CROP BREEDING AND APPLIED BIOTECHNOLOGY

NOTE

Resistance to bacterial halo blight in Arabica coffee lines derivative from the genotype C1195-5-6-2 under natural infection conditions

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Abstract: *The aim of this study was to identify resistance to bacterial halo blight in Arabica coffee lines carrying Coffea racemosa genes. Eighteen Arabica coffee lines derivative from the genotype C1195-5-6-2, and the cultivars IAPAR 59 and IPR 99 were evaluated for resistance to bacterial halo blight in two trials carried out in field conditions, in Londrina, PR, Brazil. The cultivars Mundo Novo IAC 376-4 and Catuaí Vermelho IAC 81 were included as susceptible controls. Ten lines and the cultivar IAPAR 59 showed resistance to bacterial halo blight. The cultivar IPR 99 presented intermediate reaction, and the controls were very susceptible. This is the first study to show that lines derivative from the genotype C1195-5-6-2, which has C. racemosa genes, could be a source of resistance to bacterial halo blight in coffee breeding programs.*

Key words: *Aramosa, bacteriosis, breeding, Coffea arabica, Pseudomonas syringae pv. garcae.*

INTRODUCTION

Bacteria are one of the major causal agents of plant diseases. Species of the genus *Pseudomonas*, particularly *P. syringae*, are known as pathogens of several crops. Species of the genera *Pseudomonas* sp., especially *P. syringae* pv. *garcae,* are known for being a pathogen of different crops (Bedendo 2011). The strain *P. syringae* pv. *garcae* was identified for the first time in coffee crops in the municipality of Garça - SP, Brazil, and the disease was named bacterial halo blight (Amaral et al. 1956). The disease occurs in the main producing regions of Brazil, such as Minas Gerais, São Paulo and Paraná (Mohan et al. 1978, Petek et al. 2006, Ito et al. 2008, Zoccoli et al. 2011), and in other countries, such as Kenya (Ithiru et al. 2013). Coffee crops cultivated in high altitude regions, with mild temperatures and much rainfall, and which are exposed to strong and/or constant winds, and to occasional frosts are more likely to be attacked by these bacteria (Zoccoli et al. 2011). Thus, this disease may be a limiting factor to the development of these crops (Sera 2001).

Symptoms of bacterial halo blight are found on leaves, flowers, fruit and young branches (Costa and Silva 1960). Lesions on the leaves are irregular, dark-brown colored, with a yellow halo around. The lesions are more frequent on the edges of the leaves, being easier for the bacteria to penetrate, due to

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mechanical damage (Reis and Olivares 2006). However, the lesions can extend to the leaf surface. In more severe attacks, branches and fruits necrosis may occur. Young crops are more sensitive, and may suffer general defoliation, die-back, overbudding, and delaying the initial vegetation growth (Amaral et al. 1956, Costa et al. 1957, Costa and Silva 1960). In nurseries, the symptoms are very similar, but the spread is facilitated by the density of the plants, which provides ideal environment for the development of the disease, causing necrosis of new leaves and die-back, and in many cases, leading to the death of plants (Rodrigues et al. 2013).

The preventive control by means of wind breaks at the implementation of the crops is essential. Chemical control by copper fungicides and antibiotics is difficult (Patrício et al. 2008). Thus, the most appropriate disease control is the use of resistant cultivars (Sera 2001).

Sources of resistance to bacterial halo blight have been found in genotypes carrying the S_H1 resistance factor, such as Harar, Dilla and Alghe, S12 KAFFA and Geisha. This resistance factor is classically known for providing resistance to some physiological races of coffee leaf rust (*Hemileia vastatrix* Berk. and Br.) (Moraes et al. 1975). Other resistance sources have also been reported, such as in Icatu, Híbrido de Timor, *C. eugenioides*, *C. stenophylla* (Mohan et al. 1978), *C. congensis* (Moraes et al. 1975), and coffee plants derived from Icatu x Catuaí (Petek et al. 2006, Ito et al. 2008). In addition, hybrids of *C. arabica* have been reported as carrying *C. racemosa* genes; however, this data must be confirmed (Andreazi et al. 2015).

Results obtained in a trial carried out in fields naturally infested with *P. syringae* pv. *garcae* had 95% of complete resistant plants in cultivar IPR 102, and partial resistance in IPR 103, IPR 104, IPR 108 and IAPAR 59, indicating the possible presence of qualitative and quantitative resistance in these genotypes (Ito et al. 2008).

There are few Arabica coffee cultivars available with high levels of resistance to bacterial halo blight. The breeding program of IAPAR has Arabica coffee lines obtained from crossings with the genotype C1195-5-6-2, which has *C. racemosa* genes, and they could present resistance to bacterial halo blight. Thus, the aim of this study was to identify resistance to bacterial halo blight in Arabica coffee lines derivative from the genotype C1195-5-6-2 of the IAPAR breeding program.

MATERIAL AND METHODS

The trials were carried out in the field Experimental Station of IAPAR (lat 23º 22' S, long 51° 10' W, alt 585 m asl), in Londrina, PR, Brazil. The climate is Cfa (Humid Subtropical Climate), according to the Köppen classification. The average annual temperature is 21.1 °C; the average annual relative humidity ranges between 75 and 80%; and the average annual rainfall ranges between 1400 and 1600 mm $year^{-1}$.

The first and the second trials evaluated 11 and seven F_2RC_5 *Coffea arabica* lines, respectively. Lines were derivative from backcrosses (BCs) of different Arabica coffee genotypes with an F_2 plant (IAPAR 81185) of the F_1RC_2 (C1195-5-6-2 c.950 Ep209) genotype (Tables 1 and 2). The genotype C1195-5 is a natural hybrid between *C. arabica* and *C. racemosa* (C1195). The hybrid C1195-5 was twice naturally backcrossed with *C. arabica*, originating the F_1RC_2 progeny, which was denominated C1195-5-6-2. Thus, three other backcrosses were carried out with different genotypes, resulting in the F_1RC_5 line. Only plants with no symptoms of bacterial halo blight were used in these three backcrosses. These lines were advanced to F_2, generating the F_2RC_5 lines, which were studied in this work. The cultivars Mundo Novo IAC 376 and Catuaí Vermelho IAC 81 were used as susceptible controls in the first and in the second trials, respectively.

The first trial was installed in April 2006, in randomized blocks statistical design, with three replications and five plants per plot. The second trial was installed in November 2007, in randomized blocks statistical design, with three replications and five plants per plot. Plants were spaced 2.5 m between lines, and 0.5 m between plants. Soil correction was performed according to the result of chemical analysis and fertilization, and cultural practices were carried out as recommended for coffee crop.

Severity was evaluated in January 2013, in natural infection condition, using a score scale, ranging from 1 to 5, in which: 1 = no necrotic lesions; 2 = 0.01 to 3% of the leaves with small necrotic lesions, with yellowish halo (up to 0.5 cm); 3 = 3.01 to 15% of the leaves with small and medium lesions (up to 1 cm), with possible presence of 1% large lesions (greater than 1 cm); 4 = 15.01 to 30% of the leaves with small to large lesions; 5 = more than 30% of the leaves with small to large lesions, with possible die-back of the branches. Disease severity was evaluated in the whole plant,

from the upper third to the lower third of the plant canopy. However, only unexpanded young leaves to sixth pair of fully expanded leaves were considered. Plants with scores 1 and 2 were considered resistant (R), and those with scores 3 or higher were considered susceptible (S).

Table 1. Arabica coffee F_2RC_5 lines carrying *Coffea racemosa* genes, evaluated in January 2013 for resistance to bacterial halo blight, in trials 1 and 2, installed in April 2006 and November 2007, respectively

Trial	Genealogy[1]	Genotype
1	Acaiá x (Tupi x (IAPAR 81185 x Tupi))	H0105-04
1	IPR 98 x (Tupi x (IAPAR 81185 x Tupi))	H0106-11
1	IPR 107 x (Tupi x (IAPAR 81185 x Tupi))	H0107-10
1	(IAPAR 59 x "Catuaí Erecta") x (Tupi x (IAPAR 81185 x Tupi))	H0110-13
1	(IAPAR 59 x "Catuaí Erecta") x (Tupi x (IAPAR 81185 x Tupi))	H0110-09
1	("Etiópia SH1" x Catuaí) x (Tupi x (IAPAR 81185 x Tupi))	H0111-20
1	("Etiópia SH1" x Catuaí) x (Tupi x (IAPAR 81185 x Tupi))	H0111-06
1	Tupi x (Tupi x (IAPAR 81185 x Tupi))	H0112-11
1	IPR 104 x (Tupi x (IAPAR 81185 x Tupi))	H0113-20
1	IPR 104 x (Tupi x (IAPAR 81185 x Tupi))	H0113-08
1	("Etiópia SP" x IPR 98) x (Tupi x (IAPAR 81185 x Tupi))	H0116-02
1	"Villa Sarchi CIFC 971/10" x "Híbrido de Timor CIFC 832/2"	'IAPAR 59'
1	"Villa Sarchi CIFC 971/10" x "Híbrido de Timor CIFC 832/2"	'IPR 99'
1	"Bourbon" x "Sumatra"	'Mundo Novo IAC 376-4'
2	IAPAR 59 x (Tupi x (IAPAR 81185 x Tupi))	H0101-18
2	IAPAR 59 x (Tupi x (IAPAR 81185 x Tupi))	H0101-20
2	Tupi x (Tupi x (IAPAR 81185 x Tupi))	H0102-16
2	Icatu 3282 x (Tupi x (IAPAR 81185 x Tupi))	H0103-11
2	Catuaí x (Tupi x (IAPAR 81185 x Tupi))	H0104-11
2	Catuaí x (Tupi x (IAPAR 81185 x Tupi))	H0104-12
2	Catuaí x (Tupi x (IAPAR 81185 x Tupi))	H0104-02
2	"Villa Sarchi CIFC 971/10" x "Híbrido de Timor CIFC 832/2"	'Iapar 59'
2	"Caturra" x "Mundo Novo"	'Catuaí IAC 81'

[1] IAPAR 81185 = F2 plant of the genotype F1RC2 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'; Acaiá = 'Acaiá IAC 474/4'.

Table 2. Mean severity of bacterial halo blight in F_2 Arabica coffee lines carrying *Coffea racemosa* genes, in trials 1 and 2

Trial 1		Trial 2	
Genotype	RBHB[1]	Genotype	RBHB[1]
H0110-09	1.7 a	H0104-11	1.5 a
H0111-20	1.7 a	H0102-16	1.6 a
H0113-20	1.8 a	H0104-02	1.7 a
H0111-06	1.9 a	H0101-18	2.0 a
H0110-13	2.0 a	H0104-12	2.0 a
H0113-08	2.1 a	H0103-11	2.2 a
H0105-04	2.2 a	'IAPAR 59'	2.4 a
H0112-11	2.2 a	'Catuaí Vermelho IAC 81'	3.1 b
H0116-02	2.2 a	H0101-20	3.7 b
IAPAR 59	2.4 a	Mean	2.24
H0107-10	2.5 a	CV (%)	17.17
IPR 99	2.8 b		
H0106-11	2.9 b		
Mundo Novo IAC 376-4	3.7 c		
Mean	2.29		
CV (%)	13.61		

[1] Score 1= no lesions; score 5 = several lesions. RBHB = Reaction to bacterial halo blight. Means followed by the same letter do not differ by the Scott Knott test at 5% probability

Data were subjected to analysis of variance, and means were clustered by the Scott Knott test at 5% significance level. Analyses were performed using the statistical software Sisvar (Ferreira 2011).

RESULTS AND DISCUSSION

In the first trial, by using the ANOVA, the F values for treatments and blocks were 9.161 ($p < 0.000$) and 0.035 ($p < 0.9657$), respectively. The susceptible control Mundo Novo IAC 376-4 showed the highest disease severity. The genotype IPR 99 (2.8) presented behavior intermediate to Mundo Novo and IAPAR 59 (2.4). Among the 11 lines, 10 of them did not differ from IAPAR 59 (Table 2). Mundo Novo (susceptible control) presented 100% susceptible plants. IAPAR 59 and IPR 99 showed 66.7 and 33.3% resistant plants, respectively. Among the 11 lines, nine had more than 60% R plants, and H0111-20 stood out for presenting100% R plants (Table 3).

In the second trial, by using the ANOVA, the F values for treatments and blocks were 11.159 ($p < 0.000$) and 0.492 ($p < 0.6204$), respectively. The susceptible control Catuaí Vermelho IAC 81 showed 86.7% susceptible plants, while IAPAR 59 had 40% susceptible plants (Table 4). IAPAR 59 differed from the susceptible control Catuaí. Among the seven lines evaluated, six of them had means significantly lower than that of Catuaí, and did not differ from IAPAR 59 (Table 2). Among the evaluated lines, only H0102-16 had 100% R plants, and H0101-20 was the only one that showed few R plants (Table 4).

In both trials, all lines, except for H0106-11 and H0101-20, differed statistically from the susceptible controls; however,

Table 3. Percentage of plants according to the severity score scale of bacterial halo blight in F_2RC_5 Arabica coffee lines carrying *Coffea racemosa* genes in trial 1

	Score				
Genotypes	**1**	**2**	**3**	**4**	**5**
H0105-04	0.0	80.0	20.0	0.0	0.0
H0106-11	0.0	20.0	73.3	6.7	0.0
H0107-10	0.0	46.7	53.3	0.0	0.0
H0110-09	46.7	40.0	13.3	0.0	0.0
H0110-13	13.3	73.3	13.3	0.0	0.0
H0111-06	13.3	80.0	6.7	0.0	0.0
H0111-20	26.7	73.3	0.0	0.0	0.0
H0112-11	13.3	53.3	33.3	0.0	0.0
H0113-08	0.0	93.3	6.7	0.0	0.0
H0113-20	26.7	66.7	6.7	0.0	0.0
H0116-02	0.0	80.0	20.0	0.0	0.0
'IAPAR 59'	0.0	66.7	26.7	6.7	0.0
'Mundo Novo IAC 376-4'	0.0	0.0	33.3	66.7	0.0
'IPR 99'	0.0	33.3	60.0	0.0	6.7

Table 4. Percentage of plants according to the severity score scale of bacterial halo blight in F_2RC_5 Arabica coffee lines carrying *Coffea racemosa* genes in trial 2

Genotype	Score				
	1	**2**	**3**	**4**	**5**
H0101-18	13.3	73.3	13.3	0.0	0.0
H0101-20	0.0	13.3	20.0	46.7	20.0
H0102-16	40.0	60.0	0.0	0.0	0.0
H0103-11	0.0	80.0	20.0	0.0	0.0
H0104-11	60.0	33.3	6.7	0.0	0.0
H0104-02	46.7	40.0	13.3	0.0	0.0
H0104-12	20.0	60.0	20.0	0.0	0.0
'IAPAR 59'	0.0	60.0	40.0	0.0	0.0
'Catuaí Vermelho IAC 81'	0.0	13.3	66.7	20.0	0.0

they did not differ from IAPAR 59, which showed resistance in previous studies (Petek et al. 2006, Ito et al. 2008). 'IAPAR 59' presented resistance, but it is still segregating for this characteristic, since it presented more than 33% and 40% S plants in trials 1 and 2, respectively (Tables 3 and 4), corroborating the study of Ito et al. (2008). One of the parents of 'IAPAR 59' was "Hibrido de Timor CIFC 832/2", which can be the resistance source, since according to the study of Mohan et al. (1978), three genotypes derivative from Híbrido de Timor presented different levels of resistance. Villa Sarchi CIFC 971/10 is the other parent of IAPAR 59, but no studies on resistance of this parent have been carried out yet. 'Tupi IAC 1669-33', which makes part of all the crosses that originated the lines of this study, despite being a "Sarchimor", just like 'IAPAR 59', presented no resistance to bacterial halo blight (Andreazi et al. 2015). Similarly, 'IPR 99' also has its origin in the "Sarchimor" germplasm; however, it was more susceptible to bacterial halo blight. 'Mundo Novo' was more susceptible than 'IPR 99'. Other authors have also identified susceptibility in 'IPR 99' (Ito et al. 2008), 'Catuaí' and 'Mundo Novo' (Mohan et al. 1978, Ito et al. 2008). The percentages of 33.3% and 13.3% of R plants, respectively, for IPR 99 and Catuaí were due to scape in the trial, and it is likely that there cultivars do not have resistance. In addition, it is possible that IPR 99 is still segregating, likewise IAPAR 59, since it presented 20% more resistant plants than Catuaí. In H0101-20, this percentage was of 13.3% R plants, and this value could also be explained by the scape; another possibility is that these plants are resistant.

The parents of the resistant genotypes H0105-04, H0107-10, H0112-11, H0102-16, H0103-11, H0104-11, H0104-12, H0104-02 were IAPAR 81185 and the susceptible genotypes 'IPR 98', 'IPR 107', 'Catuaí Vermelho IAC 81', 'Tupi IAC 1669-33' (Ito et al. 2008, Andreazi et al. 2015) and 'Acaiá IAC 474/4' (Mohan et al. 1978). The resistance observed in these studies might have originated from IAPAR 81185; however, the resistance of this genotype must be confirmed by artificial inoculations.

In the lines H0110-09, H0110-13, H0101-18, H0113-08, and H0113-20, resistance may have come from IAPAR 81185, or from the cultivars IAPAR 59 and IPR 104, which showed moderate resistance in the study of Ito et al. (2008).

The resistance observed in the lines H0111-06, H0111-20 and H0116-02 may have been originated either from IAPAR 81185 or from Ethiopian accessions, which were identified as resistance sources by Moraes et al. (1975) and Mohan et al. (1978).

As previously reported, IAPAR 81185 is an F_2RC_2 plant derivative from C1195-5-6-2 genotype, which was twice naturally backcrossed with *C. arabica*. "C1195-5-6-2" is an important genotype used in Brazilian breeding programs, aiming at transferring resistance to leaf miner (*Leucoptera coffeella*) to other genotypes (Medina-Filho et al. 1977a, Medina-Filho et al. 1977b), as well as tolerance to drought (Medina-Filho et al. 1977a), which are characteristics inherited from *Coffea racemosa.* Investigation in *C. racemosa* and *C. arabica* cv. Blue Mountain by means of artificial inoculations is necessary, in order to confirm if the resistance to bacterial halo blight of C1195-5-6-2 was originated from *C. racemosa*, which has not been tested yet.

Since the lines are still in the F_2 generation, genes resistant to bacterial halo blight are in heterozygous condition. H0111-20 is the only line that does not present segregation for susceptibility; however, it might not be in homozygosity, since it has Arabica coffee from Ethiopia and the genotype IAPAR 8118 as resistance source. Probably, the resistance to bacterial halo blight observed in most evaluated lines is due to the backcrossings and self-pollinations carried out with plants resistant to *P. syringae* pv. *garcae*.

To date, only the cultivar IPR 102 has been reported as presenting homozygous resistance to bacterial halo blight (Ito et al. 2008). Individual plants of the F_2RC_5 lines will be advanced to next self-pollination generation aiming to identify F_3RC_5 lines with homozygous resistance and other desirable agronomic traits, such as high yield, early ripening cycle, resistance to leaf rust and leaf miner, and tolerance to drought.

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