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Multiple resistance to bacterial halo blight and bacterial leaf spot in *Coffea* spp.*

Resistência múltipla à mancha aureolada e à mancha foliar bacteriana em **Coffea** *spp.*

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ABSTRACT: Breeding for genetic resistance is an important method of crop disease management, due to the numerous benefits and low cost of establishment. In this study, progenies of 11 *Coffea* species and 16 wild *C. arabica* accessions were tested for their response to *Pseudomonas syringae* pv. *garcae*, the causal agent of bacterial halo blight, a widespread disease in the main coffee-producing regions of Brazil and considered a limiting factor for cultivation in pathogen-favorable areas; and also to *P. syringae* pv. *tabaci*, causal agent of bacterial leaf spot, a highly aggressive disease recently detected in Brazil. Separate experiments for each disease were carried out in a greenhouse, with artificial pathogen inoculations and ideal moisture conditions for disease development. The results showed that *C. canephora*, *C. congensis*, *C. eugenioides*, *C. stenophylla*, and *C. salvatrix* progenies, the wild *C. arabica* accessions Dilla & Alghe and Palido Viridis, and cultivar IPR 102 contain satisfactory levels of simultaneous resistance against bacterial halo blight and bacterial leaf spot. These results are useful in breeding programs for durable resistance to multiple biotic agents, providing new combinations of resistance alleles by hybridization, as well as for phytopathological studies, to identify infraspecific variability of the pathogens.

KEYWORDS: germplasm bank; resistant accessions; *Pseudomonas syringae* pv. *garcae*; *Pseudomonas syringae* pv. *tabaci*.

RESUMO: O melhoramento de plantas para resistência genética é um método importante para o manejo de doenças, pelos inúmeros benefícios e baixo custo de implementação. No presente estudo, progênies de 11 espécies de *Coffea* e 16 acessos selvagens de *C. arabica* foram testados quanto à resposta a *Pseudomonas syringae* pv. *garcae*, agente causal da mancha aureolada, doença disseminada nas principais regiões produtoras de café do Brasil e considerada fator limitante para o cultivo em áreas favoráveis a patógenos; e também para *P. syringae* pv. *tabaci*, agente causal da mancha foliar bacteriana, doença altamente agressiva detectada recentemente no Brasil. Experimentos separados para cada doença foram realizados em estufa, por meio da inoculação artificial dos patógenos em condições ideais de umidade para o desenvolvimento das doenças. Os resultados mostraram que as progênies *Coffea canephora*, *C. congensis*, *C. eugenioides*, *C. stenophylla* e *C. salvatrix*, além dos acessos selvagens de *C. arabica* Dilla & Alghe e Palido Viridis e da cultivar IPR 102, possuem níveis satisfatórios de resistência simultânea contra mancha aureolada e mancha foliar bacteriana. Os resultados descritos são úteis em programas de melhoramento para resistência duradoura a múltiplos agentes bióticos, fornecendo novas combinações de alelos de resistência por hibridização, bem como para estudos fitopatológicos, para identificar a variabilidade infraespecífica dos patógenos.

PALAVRAS-CHAVE: banco de germoplasma; acessos resistentes; *Pseudomonas syringae* pv. *garcae*; *P. syringae* pv. *tabaci*.

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INTRODUCTION

The current taxonomy of the genus *Coffea* comprises 125 species (KRISHNAN et al., 2015), but only grains of *C. arabica* Lineu and *C. canephora* Pierre ex A. Froehner species are commercially exploited, corresponding to 55 and 45%, respectively, of the world production (USDA, 2014). According to DAVIS et al. (2006), *C. liberica* Bull. ex Hiern can also be cited, although commercially it plays a marginal role.

In spite of the wide diversity of the genus, few species have been used in breeding programs of *C. arabica* and *C. canephora*, partly because of the genetic barriers that hamper the development of hybrid plants (CARVALHO et al., 1984), but mainly due to the difficulty in reestablishing the original genome of the cultivated varieties and, consequently, the selection of a stable cultivar.

Some species such as *C. eugenioides* S. Moore (NAGAI et al., 2008), *C. liberica* (BETTENCOURT; CARVALHO, 1968; FORTUNATO et al., 2010), and *C. racemosa* Lour. (GUERREIRO FILHO, 2007), respectively, were already used as allele donor parents of genes related to caffeine synthesis, resistance to leaf rust *Hemileia vastatrix* Berkeley & Broome and resistance to coffee leaf miner *Leucoptera coffeella* Guérin-Ménéville.

For an improved use of *Coffea* species in breeding programs, mainly for tolerance to abiotic stresses and resistance to biotic stresses such as diseases and pests, traits of agronomical interest must be phenotyped. In fact, the *Coffea* germplasm banks were primarily phenotyped for the main diseases of the crop. Some of these diseases have become relevant in a number of coffee-producing countries and sources of pathogen resistance can still be found in wild genetic resources preserved in *in situ* collections.

Bacterial halo blight of coffee (BHB), caused by *Pseudomonas syringae* pv. *garcae* (AMARAL et al., 1956; YOUNG et al., 1978), can cause large-scale damage and disease outbreaks, according to COSTA et al. (1957). Outbreaks of the disease occur in areas with high inoculum potential or in nurseries, making coffee production and/or the sale of seedlings infeasible. In Brazil, BHB has already been detected in several regions of the states of São Paulo, Paraná and Minas Gerais (AMARAL et al., 1956; KIMURA et al., 1973; ZOCCOLI et al., 2011; RODRIGUES et al., 2017b). Moreover, ALMEIDA et al. (2012) reported an increase in disease incidence and severity in the main coffee-producing states of Brazil.

In the African continent, BHB has already been described in Kenya, Ethiopia and Uganda (RAMOS; SHAVDIA, 1976; KOROBKO; WONDIMIGEGNE 1997), and in Asia, only in China (XUEHUI et al., 2013).

Resistance sources to BHB were identified in *C. stenophylla* G. Don and *C. eugenioides* accessions of the germplasm bank of the Agronomic Institute of Paraná (Instituto Agronômico do Paraná — IAPAR) (MOHAN et al., 1978). Exotic varieties of *C. arabica*, *e.g.*, Dilla & Alghe, Geisha, Harar, and S. 12 Kaffa (MORAES et al., 1975), Ennarea and Semierecta (MOHAN et al., 1978), SL 28 (KAIRU, 1997), as well as 38 wild accessions from Ethiopia, mentioned in a Food and Agriculture Organization (FAO) survey of 1968 (FAO, 1968; MOHAN et al., 1978), were resistant to *P. syringae* pv. *garcae*. In addition, plants of *C. canephora*, *C. congensis* and *C. liberica* var. dewevrei species proved susceptible to this pathogen (COSTA et al., 1957).

In Brazil, all arabica cultivars registered by the Brazilian Ministry of Agriculture, Livestock and Food Supply (Ministério da Agricultura, Pecuária e Abastecimento — MAPA) are BHB-susceptible, except for cultivar IPR 102, which is resistant to the disease (SERA et al., 2017).

Bacterial leaf spot (BLS), caused by *P. syringae* pv. *tabaci* (WOLF; FOSTER, 1917) (YOUNG et al., 1978) is a less-known disease, firstly observed in a coffee seedling nursery in southern São Paulo State (DESTÉFANO et al., 2010). A recent study reported the occurrence of BLS under field conditions in the state of Paraná, in separated and mixed infections with *P. syringae* pv. *garcae* (RODRIGUES et al., 2017b). To date, BLS has been poorly studied, probably because its symptoms are easily confused with BHB, for being extremely similar.

In addition, no source of BLS resistance was described. The commercial *C. arabica* cultivars Mundo Novo, IAC 125 RN, Obatã IAC 1669-20, Obatã IAC 4739, Bourbon Amarelo, and Icatu Vermelho IAC 4045, as well as a genotype of Timor Hybrid IAC 1559-13 and cultivar Apoatã IAC 2258 of *C. canephora*, were found to be susceptible to this pathogen (RODRIGUES et al., 2009).

Unlike *P. syringae* pv. *garcae*, which is specific to coffee trees (KIMURA et al., 1973), *P. syringae* pv. *tabaci* naturally infects a wide range of hosts (BRADBURY, 1986; MALAVOLTA JUNIOR et al., 2008), which represent a source of primary inoculum for coffee plants.

In view of the epidemiological importance of BLS for coffee cultivation, the knowledge about resistance sources to this pathogen must be deepened, and useful information for breeding programs of the crop obtained, targeting the introgression of genes responsible for the expression of resistance to biotic agents in susceptible commercial cultivars.

The purpose of this study was to identify simultaneous resistance sources to BHB and BLS in *Coffea* spp., as well as in *C. arabica* accessions of the Germplasm Bank of the Agronomic Institute of Campinas (Instituto Agronômico de Campinas — IAC), Campinas, São Paulo, Brazil, which are potentially useful in breeding programs, especially for *C. arabica*.

MATERIALS AND METHODS

Germplasm accessions

Seeds of 11 species of the genus *Coffea* and 16 accessions of *C. arabica* (Tables 1 and 2), in the *Coffea* Germplasm Bank

of the IAC, were transplanted at the cotyledonary stage into 180-cm^3 tubes containing plant substrate and Osmocote® fertilizer (3 g.L^{-1}), and maintained in a greenhouse until the evaluation of BHB and BLS severity.

Bacterial strains and inoculation

The bacterial strains used in the experiments were obtained from the Phytobacteria Culture Collection of the Biological Institute (Coleção de Culturas de Fitobactérias do Instituto

Table 1. Frequency of resistance, moderately resistance and susceptible plants, according to the disease rating scale (0 – 5 points) in *Coffea* spp., in response to infection by *Pseudomonas syringae* pv. *garcae*, causal agent of bacterial halo blight, evaluated in 2012 and 2013, and against *P. syringae* pv. *tabaci*, causal agent of bacterial leaf spot, evaluated in 2015.

Germplasm	E1 - 2012							E2 - 2013							E3 - 2015						
	Pseudomonas syringae pv. *garcae*														*Pseudomonas syringae* pv. *tabaci*						
		R		MR		S			R		MR		S			R		MR		S	
	n=	nº	%	nº	%	nº	%	n=	nº	%	nº	%	nº	%	n=	nº	%	nº	%	nº	%
Coffea arabica cv. IAC 125 RN	12	0	0.0	0	0.0	12	100.0	17	0	0.0	0	0.0	17	100.0	6	0	0.0	0	0.0	6	100.0
Coffea arabica cv. IAC 376-4	9	0	0.0	0	0.0	9	100.0	-	-	-	-	-	-	-	3	0	0.0	0	0.0	3	100.0
Coffea arabica cv. IAC 81	8	0	0.0	0	0.0	8	100.0	-	-	-	-	-	-	-	4	0	0.0	0	0.0	4	100.0
Coffea anthonyi	3	0	0.0	0	0.0	3	100.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Coffea congensis	-	-	-	-	-	-	-	23	0	0.0	0	0.0	23	100.0	-	-	-	-	-	-	-
Coffea kapakata	12	0	0.0	0	0.0	12	100.0	21	0	0.0	0	0.0	21	100.0	5	0	0.0	0	0.0	5	100.0
Coffea heterocalyx	4	0	0.0	0	0.0	4	100.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Coffea humilis	12	0	0.0	1	8.4	11	91.6	2	0	0.0	0	0.0	2	100.0	5	0	0.0	0	0.0	5	100.0
Coffea liberica var. *liberica*	-	-	-	-	-	-	-	16	1	6.3	5	31.2	10	62.5	-	-	-	-	-	-	-
Coffea liberica var. *dewevrei*	-	-	-	-	-	-	-	19	2	10.5	0	0.0	17	89.5	-	-	-	-	-	-	-
Coffea liberica var. *passipagore*	10	0	0.0	3	30.0	7	70.0	-	-	-	-	-	-	-	3	0	0.0	0	0.0	3	100.0
Coffea congensis IAC 4349	8	0	0.0	5	62.5	3	37.5	16	3	18.8	2	12.4	11	68.8	5	4	80.0	1	20.0	0	0.0
Coffea congensis IAC 4350	-	-	-	-	-	-	-	20	8	40.0	3	15.0	9	45.0	-	-	-	-	-	-	-
Coffea canephora var. *robusta*	7	3	42.9	1	14.2	3	42.9	-	-	-	-	-	-	-	5	3	60.0	0	0.0	2	40.0
Coffea eugenioides	11	2	18.2	6	54.5	3	27.3	19	8	42.1	1	5.3	10	52.6	5	1	20.0	0	0.0	4	80.0
Coffea stenophylla	6	1	16.6	4	66.8	1	16.6	-	-	-	-	-	-	-	3	3	100.0	0	0.0	0	0.0
Coffea salvatrix	11	4	36.4	4	36.4	3	27.2	-	-	-	-	-	-	-	4	3	75.0	0	0.0	1	25.0

R: resistant, score 0 on the disease rating scale; MR: moderately resistant, 1 point on the disease rating scale; S: susceptible, with 2 to 5 points on the disease rating scale; -: not evaluated.

Biológico — IBSBF). A preliminary study reported that mixed *P. syringae* pv. *garcae* strains induced high levels of disease severity (RODRIGUES et al., 2017a). A mixture of the *P. syringae* pv. *garcae* strains IBSBF 75 and IBSBF 1197, considered highly aggressive, was used to evaluate BHB severity. A 1:1 ratio mixture consisting of 2 mL of each bacterial suspension was inoculated. *Pseudomonas syringae* pv. *tabaci* strain IBSBF 2249 was used to evaluate the BLS resistance of the plants.

Bacterial suspensions for coffee seedling inoculations were standardized in a spectrophotometer for approximately 3×10^8 CFU.mL^{-1} (A 600 nm = 0.3) (LELLIOT; STEAD 1987). Inoculations were performed by the abrasion technique (RODRIGUES et al., 2017a).

Experimentation

The response of *Coffea* spp. to BHB was tested in two independent experiments, in 2012 (E1) and 2013 (E2), using 11 and seven species, respectively. The experiment was arranged in a completely randomized design with three to 23 replications per species, and the experimental plots consisted of a single plant. After inoculation, the plants were maintained at a relative humidity level above 70%.

In 2014, the same coffee plants used in E1 were pruned and maintained at low relative humidity, unfavorable to pathogen growth. Subsequently, in 2015 (E3), plants with 4 – 5 expanded leaves (approximately 5 – 6 months after pruning) were tested against *P. syringae* pv. *tabaci*, strain IBSBF 2249.

In 2014 (E4), the behavior of 16 *C. arabica* accessions in response to BHB was evaluated as described above. After the end of the experimental period, 42 days after inoculation (DAI), the plants were pruned and maintained at low relative humidity, unfavorable to the pathogen. In 2015 (E5), after the development of at least three healthy internodes, the plants were tested for BLS-resistance. The experimental design was a completely randomized design with six replications per accession, and the plots represented by a single plant.

Disease evaluation

A 0 – 5-disease-rating scale was used, according to the symptoms observed in the inoculated area, in which 0 was attributed to symptom-free plants and 5 to leaves with necrosis of the entire inoculated area (RODRIGUES et al., 2017a). For the interpretation of results, the following classification

Table 2. Number of inoculated seedlings, frequency of resistance, moderately resistance and susceptible plants, according to the disease rating scale of 0 – 5 points, 42 days after inoculation of germplasm, evaluated in relation to infection by *Pseudomonas syringae* pv. *garcae* and *P. syringae* pv. *tabaci* causal agents of bacterial halo blight and bacterial leaf spot, respectively.

Germplasm	*Pseudomonas syringae* pv. *garcae* (E4)							*Pseudomonas syringae* pv. *tabaci* (E5)						
	R			MR		S		R			MR		S	
	n=	nº	%	nº	%	nº	%	n=	nº	%	nº	%	nº	%
Coffea arabica var. Abyssinica	6	0	0.0	0	0.0	6	100.0	6	0	0.0	0	0.0	6	100.0
Coffea arabica var. Crassinervia	6	0	0.0	0	0.0	6	100.0	6	0	0.0	0	0.0	6	100.0
Coffea arabica var. Dilla & Alghe	6	2	33.3	1	16.7	3	50.0	6	0	0.0	1	16.7	5	83.3
Coffea arabica var. Glaucia	6	0	0.0	0	0.0	6	100.0	6	0	0.0	0	0.0	6	100.0
Coffea arabica cv. Iarana	6	0	0.0	0	0.0	6	100.0	6	0	0.0	0	0.0	6	100.0
Coffea arabica cv. Ibaare	6	0	0.0	0	0.0	6	100.0	6	0	0.0	1	16.7	5	83.3
Coffea arabica cv. IPR 102	6	6	100.0	0	0.0	0	0.0	6	2	33.3	2	33.3	2	33.3
Coffea arabica cv. Ibairi	6	0	0.0	0	0.0	6	100.0	6	0	0.0	0	0.0	6	100.0
C. arabica var. Pacas	6	0	0.0	0	0.0	6	100.0	6	0	0.0	0	0.0	6	100.0
Coffea arabica var. Palido Viridis	6	6	100.0	0	0.0	0	0.0	6	1	16.7	5	83.3	0	0.0
Coffea arabica var. Pendula	6	0	0.0	0	0.0	6	100.0	6	0	0.0	0	0.0	6	100.0
Coffea arabica cv. São Bernardo	5	2	40.0	0	0.0	3	60.0	5	0	0.0	0	0.0	6	100.0
São Bernardo × Mundo Novo	6	0	0.0	0	0.0	6	100.0	6	0	0.0	0	0.0	6	100.0
Coffea arabica cv. Villa Lobos	6	0	0.0	0	0.0	6	100.0	6	0	0.0	0	0.0	6	100.0
Coffea arabica cv. Villa Sarchi	6	2	33.3	0	0.0	4	66.7	6	0	0.0	0	0.0	6	100.0
Coffea arabica cv. IAC 125 RN	6	0	0.0	0	0.0	6	100.0	6	0	0.0	0	0.0	6	100.0

R: resistant, score 0 on the disease rating scale; MR: moderately resistant, 1 point on the disease rating scale; S: susceptible, with 2 to 5 points on the disease rating scale.

was considered: resistant (score 0); moderately resistant (1); and susceptible (2 to 5 points on the disease-rating scale).

RESULTS

The results of the experiments E1 and E2 are shown in Table 1. In the cultivars *Coffea arabica* Mundo Novo IAC 376-4 and Catuaí Vermelho IAC 81, high susceptibility levels to *P. syringae* pv. *garcae* were observed in E1, similar to cultivar IAC 125 RN, used as susceptible control. Susceptibility of all plants of the species *C. kapakata* A. Chev., *C. anthonyi* Stoff. & F. Anthony, and *C. heterocalyx* Stoff. was also observed. However, over the experimental period, disease evolution varied among the plants of each species, unlike in the case of the *C. arabica* cultivars, with a generally uniform development. The disease evolution in susceptible plants was heterogeneous, varying among the plants of each species, especially in *C. anthonyi* and *C. heterocalyx*.

In *C. kapakata*, between 7 and 21 DAI, all inoculated leaves dropped, and, in some cases, the bacteria colonized young leaves in the expansion phase.

Segregation for BHB resistance in variable proportions was observed in the other seven evaluated *Coffea* species (Table 1).

While high percentages of BHB-susceptible plants were found in progenies of *C. humilis* A. Chev. (91.6%) and *C. liberica* var. passipagore (70%), 42.9 and 37.5%, respectively, were found for *C. canephora* and *C. congensis* A. Froehner IAC 4,349. Lowest percentages of susceptible plants were observed in *C. salvatrix* Swynn & Phillipson (27.2%), *C. eugenioides* (27.3%) and *C. stenophylla* (16.6%).

In BHB-resistant plants, distinct reactions were observed in leaf tissues around the wounds caused by inoculations. The species *C. salvatrix* and *C. canephora* showed no visible changes around the inoculation areas. In some resistant plants of *C. eugenioides* and *C. stenophylla*, darkening around the injured points was observed. Additionally, around some wounds, the presence of a yellowish halo without visual signs of pathogen colonization was observed (Fig. 1). Bacterial flow exudates from these tissues tested negative for the pathogen.

Similar results were obtained in E2 (Table 1). Lowest percentages of susceptible coffee trees were found for *C. congensis* IAC 4,350 (45%) and *C. eugenioides* (52.6%), and the highest percentage of resistant coffee trees (42.1%) (Table 1).

All plants of the species *C. kapakata* and *C. humilis* were susceptible, as well as those of the susceptible control cultivar IAC 125 RN of *C. arabica*.

Segregation for BHB resistance was observed in progenies of other species. The frequency of resistant plants was about 40% in progenies of *C. eugenioides* and *C. congensis* IAC 4,350 and less than 20% in *C. liberica* var. liberica, *C. liberica* var. dewevrei, and *C. congensis*. In these three species, the percentage of susceptible plants was 62.5, 89.5 and 68.8%, respectively.

The symptom development in E2 plants was similar as in those of the previous tests (E1). The severity in *C. kapakata* peaked seven DAI, while symptom evolution was slower on plants of the susceptible cultivar IAC 125 RN.

The severity of BLS was also evaluated in nine species of the genus *Coffea* (E3). Results of the plants of each progeny were ranked according to the level of disease resistance/susceptibility (Table 1).

The species *C. humilis*, *C. kapakata* and *C. liberica* var. passipagore were found to be as susceptible to *P. syringae* pv. *tabaci* as the evaluated *C. arabica* cultivars.

Sources of BLS-resistance were observed in the species *C. congensis*, cultivar IAC 4,349, *C. canephora*, *C. eugenioides*, *C. stenophylla*, and *C. salvatrix*. Among these, only *C. congensis* and *C. stenophylla* had no BLS-susceptible plants. In resistant coffee trees of *C. stenophylla* and in only one of *C. salvatrix*, dry lesions surrounded by a discrete yellow halo, with no visual symptoms of bacteria colonization, were observed 42 DAI

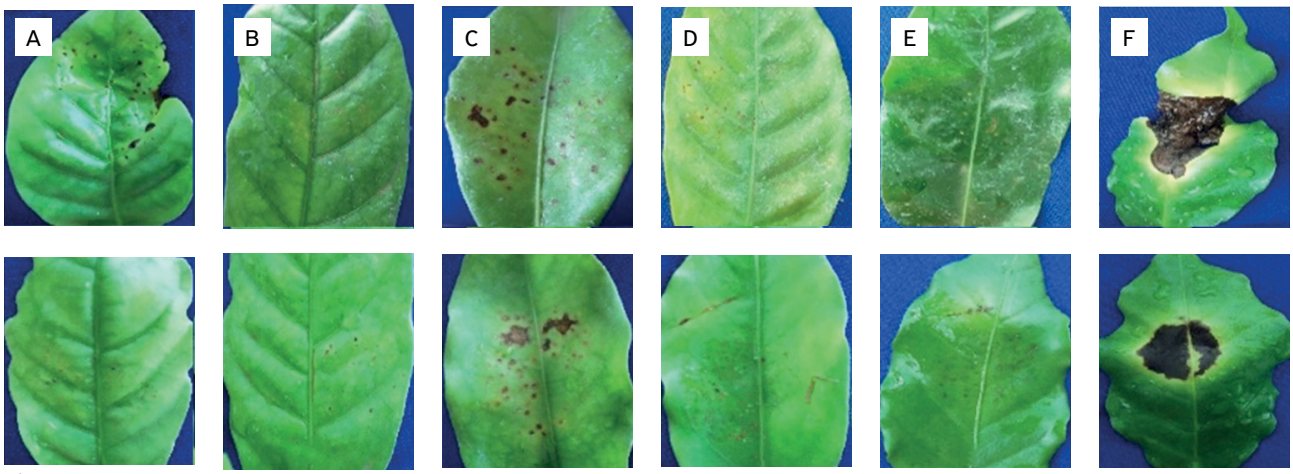

Figure 1. Reaction of *Coffea* spp. against *Pseudomonas syringae* pv. *garcae* (pictures above) and *P. syringae* pv. *tabaci* (pictures below), respectively in: (A) *Coffea canephora*; (B) *C. congensis*; (C) *C. eugenioides*; (D) *C. stenophylla*; (E) *C. salvatrix*; (F) *C. arabica*, cultivar cultivar IAC 125 RN (F), 42 days after inoculation.

(Fig. 1). Microscopic examinations of bacterial flow exudates tested negative.

The analysis of the reactions of the evaluated cultivars and botanical and exotic varieties of *C. arabica* to *P. syringae*, pathovars *garcae* (E4) and *tabaci* (E5), are shown in Table 2. All plants of cultivar IPR 102, as well as the variety Palido Viridis, were BHB-resistant. However, resistance to *P. syringae* pv. *tabaci* was only observed in Palido Viridis, classified as resistant (1) or moderately resistant (5). The plants of cultivar IPR 102 were BLS-susceptible.

Genetic variability for simultaneous resistance to *P. syringae* pathovars *garcae* and *tabaci* was identified in progenies of variety Dilla & Alghe. Of the varieties São Bernardo and Villa Sarchi, 40 and 33.3% were BHB-resistant, respectively, although BLS-susceptible. All other evaluated *C. arabica* genotypes were susceptible to both diseases.

DISCUSSION

Resistance to BHB was reported previously by MOHAN et al. (1978) in the species *C. eugenioides* and *C. stenophylla*. Our results extended the diversity of resistance sources with the inclusion of *C. liberica*, *C. canephora*, *C. congensis*, and *C. salvatrix*. Sources of simultaneous resistance to the pathovars *P. syringae garcae* and *tabaci* were identified in the species *C. canephora*, *C. congensis*, *C. eugenioides*, *C. salvatrix*, and *C. stenophylla*.

In the studied plant species, resistance reactions to *P. syringae* pv. *garcae* and *P. syringae* pv. *tabaci* occur separately or simultaneously, suggesting the existence of different genes acting in the resistance expression to both pathovars in coffee plants. Probably, there are different resistance mechanisms against these pathogens in the analyzed populations.

Different *Coffea* species, resistant to BHB and/or BLS, have a particular use in phytobacteriology, with a view to identify infraspecific variability of these pathogens, as already known for other pathovars of *P. syringae*. The transfer of the resistance genes contained in these resistant sources may also allow the development of a cultivar with intrinsic traits found only in these species.

In spite of some difficulties of this strategy, *e.g.*, the existence of genetic barriers to interspecific crosses (CARVALHO; MONACO, 1968) and the long time required for the introgression of genes of interest and recovery of the recurrent genome, a *C. arabica* cultivar resistant to *L. coffeella* was developed in Brazil, by the transfer of resistance genes from *C. racemosa* (CARDOSO et al., 2014; MENDONÇA et al., 2016). Low-caffeine content cultivars were selected by hybridization of *C. eugenioides* in recombination with the species *C. arabica* and *C. canephora* (NAGAI et al., 2008).

Other currently available methods, *e.g.*, marker-assisted selection (BERNARDO, 2008) or genome-wide selection (MEUWISSEN et al., 2001), can make the use of wild species with multiple traits of interest feasible, for example of *C. eugenioides* and *C. salvatrix*, according to our studies both resistant to bacterial diseases, resistant to leaf-miner (GUERREIRO FILHO et al., 1991) and having a low caffeine content in the endosperm (MAZZAFERA; CARVALHO, 1992). *Coffea eugenioides* was also resistant to coffee berry borer, *Hypothenemus hampei* Ferrari (SERA et al., 2010), and in *C. salvatrix* the seed oil content was high (MAZZAFERA et al., 1998).

Genome-wide selection was used by ALKIMIM et al. (2017) for the identification and selection of *C. arabica* genotypes carrying resistant genes to leaf rust and coffee berry disease, caused by *Colletotrichum kahawae* (Waller & Bridge), introgressed from *C. liberica* and *C. canephora*, respectively. The profile of the species *C. liberica* was remarkable for Arabica coffee breeding, for having plants that carry the S_H3 gene, a resistant source to all *Hemileia vastatrix* races described in Brazil so far (FAZUOLI et al., 2009), being indicated as tolerant to cold stress (PETEK et al., 2005; FORTUNATO et al., 2010), as well as having simultaneous BHB and BLS resistance, according to our results.

Among the diploid species, *C. canephora* was the most adequate BHB and BLS-resistance source for traditional *C. arabica* breeding, since hybrids between these species could be established without difficulty (CARVALHO et al., 1984), and the simultaneous resistance to these pathogens was observed in this study at a relatively high frequency in progenies of this species. The proportion of BHB-resistant (43%) and BLS-resistant (60%) coffee trees in the evaluated progenies suggests that the frequency of resistance alleles in the tested genotype *C. canephora* is high. Several *C. arabica* cultivars have been developed from interspecific hybridizations of *C. canephora* with leaf rust resistant genes, such as Icatu (Brazil) (FAZUOLI et al., 1983), Ruiru 11 (Kenya) (OMONDI et al., 2001), and Cenicafé 1 (Colombia) (FLÓREZ et al., 2016).

However, a detailed investigation of resistance in *C. canephora* is required, since plants of this species evaluated by COSTA et al. (1957) and MOHAN et al. (1978) were BHB-susceptible. These divergent results suggest considerable variability in the disease resistance of this species.

In view of the difficulties described above, the most adequate method to breed new cultivars with simultaneous and stable resistance is the exploration of a primary gene pool of *C. arabica* accessions identified as BHB and BLS-resistant.

In this context, the most promising genetic material of the evaluated germplasms is cultivar IPR 102, which is highly yielding and segregates only genes for BLS resistance. This cultivar resulted from the hybridization between *C. arabica* Bourbon Vermelho Co 667 and *C. canephora* var. Robusta Co 254. Therefore, the resistance to both pathogens is probably the result of introgression of resistance genes contained in

C. canephora. The BHB-susceptibility of cultivar Bourbon Vermelho (RODRIGUES et al., 2017a) and the frequency of resistant plants of *C. canephora* to the pathovars *garcae* and *tabaci* recorded in this study support this hypothesis.

The inoculation results of *C. arabica* variety Villa Sarchi agreed with MORAES et al. (1975) and confirmed the resistance to BHB. The mutant Palido Viridis of *C. arabica*, less productive but BHB and BLS-resistant, is also an important tool for studies related to resistance inheritance and for coffee breeding programs. A few plants of *C. arabica*, variety São Bernardo, were BHB-resistant, probably due to exogenous pollen grain fertilization, since no plants of the hybrid São Bernardo × Mundo Novo with resistance to the disease were observed.

Although the pathogens are genetically very similar, the presence of resistance to one pathogen in a plant does not mean resistance to the other. Therefore, further studies aiming at the selection of plants with multiple resistance to these agents are highly desirable, as well as an improved knowledge of the resistance mechanisms involved.

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