

Research Note

## Resistance to frost in Arabica coffee lines introgressed with *Coffea racemosa* Lour. genes

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### Abstract

The aim of this study was to assess resistance to frost in Arabica coffees with introgression of *Coffea racemosa*. The field experiment (Londrina, Paraná, Brazil) was assessed after two consecutive frosts in June 2011. Eleven F<sub>2</sub>BC<sub>5</sub> Arabica coffee lines with introgression of *C. racemosa* were assessed 52 months after planting. *C. arabica* cultivars IAPAR 59, Tupi IAC 1669-33, Catuaí Vermelho IAC 99 and Mundo Novo IAC 376-4 were used as sensitive controls. Foliar damages assessments were carried out 20 days after exposure to freezing temperatures. Foliar damages were scored using grades ranging from 1 to 5, where: grade 1 = no damage; and 5 grade = from 75.01 to 100.00% damaged leaf area. Levels of foliar damage (e.g. no damage – very severe damages) were classified according to the mean grades of the assessments. Severe leaf damage was observed in the controls, while nine lines showed moderate damage. H0107-32 line is the most resistant to frost, and is the only one which presented slight leaf damage.

**Keywords:** Aramosa, C1195-5-6-2, cold, breeding, freezing temperatures.

**Abbreviations:** BC\_ backcross; Instituto de Desenvolvimento Rural do Paraná\_IDR-Paraná.

### Introduction

Coffee crop is limited to the subtropical and tropical areas of the world, within the equatorial belt, located in latitudes between 25° N and 25° S. The cultivation in these areas is limited due to climatic factors, such as frosts, which occur at higher latitudes in the equatorial belt, but are also associated with high altitudes at lower latitudes. For every 100 m increase in altitude, average temperature reduces 0.65°C. In general, most coffee producing countries are located in latitudes between 18° N and 18° S, where frosts are restricted to high altitudes (Paes de Camargo and Paes de Camargo, 2009).

Frost easily causes damage to coffee plants, and has possibly caused the death of plants, since it has occurred over the time both in the southern Brazil, and in regions close to the equator line, at altitudes of about 2000m and above (Damatta and Carvalho, 2006). Frost (freezing temperatures) may compromise the economic viability of the culture, since it causes damage in leaves and fruits in the year of occurrence, but it can also affect subsequent productions (Androcioli-Filho et al., 1986).

When plants are subjected to freezing temperatures for long periods, the formation of extracellular ice crystals generates a continuous process of water transfer from the intracellular

region to the extracellular region, causing protoplast dehydration and freezing of the intracellular content. The formation of ice crystals inside the cells causes extensive damages, which is usually lethal (Taiz and Zeiger, 2009).

Genetic variability has been observed in *Coffea* sp., and *C. canephora* Pierre ex A. Froehner was much more sensitive to cold than *C. arabica* L. (Jouve et al., 1993). The assessment of the photoprotective mechanism of coffee showed greater ability to withstand cold stress in *C. arabica* cv. IPR 102 cultivar than in clones of *C. canephora* var. *kouillou* (Partelli et al., 2009).

Coffee plants assessed in the field, after the frost which occurred in the year of 2000 in Londrina-PR, presented genetic variability for resistance to freezing temperatures. It was observed high resistance to freezing temperatures in *C. racemosa* Lour., which is a triploid hybrid between *C. arabica* and *C. racemosa*, *C. liberica* var. *dewevrei* and in a "Piatã" genotype ((*C. liberica* var. *dewevrei* x *C. arabica*) x *C. arabica*). It was also noted that "Arabusta" (*C. arabica* x *C. canephora* var. *robusta*) and *C. canephora* var. *kouillou* showed the greatest damage, and the latter was the most sensitive genotype (Petek et al., 2005a, b).

So far, there are no Arabica coffee cultivars with good level of resistance to freezing temperatures. Instituto de Desenvolvimento Rural do Paraná (IDR-Paraná) has Arabica coffee lines carrying *C. racemosa* genes with probable resistance to frost. Therefore, the aim of this study was to assess resistance to frost in Arabica coffee lines carrying *C. racemosa* genes.

## Results and discussion

In all the controls, mean leaf damage was severe, and significantly differed from all  $F_2RC_5$  lines. Eight lines were more tolerant than controls, and showed moderate leaf damage. H0107-32 line stood out for statistically differing from these eight lines, besides presenting slight foliar damage (Table 1).

All sensitive controls present 100% of plants with a score greater than or equal to 3.0, that is, with a percentage of the damaged leaf area greater than 25%, with the exception of 'Mundo Novo' with 8.33% of plants with a score of 2.0. All Catuaí Vermelho IAC 81 plants had a 4.0 score (Table 2).

Genotypes with more than 50% plants with grades lower or equal to 2.0 were H0103-6, H0115-6, H0109-11 and H0107-32, especially the latter, which had 60% plants with grade 1 (no damage).

In other study under field conditions, it was observed that the first symptoms of damage appeared in leaves when temperature reached  $-2.0\text{ }^\circ\text{C}$ , while severe and general damage occurred only when the minimum temperature was below  $-3.0\text{ }^\circ\text{C}$ . When the temperature reached  $-4.5\text{ }^\circ\text{C}$ , there was total damage of plants (Paes de Camargo and Salati, 1966). Similar results were obtained in a controlled environment by Manetti-Filho and Caramori (1986), who observed in Catuaí Vermelho IAC 81 slight to moderate damage at  $-2\text{ }^\circ\text{C}$ ; moderate to severe damage at  $-3^\circ\text{C}$ ; and severe damage at  $-4\text{ }^\circ\text{C}$ . Furthermore, they demonstrated that the effect of freezing temperatures is aggravated with an increase in the exposure time. Therefore, since in the sensitive controls the foliar damage is severe, it is probable that the temperature in the experiment of the present study was equal to or less than  $-3^\circ\text{C}$ , although it was registered  $-7^\circ\text{C}$  in the grass.

The controls Catuaí and Tupi showed severe damage, while slight damage was observed in H0107-32, which originated from two backcrosses of Tupi and one backcross of Catuaí with IAPAR 81185. Thus, it can be inferred that IAPAR 81185 is the resistance source, and that it is an  $F_2$  plant originated from the genotype  $F_1RC_2$  C1195-5-6-2 c.950 Ep209. *Coffea racemosa* may have been source of resistance to frost of IAPAR 81185, since some studies suggest that this species is resistant. In a field study carried out in July 2000, when there was a severe frost (minimum temperature in the meteorological shelter of  $-1.3^\circ\text{C}$ ) in Londrina, *C. racemosa* and a triploid hybrid (*C. arabica* x *C. racemosa*) presented high resistance to cold (Petek et al., 2005b). "C1195-5-6-2" is an important genotype used in Brazilian breeding programs, aiming at transferring resistance to leaf miner (*Leucoptera coffeella*) (Medina-Filho et al., 1977a, b), resistance to bacterial-halo-blight (Andreazi et al., 2018), tolerance to drought (Medina Filho et al., 1977a; Carvalho et al., 2017), besides resistance to frost, as observed in the present study. All the traits are inherited from *C. racemosa* species. There is the possibility that, in this species, the mechanism that promotes resistance to freezing temperatures is the same as that of tolerance to drought. Physiological studies should be

carried out to elucidate the mechanism that promotes resistance to frost in *C. racemosa*.

The frosts that frequently occurred in most coffee producing areas in Brazil, especially in the state of Paraná, was a factor that encouraged farmers to quit the activity (Caramori and Manetti Filho, 1993; Paraná, 2014). Cultivars of the Catuaí and Mundo Novo groups, besides IAPAR 59 and Tupi IAC 1669-33, are widely planted in Paraná, and all of them were sensitive to frost. Therefore, it is extremely important to obtain cultivars that are resistant to frost from these Arabica coffee lines carrying *C. racemosa* genes. Since the lines are still in the  $F_2$  generation, genes of resistance to frost are in heterozygous condition. Segregating sensitive plants within these lines increased the mean leaf damage (Table 2). It is possible that some lines, such as H0107-32, have greater number of genes of resistance to frost, which allowed lower mean damage than the other lines (Table 1). In H0107-32, 60.00% plants had grade 1. In the other lines, it was observed 6.67% plants, at maximum, with grade 1 (Table 2). Individual plants within these  $F_2RC_5$  lines will be selected for generation advance, in order to identify resistant  $F_2RC_5$  lines, and to obtain homozygous cultivars.

## Materials and methods

### Plant materials

We assessed 11  $F_2BC_5$  lines derived from backcrosses (BCs) of different Arabica coffee genotypes, with an  $F_2$  plant of the C1195-5-6-2 genotype (Table 3). C1195-5 coffee was originated from a spontaneous hybridization between *C. arabica* and *C. racemosa* (C1195). C1195-5 was backcrossed twice with *C. arabica*, originating the  $F_1BC_2$  progeny, which was denominated C1195-5-6-2. At IDR-Paraná, it was carried out several backcrosses of different cultivars with an  $F_2$  plant (IAPAR 81185) of the  $F_1BC_2$  genotype (C1195-5-6-2 Ep209 c.950) (Fig. 1), identified as resistant to frost between 1980s and 1990s.

Cultivars of *C. arabica* IAPAR 59, Tupi IAC 1669-33 (Tupi), Catuaí Vermelho IAC 99 (Catuaí), and Mundo Novo IAC 376-4 (Mundo Novo) were used as controls. These cultivars are sensitive to freezing temperatures.

### Field trial

The field trial was installed in April 2006 at IDR-Paraná's experimental station (lat  $23^\circ21'28.61''\text{S}$ , long  $51^\circ09'36.19''\text{W}$ ; 575m asl), in Londrina, Paraná State, Brazil. The climate is classified as Cfa, according to Köppen. The average annual temperature in Londrina is  $21.1^\circ\text{C}$  and the annual average precipitation varies from 1400 to 1600 mm per year.

The trial was planted at the 2.50m spacing inter-row and 0.50m spacing within the row. The randomized blocks design with three replications and five plants per plot was used.

Soil fertilization and corrections, besides agricultural practices, were made according to the recommendation for coffee crop (Matiello et al., 2016).

Two consecutive frosts occurred on June 27<sup>th</sup> and 28<sup>th</sup> 2011, 52 months after planting, with temperature of  $1.6\text{ }^\circ\text{C}$  in the meteorological shelter (1.5m high), and with temperature of  $-7\text{ }^\circ\text{C}$  in the lawn.

### Resistance assessment

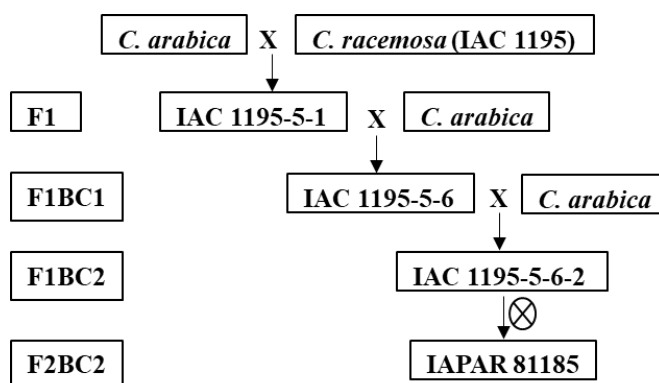
Assessments of foliar damages caused by frosts were carried out 20 days after exposure to freezing temperatures. Symptoms on the leaves were dark brown in color due to

**Table 1.** Mean grades of foliar damage and damage level in Arabica coffee genotypes with *Coffea racemosa* introgression, assessed 20 days after two consecutive frosts, which occurred on June 27<sup>th</sup> and 28<sup>th</sup>, 2011, in Londrina, PR, Brazil.

Genotypes	Means <sup>(1)</sup>	Damage
H0107-32	1.40 a	Slight
H0103-6	2.13 b	Moderate
H0115-6	2.27 b	Moderate
H0109-11	2.33 b	Moderate
H0105-11	2.60 c	Moderate
H0118-20	2.67 c	Moderate
H0108-4	2.80 c	Moderate
H0102-16	2.78 c	Moderate
H0121-40	2.80 c	Moderate
H0120-11	2.93 c	Moderate
H0117-8	3.13 c	Severe
'Mundo Novo IAC 376-4' <sup>(2)</sup>	3.33 d	Severe
'IAPAR 59' <sup>(2)</sup>	3.32 d	Severe
'Tupi IAC 1669-33' <sup>(2)</sup>	3.53 d	Severe
'Catuaí Vermelho IAC 81' <sup>(2)</sup>	4.00 d	Severe
Mean	2.80	
CV	14.29%	

<sup>(1)</sup> Means followed by the same letter do not differ at 5% probability by the Scott Knott test. Data processed in log (x).

<sup>(2)</sup> Controls sensitive to freezing temperatures.



**Fig 1.** Pedigree of the coffee genotype IAPAR 81185. BC = backcross.

**Table 2.** Percentage of plants according to the grades (1 to 5) of foliar damage in F<sub>2</sub>BC<sub>5</sub> lines of Arabica coffee carrying *Coffea racemosa* genes, assessed after two consecutive frosts in June 2011 (Londrina, PR, Brazil).

Genotypes	Plants according to the grades of leaf damage%				
	1	2	3	4	5
H0102-16	-	21.43	78.57	-	-
H0103-6	-	86.67	13.33	-	-
H0105-11	-	40.00	60.00	-	-
H0107-32	60.00	40.00	-	-	-
H0108-4	-	33.33	53.34	13.33	-
H0109-11	6.67	53.33	40.00	-	-
H0115-6	6.67	60.00	33.33	-	-
H0118-20	6.67	26.66	60.00	6.67	-
H0120-11	-	6.67	93.33	-	-
H0117-8	-	20.00	46.67	33.33	-
H0121-40	-	20.00	80.00	-	-
'IAPAR 59' <sup>(1)</sup>	-	-	71.43	28.57	-
'Catuaí V. IAC 81' <sup>(1)</sup>	-	-	-	100.00	-
'Tupi IAC 1669-33' <sup>(1)</sup>	-	-	50.00	50.00	-
'Mundo Novo IAC 376-4' <sup>(1)</sup>	-	8.33	50.00	41.67	-

<sup>(1)</sup> Controls sensitive to freezing temperatures.

**Table 3.** *Coffea arabica* genotypes with *C. racemosa* introgression and sensitive controls assessed for resistance to frost in Londrina, PR, Brazil, 2011.

Genotypes	Description <sup>(1)</sup>
H0102-16	F <sub>2</sub> of 'Tupi' x ['Tupi' x (IAPAR 81185 x 'Tupi')]
H0103-6	F <sub>2</sub> of 'Icatu 3282' x ['Tupi' x (IAPAR 81185 x 'Tupi')]
H0105-11	F <sub>2</sub> of 'Catuaí IAC 81' x ['Tupi' x (IAPAR 81185 x 'Tupi')]
H0107-32	F <sub>2</sub> of 'Catuaí IAC 81' x ['Tupi' x (IAPAR 81185 x 'Tupi')]
H0108-4	F <sub>2</sub> of 'Acaia' x ['Tupi' x (IAPAR 81185 x 'Tupi')]
H0109-11	F <sub>2</sub> of 'IPR 98' x ['Tupi' x (IAPAR 81185 x 'Tupi')]
H0115-6	F <sub>2</sub> of ("Etiópia" x "Catuaí") x ['Tupi' x (IAPAR 81185 x 'Tupi')]
H0118-20	F <sub>2</sub> of ("Etiópia" x "Catuaí") x ['Tupi' x (IAPAR 81185 x 'Tupi')]
H0120-11	F <sub>2</sub> of 'Tupi' x ['Tupi' x (IAPAR 81185 x 'Tupi')]
H0117-8	F <sub>2</sub> of 'IPR 104' x ['Tupi' x (IAPAR 81185 x 'Tupi')]
H0121-40	F <sub>2</sub> of 'IPR 104' x ['Tupi' x (IAPAR 81185 x 'Tupi')]
'IAPAR 59'	"Villa Sarchi CIFC 971/10" x "Híbrido de Timor CIFC 832/2"
'Catuaí Vermelho IAC 81'	"Caturra" x "Mundo Novo"
'Tupi IAC 1669-33'	"Villa Sarchi CIFC 971/10" x "Híbrido de Timor CIFC 832/2"
'Mundo Novo IAC 376-4'	"Bourbon" x "Sumatra"

<sup>(1)</sup> IAPAR 81185 = F<sub>2</sub> plant of the genotype F<sub>1</sub>BC<sub>2</sub> C1195-5-6-2 c.950 Ep209, originated from the crossing [(*Coffea arabica* x *C. racemosa* C1195) x *C. arabica*] x *C. arabica*; 'Tupi' = 'Tupi IAC 1669-33'; 'Catuaí IAC 81' = 'Catuaí Vermelho IAC 81'; 'Icatu 3282' = 'Icatu Precoce IAC 3282'; 'Acaia' = 'Acaia IAC 474/4'.

cell damage from frost. Foliar damages were scored using grades ranging from 1 to 5 (modified Manetti-Filho and Caramori, 1986), where: grade 1 = no damage; grade 2 = from 0.01 to 25.00% damaged leaf area; grade 3 = from 25.01 to 50.00% damaged leaf area; grade 4 = from 50.01 to 75.00% damaged leaf area; and 5 grade = from 75.01 to 100.00% damaged leaf area. Levels of foliar damage were classified according to the mean grade of the assessments, where: 1.00 = no damage; 1.01 to 2.00 = slight damage; 2.01 to 3.00 = moderate damage; 3.01 to 4.00 = severe damage; 4.01 to 5.00 = very severe damage, with death of plants (modified Caramori et al., 2002).

### Statistical analysis

Data of foliar damage were subjected to analysis of variance (ANOVA) and mean clustering by the Scott-Knott 5% test, using the SISVAR statistical program (Ferreira, 2011). Data were transformed into log (x) to meet the assumptions of homogeneity of variance and normality of data.

### Conclusion

Arabica coffee lines with introgression of *C. racemosa* derivatives from C1195-5-6-2 were more resistant to frost than Arabica coffee cultivars IAPAR 59, Tupi IAC 1669-33, Catuaí Vermelho IAC 99 and Mundo Novo IAC 376-4.

Leaf damages were slight for the most resistant line H0107-32, while in sensitive controls the damages were severe.

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