3°C, −4°C, and −5°C, in addition to the control (environment).

Genotypes	Temperatures (°C)									
	After 24 h									
	Environment		−2		−3		−4		−5	
Mundo Novo	1.00	Ba*	1.00	Ba	1.00	Bb	3.50	Aa	4.00	Aa
Catuaí Vermelho	1.00	Ca	1.00	Ca	1.00	Cb	3.25	Ba	4.00	Aa
H147/1	1.00	Ca	1.00	Ca	1.75	Ba	3.50	Aa	4.00	Aa
E0706 5-1-1	1.00	Ba	1.00	Ba	1.00	Bb	3.50	Aa	4.00	Aa
E0706 9-1-10	1.00	Ba	1.00	Ba	1.00	Bb	4.00	Aa	4.00	Aa
E0706 13-1-3	1.00	Ba	1.00	Ba	1.00	Bb	3.50	Aa	4.00	Aa
E0706 27-2-2	1.00	Ba	1.00	Ba	1.00	Bb	3.75	Aa	4.00	Aa
Coffea racemosa	1.00	Ba	1.00	Ba	1.00	Bb	1.50	Bb	3.00	Ab
IPR 105	1.00	Ca	1.00	Ca	1.00	Cb	3.25	Ba	4.00	Aa
IPR 100	1.00	Ba	1.00	Ba	1.00	Bb	3.00	Aa	4.00	Aa
Coefficient of variation 17.3%										
	After 15 days									
	Environment		- 2		- 3		- 4		- 5	
Mundo Novo	1.00	Da	2.50	Cb	1.00	Dc	3.50	Bb	5.00	Aa
Catuaí Vermelho	1.00	Da	2.25	Cb	1.00	Dc	3.75	Bb	5.00	Aa
H147/1	1.00	Ca	3.00	Ba	2.75	Ba	4.50	Aa	5.00	Aa
E0706 5-1-1	1.00	Ea	3.00	Ca	2.00	Db	3.75	Bb	5.00	Aa
E0706 9-1-10	1.00	Da	2.50	Cb	1.00	Dc	4.00	Ba	5.00	Aa
E0706 13-1-3	1.00	Da	3.00	Ba	2.00	Cb	3.50	Bb	5.00	Aa
E0706 27-2-2	1.00	Ea	3.00	Ca	2.00	Db	3.75	Bb	5.00	Aa
Coffea racemosa	1.00	Ca	3.00	Aa	2.00	Bb	1.50	Bc	3.00	Ab
IPR 105	1.00	Da	3.00	Ba	2.00	Cb	3.50	Bb	5.00	Aa
IPR 100	1.00	Ea	3.00	Ca	2.00	Db	4.00	Ba	5.00	Aa
Coefficient of variation: 14.4%										

*Means followed by the same uppercase letter in the row and lowercase letter in the column do not differ from each other according to the Scott-Knott test at 5% probability.

The average scores of leaf damage were higher after 15 days of stress than after 24 h. At -2°C and -3°C, the genotypes that did not show visual damage 24 h after being subjected to negative temperatures showed mild to moderate damage after 15 days. When the plants were exposed to −4°C, only *C. racemosa* was resistant. At -5°C, *C. racemosa* remained with less visual damage, whereas the other species reached a score of 5, with 100% of leaf blade death.

Similar results were obtained by Caramori and Manetti (2002), who observed in Mundo Novo IAC 376-4 light to moderate damage at -2°C, moderate to severe damage at -3°C, and severe damage at -4°C. The results of the present analysis showed that -4°C and -5°C were the most suitable temperatures to identify resistant genotypes, as leaf damage was, respectively, severe and very severe in sensitive coffee plants, and mild and moderate in *C. racemosa.*

Genotypes H 147/1, IPR 100, and IPR 105 are Arabica coffee plants with genes for *C. liberica* var. *liberica;* they were all sensitive to cold, especially H 147/1, which showed greater susceptibility to negative temperatures. Thus, *C. liberica* var. *liberica* may be indeed sensitive to cold, unlike the resistant *C. liberica* var. *dewevrei* (Matta et al., 1997).

Coffea racemosa was the most resistant to negative temperatures in the visual analysis at 24 h and 15 d after exposure, with light damage occurring only at -4°C, whereas *C. arabica* had severe damage. These results corroborate those from field evaluations carried out in July 2000, when severe frost (minimum temperature in the meteorological shelter of -1.3°C) occurred in Londrina-PR and *C. racemosa* and the triploid hybrid (*C arabica* x *C. racemosa*; interspecific cross) were resistant to cold (Petek et al., 2005).

After two consecutive frosts in June 2011 in Londrina-PR, F2 progenies of *C. arabica* with introgression of *C. racemosa* were identified from the same origin as that of the F3 progenies in our study, and presented moderate and light damage, while the cultivars Catuaí IAC 81 and Mundo Novo IAC 376-4 were severely damaged (Mariucci et al., 2022). In the present study, the *C. arabica* progeny with introgression of *C. racemosa* was resistant to negative temperatures, probably because they were not selected for frost resistance. Another hypothesis is that in our study, the plants had little time (24 h) for acclimatization at 5°C, whereas in the field conditions of Mariucci et al. (2022), the plants had more time to acclimatize to the low temperature, possibly allowing the activation of cold resistance mechanisms.

The photosynthetic apparatus of the studied coffee seedlings were damaged at -2°C, with E0706 9-1-10 and *C. racemosa* suffering less stress, presenting values statistically equal to those of the control treatment. *Coffea racemosa* showed higher photosynthetic activity after exposure to −3°C, differing from the other genotypes. At -4°C and -5°C, no significant differences were found among the genotypes, as the photosynthetic activity was mostly close to zero and had no significant values (Table 3). This corroborates the results of Matta et al. (1997), who observed that temperatures between -3°C and -4°C caused tissue death in coffee plants.

Table 3
Photosynthesis (μmol m^{-2} s^{-1}) of coffee seedlings of different genotypes 24 h after being submitted to temperatures of −2°C, −3°C, −4°C, and −5°C, in addition to the control (environment).

Genotypes	Temperatures (°C)									
	Environment		−2		−3		−4		−5	
Mundo Novo	8.11	Aa*	5.50	Bc	0.49	Cb	0.00	Ca	0.00	Ca
Catuaí Vermelho	7.64	Ab	4.48	Bd	0.47	Cb	0.00	Ca	0.00	Ca
H147/1	7.39	Ab	5.69	Bc	0.52	Cb	0.00	Ca	0.00	Ca
E0706 5-1-1	8.26	Aa	5.79	Bc	0.49	Cb	0.00	Ca	0.00	Ca
E0706 9-1-10	8.10	Aa	7.70	Aa	0.43	Cb	0.00	Ca	0.00	Ca
E0706 13-1-3	7.60	Ab	6.48	Bb	0.38	Cb	0.00	Ca	0.04	Ca
E0706 27-2-2	8.61	Aa	6.83	Bb	0.72	Cb	0.00	Ca	0.03	Ca
Coffea racemosa	8.39	Aa	7.35	Aa	3.73	Ba	0.00	Ca	0.09	Ca
IPR 105	7.51	Ab	6.48	Bb	0.60	Cb	0.00	Ca	0.13	Ca
IPR 100	7.14	Ab	6.20	Bb	0.32	Cb	0.00	Ca	0.08	Ca
Coefficient of variation: 15.5%										

*Means followed by the same uppercase letter in the row and lowercase letter in the column do not differ from each other according to the Scott-Knott test at 5% probability.

The results of the present study are also comparable to those of Siebeneichler et al. (2000), who observed a decrease in net photosynthesis in two bean cultivars under low temperature stress. Photosynthesis in plants under low temperature can be inhibited by the accumulation of carbohydrates in leaves via a feedback mechanism, because of the reduction of their mitochondrial activity or low diffusive conductance (Fürtauer et al., 2019).

Although cold stress is considered to improve the efficiency of Rubisco activity because of the increase in CO_2 solubility compared to that of O_2 (Douce & Heldt, 2000), Hendrickson et al. (2004) observed that in vines under cold stress, Rubisco activity was inefficient in carboxylation.

The photochemical efficiency (i.e., ratio between the variable fluorescence and the maximum PSII; Fv/Fm) was statistically equal under -2°C and -3°C, varying between 0.72 and 0.76, which indicates the absence of photoinhibition, as reported by Ribeiro et al. (2009) (Table 4). Genotype responses were evident at -4°C, with a decrease in the Fv/Fm ratio from this temperature onwards. All materials except the progeny E0706 9-1-10 presented lower values than those obtained at higher temperatures. At -5°C, the cultivars Mundo Novo, IPR 105, and progeny E0706 9-1-10 showed low photochemical efficiency values, indicating that photoinhibition occurs because of the exposure of plants to low temperatures (Yusuf et al., 2010). In this situation, plants reduce their photosynthetic capacity, increase the probability of PSII excitation, and reduce their recovery capacity. The D1 protein synthesis becomes less efficient in the reaction centers, which reduces the protection against photoinhibition. As

reported by Björkman and Demming (1987), the Fv/Fm ratio of a C3 plant with an intact photosynthetic apparatus varies between 0.75 and 0.85, and a decrease in this ratio indicates photoinhibitory damage in the centers of the PSII reaction. Similar results were obtained by Yusuf et al. (2010), who evaluated abiotic stress in *Brassica juncea,* and Rapacz et al. (2007) in wheat plants, who also found that the chlorophyll fluorescence response was manifested by the reduction of Fv/Fm when subjected to low-temperature stress.

Table 4
Photochemical efficiency (ratio between variable and maximum fluorescence (Fv/Fm)) of coffee seedlings of different genotypes 24 h after being submitted to minimum temperatures of −2°C, −3°C, −4°C, and −5°C, in addition to control (environment).

Genotypes	Temperatures (°C)									
	Environment		−2		−3		−4		−5	
Mundo Novo	0.750	Aa*	0.730	Aa	0.730	Aa	0.660	Ba	0.600	Cb
Catuaí Vermelho	0.750	Aa	0.730	Aa	0.740	Aa	0.650	Ba	0.680	Ba
H147/1	0.730	Aa	0.740	Aa	0.720	Aa	0.630	Ba	0.670	Ba
E0706 5-1-1	0.750	Aa	0.730	Aa	0.740	Aa	0.620	Ba	0.610	Ba
E0706 9-1-10	0.740	Aa	0.750	Aa	0.740	Aa	0.690	Aa	0.550	Cb
E0706 13-1-3	0.730	Aa	0.750	Aa	0.730	Aa	0.660	Ba	0.660	Ba
E0706 27-2-2	0.740	Aa	0.730	Aa	0.740	Aa	0.620	Ba	0.620	Ba
Coffea racemosa	0.740	Aa	0.750	Aa	0.730	Aa	0.600	Ba	0.600	Ba
IPR 105	0.740	Aa	0.750	Aa	0.720	Aa	0.640	Ba	0.560	Cb
IPR 100	0.760	Aa	0.750	Aa	0.740	Aa	0.630	Ba	0.640	Ba
Coefficient of variation: 6.3%										

*Means followed by the same uppercase letter in the row and lowercase letter in the column do not differ from each other according to the Scott-Knott test at 5% probability.

The reduction in fluorescence values with decreasing temperature observed in the present study shows that the plants were affected by negative temperatures, as PSII is one of the main targets of low temperature stress (Bertamini et al., 2007). This occurs because the fluorescence emission shows the level of excitation energy in the pigment/protein complexes, which controls photosynthesis. Through fluorescence, detailed information on the structure, energy distribution, and function of the photosynthetic apparatus, especially photosystem II, can be obtained. Chlorophyll fluorescence has been widely used for evaluating damage to photosynthetic metabolism under conditions of stress (Stirbet, 2011).

Progeny E0706 9-1-10 and cultivar IPR 100 had the highest electrical conductivity values at all measured temperatures. At -3°C, genotypes E0706 9-1-10, IPR 105, and IPR

100 had the highest electrical conductivity values, whereas no difference was observed at -5°C (Table 5). The high conductivity values indicate that the membranes were damaged, as negative temperatures freeze the liquid in intercellular spaces, causing membrane rupture and electrolyte leaching (Sanghera et al., 2011).

Table 5
Electrical conductivity (µmhos cm^{-1}) of the imbibition solution with leaf disks from coffee seedlings of different genotypes 24 h after being submitted to minimum temperatures of −2°C, −3°C, −4°C, and −5°C, in addition to control (environment).

Genotypes	Temperatures (°C)									
	Environment		−2		−3		−4		−5	
Mundo Novo	9.83	Bb	9.67	Bb	9.94	Bb	9.41	Ba	18.89	Ab
Catuaí Vermelho	12.19	Bb	12.19	Bb	11.83	Ba	11.20	Ba	22.28	Aa
H147/1	11.88	Bb	12.88	Bb	11.60	Ba	10.34	Ba	17.00	Ab
E0706 5-1-1	12.94	Bb	12.94	Bb	10.11	Bb	12.64	Ba	18.14	Ab
E0706 9-1-10	18.52	Ba	17.08	Ba	11.45	Ca	10.45	Ca	22.52	Aa
E0706 13-1-3	11.91	Bb	11.97	Bb	10.31	Bb	10.75	Ba	18.22	Ab
E0706 27-2-2	12.09	Bb	12.09	Bb	13.29	Ba	13.54	Ba	18.70	Ab
IPR 105	13.19	Bb	12.74	Bb	12.66	Cb	10.43	Ca	22.15	Aa
IPR 100	17.79	Ba	17.90	Ba	13.51	Cb	14.68	Ca	22.84	Aa
Coefficient of variation: 15.1%										

*Means followed by the same uppercase letter in the row and lowercase letter in the column do not differ from each other according to the Scott-Knott test at 5% probability.

The total protein content (Table 6) differed among genotypes at the tested temperatures. An increase in average protein content at -3°C was observed in H147/1, differing from the other materials evaluated, whereas progeny E0706 5-1-1 showed a lower value. As the temperature decreased, the total protein values decreased; at -5°C, *C. racemosa* had the highest value, significantly different from that of other genotypes, followed by progenies E0706 27-2-2 and E0706 5-1-1, and the Mundo Novo variety.

Table 6
Total protein content (mg protein g^{-1} of fresh matter) from coffee leaves of different genotypes submitted to minimum temperatures of −2°C, −3°C, −4°C, and −5°C, in addition to the control (environment).

Genotypes	Temperatures (°C)									
	Environment		−2		−3		−4		−5	
Mundo Novo	5.27	Aa*	3.57	Ba	3.71	Bb	2.37	Ca	1.99	Cb
Catuaí Vermelho	4.11	Ab	3.90	Aa	4.10	Ab	2.53	Ba	0.67	Cc
H147/1	4.60	Aa	3.32	Ba	5.39	Aa	2.69	Ba	1.42	Cc
E0706 5-1-1	3.23	Bb	4.37	Aa	2.50	Cc	1.99	Cb	1.64	Cb
E0706 9-1-10	4.93	Aa	3.91	Aa	4.37	Ab	2.67	Ba	0.45	Cc
E0706 13-1-3	5.52	Aa	3.94	Ba	4.38	Bb	2.74	Ca	1.22	Dc
E0706 27-2-2	3.94	Ab	4.05	Aa	4.36	Ab	1.47	Bb	1.67	Bb
Coffea racemosa	4.05	Ab	3.49	Aa	3.95	Ab	1.19	Cb	2.87	Ba
IPR 105	3.76	Ab	3.91	Aa	4.20	Ab	1.90	Bb	0.96	Cc
IPR 100	3.92	Ab	3.57	Aa	4.10	Ab	2.88	Ba	1.18	Cc
Coefficient of variation: 18.7%										

*Means followed by the same uppercase letter in the row and lowercase letter in the column do not differ from each other according to the Scott-Knott test at 5% probability.

For most genotypes, the protein content was statistically similar between -3°C and the control, except for cultivar Mundo Novo and progenies E0706 5-1-1 and E0706 13-1-3. Protein content maintains membrane fluidity during cold stress, as the freezing point decreases with the increase in osmotic potential of the solution (Sanghera et al., 2011). In the present study, no protein production occurred under negative temperatures, but the existing levels were maintained; i.e., the plant spent no energy producing metabolites after stress. From -4°C onward, all genotypes showed lower protein content than the control; this indicates that the cells froze and the protein molecules ruptured. At -5°C, only *C. racemosa* differed significantly from all materials, indicating its greater resistance to cold. The higher protein content of *C. racemosa* at -5°C may indicate resistance to negative temperatures, as the osmotic potential of the cytoplasm increases with the increased protein content of the cell, thus, decreasing the cell freezing temperature (Taiz et al., 2017). Moreover, changes in proteins are a feature inherent to cold resistance (Guy & Haskell, 1988; Tseng & Li, 1990).

The results of the electrical conductivity test were not correlated with those of the other variables (temperature and averages of the genotypes), which indicates that electrical conductivity is unsuitable for evaluating the response of coffee plants at negative temperatures. All the other tests and criteria can be used to identify the minimum temperatures that damage coffee plants (Table 7). Moreover, the significant positive correlations among protein content, photosynthesis, and fluorescence indicate that the photosynthetic apparatus in plants

that maintained their protein content was not damaged, whereas the negative correlation of the visual analysis indicates that increased protein content results in less damage to the plant. Therefore, the genotypes that maintained high protein levels at the lowest temperatures (Table 6) are the most resistant to the negative temperatures herein tested.

Table 7
Pearson correlation coefficient (r) between the means of the total protein content, electrical conductivity, visual analysis after 24 h and 15 days, and photochemical efficiency (fluorescence - Fv/Fm) and photosynthesis for the coffee genotypes subjected to low temperatures.

	Protein content	Electrical conductivity	Visual analysis		Fluorescence
			24 h	15 days	Fv/Fm
Electrical conductivity	−0.63 ns				
Visual analysis - 24 h	−0.99*	0.58 ns			
Visual analysis - 15 days	−0.95*	0.64 ns	0.89*		
Fluorescence - Fv/Fm	0.98*	−0.53 ns	−0.99*	−0.87*	
Photosynthesis	0.73*	−0.15 ns	−0.75*	−0.68 ns	0.78*

*Significant at the 5% probability level. ns not significant.

Conclusions

The analyzed parameters visual damage, photochemical efficiency, and protein content were efficient for evaluating the resistance of coffee plants to negative temperatures.

Negative temperatures between -4°C and -5°C are the most suitable for testing cold resistance in coffee genotypes.

Visual evaluation of leaf damage was more efficient when performed 15 days after exposure to negative temperatures.

Coffea racemosa is resistant to negative temperatures and can, thus, be useful in breeding programs.

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