Research Note

Lines derived from coffee BA-10 with resistance to *Pseudomonas syringae* under field conditions with simultaneous natural infections of the pathovars *garcae* and *tabaci*

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Abstract

Bacterial-halo-blight and bacterial-leaf-spot are important coffee diseases caused by *Pseudomonas syringae* pv. *garcae* and pv. *tabaci*, respectively. The most suitable method to control these diseases is the use of resistant cultivars. There are no studies on resistance to *Pseudomonas syringae* (PS) in coffee derived from BA-10 genotypes. Therefore, the aim of this study was to evaluate the resistance to PS in lines derived from BA-10 under field conditions with simultaneous natural infections of the pathovars *garcae* and *tabaci*. 38 F_4 and two F_5 lines derived from BA-10 were evaluated in a field trial in Londrina, PR, Brazil. The Catuaí Vermelho IAC 81 and IAPAR 59 were the susceptible and intermediate resistant controls, respectively. Resistance to PS was evaluated in January 2017 after 45 months of planting. The grading scale varied from 1 to 5, where grade 1 was plants with more resistance and 5 plants more susceptible. Two F_5 lines showed 100% of resistant plants (grades 1 and 2) and the F_4 line IAPAR 12201 showed 60% of plants with a high level of resistance to PS (grade 1), while the control Catuaí showed no resistant plant. IAPAR 59 and several F_4 lines showed high frequency of plants with intermediate resistance to PS.

Keywords: bacteria, bacterial-halo-blight, bacterial-leaf-spot, *Coffea arabica*, *Coffea liberica*, IAC 1110-8.
Abbreviations: BHB_Bacterial-halo-blight; BLS_bacterial-leaf-spot; TH_Timor Hybrid; IAPAR_Instituto Agronômico do Paraná; PS_*Pseudomonas syringae*; IAC_Instituto Agronômico.

Introduction

Bacterial-halo-blight (BHB) is an important coffee disease caused by the *Pseudomonas syringae* pv. *garcae* (Amaral et al., 1956), which occurs more frequently in crops of high altitude regions, with mild temperatures, high rainfall, which are exposed to strong and or constant winds, and occasional frost (Zoccoli et al., 2011). In these more favorable environmental conditions, the BHB causes losses in production in important coffee producing states of Brazil such as Minas Gerais, São Paulo and Paraná (Moraes et al., 1975; Petek et al., 2006; Ito et al., 2008; Zoccoli et al., 2011; Rodrigues et al., 2017). Besides Brazil, this disease has already been found in Kenya (Ramos and Shavdia, 1976; Ithiru et al., 2013), Ethiopia (Korobko and Wondimagegne, 1997), Uganda (Ramos and Shavdia, 1976) and China (Xuehui et al., 2013).
The BHB symptoms occur in leaves, young fruits and branches. The lesions on the leaves are irregular and of a brownish-brown color with yellowish halo around them. The

lesions are more common on the edges of the leaves, where it is easier for bacteria to penetrate due to mechanical damages. However, they can extend throughout the leaf surface. In more severe attacks necrosis of branches and young fruits may occur. Young plants are more susceptible and may suffer leaf shedding, die-back of the branches, over sprouting and delayed early development (Amaral et al., 1956). Recently, *P. syringae* pv. *tabaci* was reported as a pathogenic agent of the bacterial-leaf-spot (BLS), causing symptoms in coffee leaves very similar to that of the BHB (Destéfano et al., 2010; Rodrigues et al., 2017). In the state of Paraná, the simultaneous occurrence of pathovars *garcae* and *tabaci* (Rodrigues et al., 2017) was identified.
The occurrence of these two bacterial diseases, especially the BHB, has caused significant losses in the coffee production, since the chemical control is not so efficient and there are few cultivars identified with resistance.

The most suitable method of control is the use of resistant cultivars to *P. syringae* pv. *garcae* and *P. syringae.* pv. *tabaci*, since it is efficient and avoids the use of chemical control in coffee. Few Arabica coffee cultivars were reported with a high level of resistance to BHB. Under field conditions with natural occurrence of BHB and BLS, the susceptible cultivars were Catuaí V. IAC 81, Catucaiam 24137, Catucaí Amarelo 2SL, Japy, IPR 99, Arara, IPR 107, Acauã, Sabiá e Mundo Novo IAC 376-4. The last has been classified as highly susceptible (Fernandes et al., 2020). IPR 98, IPR 100, Tupi IAC 1669-33 (Ito et al., 2008; Andreazi et al., 2015), Bourbon, Topázio MG 1190 e IAC Ouro Verde also are susceptible to BHB (Matiello et al., 2016). Results obtained in field trials with natural occurrence of BHB and BLS showed that *C. arabica* cv. IPR 102 had a high level of resistance (Ito et al., 2008; Sera et al., 2017; Fernandes et al., 2020). Intermediate resistance was found in IAPAR 59, IPR 103, IPR 104, IPR 106, IPR 108, Catiguá MG1, Catiguá MG2, Catucaiam 2015479, IBC Palma 2 and Japiam (Ito et al., 2008; Fernandes et al., 2020). In a study with coffee seedlings inoculated with BHB and BLS, IPR 102 was reported having simultaneous resistance to both diseases, with 100% of plants resistant to BHB, but presented 33.3% of segregating plants susceptible to BLS (Rodrigues et al., 2019). In the same study, the varieties Palido Viridis and Dilla and Alghe were also reported resistant to BHB and BLS. However, the latter showed susceptible plants segregating to both diseases.

Sources of resistance to BHB were identified in Arabica coffees from Ethiopia and in other species such as *C. canephora*, *C. congensis*, *C. salvatrix*, *C. eugenioides* and *C. stenophylla* (Moraes et al., 1975; Mohan et al., 1978; Rodrigues et al., 2019). Resistance to BHB was also observed in Arabica coffees with introgression of *C. canephora* genes such as in Icatu and Timor Hybrid (TH) derivatives (Mohan et al., 1978; Petek et al., 2006; Ito et al., 2008).

The genotype S288-23 was originated from the cross between the species *C. arabica* and *C. liberica.* It was classified as moderately resistant to BHB (Moraes et al., 1975), whereas this resistance probably was originated from *C. liberica*.

Coffee trees derived from BA series are tetraploids and they were also derived from the cross between *C. arabica* and *C. liberica* var. *liberica* and they were developed at the Balehonnur Experimental Station in India (Bettencourt, 1981). The Instituto Agronômico (IAC) received 10 selections from the BA series (Bettencourt, 1981), which were used in its breeding program by hybridizations with *C. arabica* cultivars such as Mundo Novo, Acaiá and Catuaí (Fazuoli et al., 2005). From these hybrids, coffee lines with *SH3* rust resistance gene, drought-tolerant and high yield were produced. The first two traits were originated from the BA series (Fazuoli et al., 2005). F_3 lines derived from these BA coffees were produced by IAC to IAPAR in 1977, one of which originated from the cross between Catuaí and (Catuaí x BA-10), was advanced by IAPAR to the F_5 generation and called PRFB E9705-9. The latter had simultaneous resistance to leaf rust and BHB and it was crossed with the cultivar IPR 100, which also derived from BA-10, but susceptible to these two diseases and resistant to nematodes. F_4 lines derived from this crossing were obtained by selection of coffee plants that showed high yield, in addition to resistance to rust and nematodes.

There are no studies about resistance to *Pseudomonas syringae* (PS) in coffee genotypes derived from BA-10. Therefore, the aim of this study was to evaluate the resistance to PS in lines derived from BA-10, under field conditions with simultaneous natural infections of the pathovars *garcae* and *tabaci*.

Results and discussion

Resistance of lines
The mean grades of PS severity of Catuaí, IPR 100 and IAPAR 59 were 4.93, 4.70 and 3.30, respectively, confirming the high susceptibility of the first two and the intermediate resistance of the last. Catuaí and IPR 100 had 100% susceptible plants with grades 4 and 5, showing that in the experiment the PS severity was very high. It was possible to classify the lines into four groups by the Scott-Knott test: group d = 20 F_4 lines did not differ from Catuaí and IPR 100; group c = 17 F_4 lines differed from Catuaí and did not differ from the IAPAR 59; group b = F_4 line IAPAR 12201 was more resistant than IAPAR 59; group a = F_5 lines, PRFB E9705-9 and IAPAR 11390, were the most resistant (Table 1).

The most resistant F_4 line IAPAR 12201 presented a mean grade of 2.20 and 60% of resistant plants with grade 1, but 40% of the plants were susceptible (Table 1). The intermediate resistance check, IAPAR 59, presented 43.75% of plants with grades 4 and 5. As we detected a mixture of the *garcae* and *tabaci* pathovars in the experiment, it is not possible to state whether IAPAR 12201 and IAPAR 59 are homozygous or heterozygous for resistance. One of the hypotheses is that 40% of plants with grades 3 and 5 observed in the line IAPAR 12201 and 43.75% of plants with grades 4 and 5 of IAPAR 59 may have occurred because these two genotypes have simultaneous resistance to both bacteria and are in heterozygous condition. The second hypothesis is that they have resistance in homozygosis only to one of the bacteria (e.g. patovar *garcae*) and are susceptible to the other (e.g. patovar *tabaci*). The third hypothesis is that the intermediate resistance observed in IAPAR 59 can be quantitative type, governed by many genes. This type of resistance is highly influenced by the environment, so it is possible that plants with intermediate resistance could have an increase in the intensity of the disease, when exposed to an environment very favorable to the pathogen. This could be the explanation for the observation of 12.50% of plants with grades 5 in IAPAR 59. But this percentage was much lower than those observed in the cultivars Catuaí and IPR 100with 70 and 93.75%, respectively.

Currently, only the cultivar IPR 102 was identified with a high level of resistance to BHB (Ito et al., 2008; Sera et al., 2017; Rodrigues et al., 2019; Fernandes et al., 2020) and BLS (Rodrigues et al., 2019). IAPAR 12201 is the F_4 line that has the highest potential to become a cultivar with a high level of resistance to *Pseudomonas syringae*; and therefore, will be advanced to the next generation of self-pollination. PRFB E9705-9 and IAPAR 11390 presented 100% resistant plants, which probably have homozygous resistance for both PS patovars. PRFB E9705-9 and IAPAR 11390 are in the F5 generation and also have the potential to become new cultivars with resistance to PS or can be used as sources of resistance in breeding programs.

Both Catuaí and IPR 100 did not have any susceptible plants with grade 3, predominating those of grade 5, while the F4 lines presented a frequency of plants with grade 3 ranging from 6.25 to 87.50%, indicating that these have an intermediate resistance, which can be quantitative. In the same way as was previously cited for IAPAR 59, these lines

Table 1. Means of *Pseudomonas syringae* (PS) severity and plant frequency according to the grade scale from 1 to 5 of PS, in 38 F_4 lines derived from the crossing "PRFB E9705-9" x IPR 100 and two F_5 lines derived from 'Catuaí V. IAC 81' x (Catuaí V. IAC 81 x IAC 1110-8), evaluated under field conditions with simultaneous natural infections of the pathovars *garcae* and *tabaci*, in Londrina (PR, Brazil).

Lines	Plant frequency (%) according to PS severity grades[1]					PS severity [2]	
	1	2	3	4	5		
IAPAR 11390 [3] (F_5)	86.67	13.33	0.00	0.00	0.00	1.13	a
"PRFB E9705-9" [4] (F_5)	73.33	26.67	0.00	0.00	0.00	1.27	a
IAPAR 12201 (F_4)	60.00	0.00	20.00	0.00	20.00	2.20	b
IAPAR 12168 (F_4)	6.67	13.33	66.67	13.33	0.00	2.87	c
IAPAR 12169 (F_4)	0.00	25.00	56.25	18.75	0.00	2.93	c
IAPAR 12170 (F_4)	0.00	0.00	87.50	12.50	0.00	3.13	c
IAPAR 12205 (F_4)	0.00	17.64	41.18	41.18	0.00	3.20	c
IAPAR 12203 (F_4)	0.00	20.00	40.00	40.00	0.00	3.20	c
IAPAR 12139 (F_4)	0.00	21.42	35.72	42.86	0.00	3.23	c
Cultivar IAPAR 59	0.00	25.00	31.25	31.25	12.50	3.30	c
IAPAR 12199 (F_4)	0.00	20.00	40.00	26.67	13.33	3.33	c
IAPAR 12188 (F_4)	0.00	6.67	73.33	20.00	0.00	3.33	c
IAPAR 12197 (F_4)	0.00	6.67	60.00	26.66	6.67	3.33	c
IAPAR 12202 (F_4)	0.00	13.33	40.00	46.67	0.00	3.33	c
IAPAR 12187 (F_4)	0.00	6.25	50.00	43.75	0.00	3.40	c
IAPAR 12204 (F_4)	0.00	6.25	43.75	43.75	6.25	3.50	c
IAPAR 12171 (F_4)	0.00	0.00	53.33	40.00	6.67	3.53	c
IAPAR 12172 (F_4)	0.00	18.75	18.75	50.00	12.50	3.53	c
IAPAR 12175 (F_4)	0.00	0.00	46.67	40.00	13.33	3.67	c
IAPAR 12194 (F_4)	0.00	6.25	31.25	56.25	6.25	3.67	c
IAPAR 12186 (F_4)	0.00	6.67	26.66	60.00	6.67	3.67	c
IAPAR 12196 (F_4)	5.89	0.00	29.41	47.05	17.65	3.73	d
IAPAR 12185 (F_4)	0.00	0.00	33.33	60.00	6.67	3.73	d
IAPAR 12174 (F_4)	0.00	0.00	33.33	46.67	20.00	3.87	d
IAPAR 12198 (F_4)	0.00	6.67	13.33	53.33	26.67	3.97	d
IAPAR 12189 (F_4)	0.00	0.00	12.50	68.75	18.75	4.00	d
IAPAR 12176 (F_4)	0.00	0.00	20.00	60.00	20.00	4.00	d
IAPAR 12179 (F_4)	0.00	6.25	12.50	56.25	25.00	4.03	d
IAPAR 12182 (F_4)	0.00	0.00	29.42	35.29	35.29	4.07	d
IAPAR 12177 (F_4)	0.00	0.00	7.14	78.58	14.28	4.10	d
IAPAR 12181 (F_4)	0.00	0.00	20.00	46.67	33.33	4.13	d
IAPAR 12193 (F_4)	6.25	0.00	6.25	43.75	43.75	4.17	d
IAPAR 12178 (F_4)	0.00	0.00	6.25	62.50	31.25	4.27	d
IAPAR 12184 (F_4)	0.00	0.00	13.33	40.00	46.67	4.33	d
IAPAR 12192 (F_4)	0.00	0.00	0.00	66.67	33.33	4.33	d
IAPAR 12183 (F_4)	0.00	0.00	5.88	52.94	41.18	4.37	d
IAPAR 12195 (F_4)	0.00	0.00	6.67	53.33	40.00	4.40	d
IAPAR 12191 (F_4)	0.00	0.00	13.33	33.33	53.34	4.40	d
IAPAR 12190 (F_4)	0.00	0.00	0.00	43.75	56.25	4.53	d
IAPAR 12180 (F_4)	0.00	0.00	0.00	43.75	56.25	4.57	d
IAPAR 12200 (F_4)	0.00	0.00	0.00	40.00	60.00	4.60	d
Cultivar IPR 100 [3]	0.00	0.00	0.00	30.00	70.00	4.70	d
Cultivar Catuaí V. IAC 81	0.00	0.00	0.00	6.25	93.75	4.93	d
General mean						3.67	
CV%						4.84	

[1] Scale of grades from 1 to 5 of the *P. syringae* severity, where 1 is the most resistant and 5 the most susceptible. [2] Means followed by the same letter do not differ by the Scott-Knott mean clustering test (α = 5%). Data transformed by $\sqrt{x+1}$. [3] Originated from H8721 (plant 1420 of 'Catuaí V. IAC 81' x "H7314-4"). [4] Originated from H8598 (plant 1412 of 'Catuaí V. IAC 81' x "H7314-5").
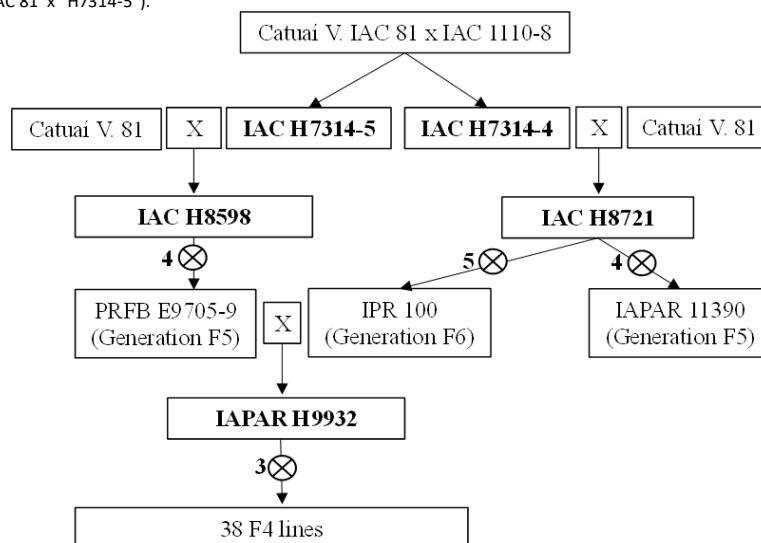
Fig 1. Pedigree of F4 lines derived from IAPAR H9932 and F5 lines named PRFB E9705-9 and IAPAR 11390. = self-pollination; X = hybridization

may have an intermediate resistance in heterozygous for the two bacteria or homozygous for one of them. Under a natural occurrence of BHB and BLS, IPR 106 and Japiam, derived from Icatu, Catiguá MG 2, Catiguá MG 1 and IBC Palma 2, which derived from TH, showed moderate resistance (Fernandes et al., 2020). Other studies also reported the possibility of the existence of quantitative resistance to BHB in genotypes derived from TH and Icatu (Petek et al., 2006; Ito et al., 2008).

Source of resistance

F_4 lines were derived from the crossing "PRFB E9705-9" x IPR 100 and the likely source of resistance was "PRFB E9705-9", as the latter showed 100% of resistant plants, while IPR 100 and Catuaí V. IAC 81 were susceptible (Table 1). "PRFB E9705-9" was originated from the hybrid H8598 (plant 1412 of 'Catuaí V. IAC 81' x "H7314-5") and H7314 is a hybrid between 'Catuaí V. IAC 81' and IAC 1110-8. IAPAR 11390 and IPR 100 were originated from H8721 (plant 1420 of 'Catuaí V. IAC 81' x "H7314-4") (Fig 1). However, the first presented 100% of resistant plants and the second 100% of susceptible plants (Table 1). Although these two were originated from the same F_1 plant of H8721, only the F_5 line IAPAR 11390 was resistant, because in previous segregant generations resistant plants were selected, whereas for IPR 100 this was not done. As "PRFB E9705-9" and IAPAR 11390 were resistant, PS resistance was originated from H7314-4 and H7314-5, which had the resistance originated from IAC 1110-8. The latter, also called BA-10, is a *Coffea arabica* with introgression of *C. liberica* genes, which may have been the source of original resistance. Rodrigues et al. (2019) studied the seedlings of *C. liberica* var. *liberica*, and artificially inoculated them with BHB. They observed 6.3% of resistant plants, 31.2% moderately resistant and 62.5% of susceptible. The main source of resistance to BHB used by breeding programs is wild Ethiopian Arabica coffees (Sera, 2001). Our study is the first to report resistance to PS in coffee genotypes derived from BA-10. In another study, the genotype S288-23, also carrier of *C. liberica* genes, presented moderate resistance to BHB (Moraes et al., 1975).

Materials and methods

Plant materials

A total of 38 F_4 lines derived from the hybrid IAPAR H9932 ("PRFB E9705-9" x IPR 100) were evaluated. The cultivars Catuaí Vermelho IAC 81 and IPR 100 were the susceptible checks and IAPAR 59 was the intermediate resistant check (Ito et al., 2008). The F_5 lines "PRFB E9705-9" and IAPAR 11390 also were evaluated. "PRFB E9705-9" was originated from the hybrid IAC H8598 (plant 1412 of 'Catuaí V. IAC 81' x "IAC H7314-5"). IAPAR 11390 and IPR 100 were originated from IAC H8721 (plant 1420 of 'Catuaí V. IAC 81' x "IAC H7314-4"). IAC H7314-4 and IAC H7314-5 are hybrids between 'Catuaí V. IAC 81' and IAC 1110-8 (Fig 1). Also known as BA-10, the genotype IAC 1110-8 was originated from the coffee plants of the BA series from India, and it is a *C. arabica* with introgression of *C. liberica* var. *liberica*.

Field trial

The field trial was installed in April 2013 at the IAPAR experimental station (lat 23º21'44" S, long 51º09'49" W, alt 582m asl), in Londrina, Paraná state, Brazil. The climate is classified as Cfa, according to Köppen. The average annual temperature in Londrina is 21.1° C and the annual average precipitation varies from 1400 to 1600 mm per year. The experiment was planting at 2.50m spacing inter-row and 0.70m within row, using randomized blocks design, with three replications and five plants per plot.

Soil fertilization and corrections, besides agricultural practices, were made according to the recommendation for coffee crop (Matiello et al., 2016). In the years 2016 and 2017, antibiotics, resistance inducers and copper-based products were not applied, which could reduce the PS intensity.

Identification of the pathovars of Pseudomonas syringae

A sample composed of 60 leaves of this experiment was sent to the Biological Institute, Campinas-SP, Brazil to identify pathogens.

The obtained isolates were submitted to the LOPAT tests (levan, oxidase, protopectinase in potato disks, arginine dihydrolase, and tobacco HR) and fluorescent pigment in BK medium (King et al., 1956). All strains presented the results, + - - - + to the respective tests and variable response to fluorescent pigment, indicating the inclusion of the obtained isolates in Group I of *P. syringae*.

Subsequently, the strains were subjected to biochemical (Lelliot et al, 1966; Young and Triggs, 1994; Schaad, 2001; Rodrigues et al, 2017) and pathogenicity tests. The results determined the occurrence of *P. syringae* pv. *garcae*, causal agent of the BHB and *P. syringae* pv. *tabaci*, causal agent of BLS, in the experimental field.

Resistance assessment

An assessment of the PS severity was made in January 2017 (45 months after planting), based on the symptoms described by Amaral et al. (1956). Severity was evaluated in natural infection condition of pathovars *garcae* and *tabaci*, using a grade scale, ranging from 1 to 5 (Sera et al., 2017), 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 tenth pair of fully expanded leaves were considered. This was done because the leaves located further inside the plant had low disease severity or no lesions.

Plants with grades 1 and 2 were considered resistant and with grades 3, 4 and 5 as susceptible.

Statistical analysis

PS severity data were transformed to $\sqrt{x+1}$ at level of mean of plots. ANOVA, Bartlett test of homogeneity of variances, Shapiro-Wilk normality test and Scott-Knott mean clustering test at 5% significance were performed using the R software version 3.3.0 (R Core Team, 2016), package ScottKnott (Jelihovschi et al., 2014).

Conclusion

Two F_5 lines, derived from BA-10, showed 100% of resistant plants to PS. The F_4 line IAPAR 12201, also derived from BA-10, showed a high frequency of PS resistant plants. Several F_4 lines showed high frequency of plants with intermediate

resistance to PS. The cultivar IPR 100 was susceptible and IAPAR 59 showed intermediate resistance.

Acknowledgments

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