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Ethiopian coffee germplasm is a valuable resistance gene pool to brazilian *Pseudomonas syringae* PVS *garcae* and *tabaci*

Germoplasma de cafeeiros etíopes é uma valiosa reserva de genes de resistência a *Pseudomonas syringae* PVS *garcae* e *tabaci*

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ABSTRACT

Seven wild accessions of *Coffea arabica* from Ethiopia prospected by FAO Coffee Mission 1964-1965 were investigated concerning the resistance to 18 Brazilian strains and two Kenyan strains of *Pseudomonas syringae* pv. *garcae* and four *P. syringae* pv. *tabaci* strains, causal agents of bacterial halo blight and bacterial leaf spot, respectively. The cultivars of *C. arabica* IPR 102, resistant to the diseases, and Mundo Novo IAC 376-4, susceptible, were used as experimental controls. Our results indicated that the Ethiopian accessions presented high levels of resistance to all Brazilian strains of *P. syringae* pv. *garcae* but were susceptible to infection caused by Kenyan strains, which causes different levels of severity in wild accessions and experimental controls. Ethiopian accessions were also considered resistant to the four *P. syringae* pv. *tabaci* strains, with low susceptibility observed, one point on the severity scale, in access E-268 in response to a strain of the bacterium.

Index terms: Bacterial halo blight; bacterial leaf spot; *Coffea arabica*; source of resistance; resistant cultivars.

RESUMO

Sete acessos selvagens de *Coffea arabica* da Etiópia prospectados pela FAO Coffee Mission 1964-1965 foram investigados quanto à resistência a 18 linhagens brasileiras e duas linhagens quenianas de *Pseudomonas syringae* pv. *garcae* e quatro *P. syringae* pv. *tabaci*, agentes etiológicos da mancha-aureolada e da mancha-foliar-bacterina, respectivamente. As cultivares de *C. arabica* IPR 102, resistente às doenças, e Mundo Novo IAC 376-4, suscetível, foram utilizadas como controle experimental. Nossos resultados indicaram que os acessos etíopes apresentaram altos níveis de resistência a todas as linhagens brasileiras de *P. syringae* pv. *garcae* avaliados, mas suscetíveis à infecção causada por linhagens quenianas, que causa diferentes níveis de severidade em acessos selvagens e nos controles experimentais. Os acessos etíopes também foram considerados resistentes às quatro linhagens de *P. syringae* pv. *tabaci*, tendo sido observada baixa suscetibilidade, um ponto na escala de severidade, no acesso E-268 em resposta a uma linhagem da bactéria.

Termos para indexação: Mancha-aureolada; mancha-foliar-bacteriana; *Coffea arabica*; fontes de resistência; cultivares resistentes.

INTRODUCTION

Pseudomonas syringae pv. *garcae* (synonym *P. coronafaciens* pv. *garcae*) (Amaral; Teixeira; Pinheiro, 1956; Young et al., 1978; Dutta et al., 2018), the causal agent of bacterial halo blight in *Coffea* spp., is widespread in main coffee producing regions in Brazil, and also occurs in some African countries such as Ethiopia, Kenya and Uganda (Amaral; Teixeira; Pinheiro, 1956; Kimura; Robbs; Ribeiro, 1973; Zoccoli; Takatsu; Uesugi, 2011; Vieira Junior et al., 2015; Rodrigues et al., 2017b; Ramos; Shavdia, 1976; Korobko; Wondinagegne 1997; Hinkosa et al., 2017), as well as in China (Xuehui et al., 2013).

Cultivars from the Catuai and Mundo Novo group are the most cultivated in Brazil and have been reported to be susceptible to bacterial halo blight (Mohan; Cardoso; Paiva, 1978; Zoccoli; Takatsu; Uesugi, et al., 2011; Fernandes et al., 2020), demonstrating the importance of growing cultivars with resistance to this bacterium. In a recent paper, Rodrigues et al. (2021), demonstrate the high susceptibility to the Brazilian *Coffea canephora* varieties and germplasm to *P. syringae* pv. *garcae*, reporting the

economic importance that this disease can cause in case of an outbreak on robusta coffee.

The bacterial halo blight is characterized by irregular and translucent brown leaf lesions, usually surrounded by a large yellowish halo (Amaral; Teixeira; Pinheiro, 1956). In severe attacks and special climatic conditions with high relative humidity and mild temperatures, the bacterium can colonize conductive vessels (Rodrigues; Queiroz-Voltan; Guerreiro Filho, 2015) that result in branch dieback and plant death (Costa et al., 1957). In fruits, the bacterium causes damage to the pericarp (Costa et al., 1957) and, although there is evidence that the bacterium may be carried by the seed (Belan et al., 2016), there are no reports regarding pathogen isolation from this reproductive structure in commercial seeds samples.

On the African continent the bacterial halo blight, already known as "Elgon die-back", is currently called "bacterial blight of coffee" and exhibits symptomatology very similar to bacterial halo blight, especially concerning to infection of the plant branches, which commonly causes severe die-back (Kairu; Nyangena; Crosse, 1985).

Differential pathogenic reaction among *P. syringae* pv. *garcae* strains was initially investigated by Kairu (1997) who studied four Kenyan and four Brazilian strains observing differences in the reaction of inoculated plants of SL-28 cultivar of *C. arabica.* In his study all Kenyan strains were pathogenic, while Brazilian strains were unable to infect that cultivar. Also, although biochemical tests with 22 different substrates were not able to differentiate the strains, the Brazilian and Kenyan strains presented differences in fluorescent pigment production and bacteriocin production/sensitivity.

Studies carried out by Ovod et al. (2004) showed that *P. syringae* pv. *garcae* strains from Kenya and Brazil were differentiated by serological tests based on the structure and diversity of O-Antigen reaction. The genetic diversity between Brazilian and Kenyan strains was reported by Maciel et al. (2018). Dendrograms based on repetitive genome sequence patterns (rep-PCR) grouped separately strains from these two countries with relative low similarity.

Pseudomonas syringae pv. *tabaci* (Wolf; Foster, 1918; Young et al., 1978) is the causal agent of bacterial leaf spot in *Coffea* spp., a disease with similar symptoms to bacterial halo blight and considered emerging in Brazilian regions where coffee is largely cultivated. The occurrence of this disease should be much wider than currently estimated since the similarity of symptoms with the bacterial halo blight induces misidentifications of the pathogen (Destéfano et al., 2010).

To date, few Brazilian cultivars have been identified as being resistant to the *garcae* and *tabaci* pathovars of *P. syringae*. High resistance to the two pathovars has been confirmed in several studies in the cultivar IPR 102 (Ito et al., 2008; Sera; Sera; Fazuoli, 2017; Fernandes et al., 2020), however, for the *P. syringae* pv. *tabaci*, it has not been much well studied. Intermediate resistance, at the level of moderate resistance in field conditions, has already been found in the cultivars IAPAR 59, IPR 104 and IPR 108 (Ito et al., 2008), in addition to the cultivars IPR 106, Japiam, Catiguá MG 1, Catiguá MG 2 and IBC Palma 2 (Fernandes et al., 2020).

Resistance sources to Brazilian *P. syringae* pv. *garcae* strains were evaluated by artificial inoculation of the pathogen by Mohan et al. (1978) and the resistance found in 38 Ethiopian accessions (Meyer et al., 1958) out of 138 tested, in addition to other genotypes. However, to date, no study has evaluated any of these resistant germplasm to Brazilian strains against Kenyan strains of the pathogen.

Therefore, in addition to the knowledge of molecular and biochemical aspects previously mentioned, it is important to obtain the pathogenic knowledge of Kenyan strains in resistant germoplasm to Brazilian strains, in order to identify possible differences between them.

The economic growing impact on *P. syringae* pathovars infected crops has required the establishment of management strategies that include genetic control through the selection of resistant cultivars, to both diseases. Our study aimed to identify sources with simultaneous resistance to several strains of different origin of *P. syringae* pv. *garcae* (or *P. coronafaciens* pv. *garcae*) and *P. syringae* pv. *tabaci* in wild accessions of *C. arabica* species and verify the possible differential reaction in the interaction between bacterial isolates, including pathovars, with resistant and susceptible plants.

MATERIAL AND METHODS

Germplasm

This study was carried out with open-pollinated progenies from S_2 generation of seven Ethiopian accessions from FAO Coffee Mission to Ethiopia 1964-1965 (Meyer et al., 1968), classified as resistance to a *P. syringae* pv. *garcae* strain (Mohan; Cardoso; Paiva, 1978), deposited at the *Coffea* Active Germplasm Bank of the Agronomic Institute (IAC), Campinas, SP, Brazil. The accessions were from the old provinces of Kaffa (E-233, E-268, E-287, E-338 and E-351), Illubabor (E-442) and Harar (E-7). Plants were growth in fertilized organic substrate in 180cm^3 pots.

Six plants per progeny, per bacterial strain, were evaluated. The resistant and susceptible cultivars, IPR 102 and Mundo Novo IAC 376-4, respectively (Sera; Sera; Fazuoli, 2017; Mohan; Cardoso; Paiva, 1978) were used as control.

Bacterial strains

Twenty strains of *P. syringae* pv. *garcae* isolated from Brazil (18) and Kenya (2) and four strains of *P. syringae* pv. *tabaci* from different geographical regions (Table 1; Figure 1), obtained from the Phytobacteria Culture Collection of the Biological Institute (IBSBF), Campinas, SP, Brazil, were used in our study. Biochemical identification of the bacterial halo blight and bacterial leaf spot agents was done by LOPAT tests (Schaad; Jones; Lacy, 2001).

Bacterial suspensions were prepared in saline (0.85% NaCl) from 24 h culture growth at 28 °C on nutrient agar medium (0.5% peptone, 0.3% meat extract, 0.1% NaCl, 1.8% agar and pH adjusted to 7.0) and adjusted to approximately 10^8 CFU.ml^{-1} by optical density in spectrophotometer ($Å_{600}$ = 0.25), according to Lelliott and Stead (1987).

Inoculation and Evaluation

Inoculations were performed by the abrasion technique (Rodrigues et al., 2017a) in young leaves from the two first internodes. The technique consists in an inoculum embedded sand course (2cm diameter) that was gently pressed on the abaxial surface of leaves. After inoculations, the seedlings were kept in greenhouse conditions and relative humidity above 70%. This technique allows for the absence of escapes. Since all plants of the susceptible genotype used as a control showed disease development, the experiment was conducted once.

The pathogenicity of strains was evaluated at 25 days post inoculation by the type of reaction and aggressiveness, according to the inoculated area affected by the disease, using following 0 to 5 points scale: 0 = resistant plants, absence of symptoms or hypersensitive reaction; 1 = moderately resistant, the onset of water-soaking, lesions with darkened edges and/or up-to 10% of leaf area inoculated with disease symptoms, absent or weak chlorotic halo around wounds; from 2 to 5 points = susceptible plants, water-soaking up to approximately 11% to the necrosis of inoculated area (Rodrigues et al., 2017a).

RESULTS AND DISCUSSION

At three days after inoculation (DAI), exception for the IBSBF 3053, IBSBF 3069, IBSBF 2998, IBSBF 3032, IBSBF 3269 strains, all other *Pseudomonas syringae* Brazilian strains showed visual colonization in the Mundo Novo IAC 376-4 used as an experimental control. For the aforementioned strains, the first symptoms started at 5 DAI. Kenyan strains showed the first visual symptoms at 5 DAI in Mundo Novo IAC 376-4 cultivar and in *C. arabica* wild accessions. No differences in the resistant reaction was observed in *C. arabica* wild accessions to all *P. syringae* strains from Brazil, exception the weak reaction caused by IBSBF 2240 in E-268 accession (Table 1).

The Ethiopian accessions showed to be resistant (zero points in severity scale), absence of bacterial halo blight symptoms, to the Brazilian *P. syringae* pv. *garcae* strains, that was able to infect only the susceptible cultivar Mundo Novo IAC 376-4, while Kenyan strains infected all evaluated accessions. However, accession E-338 and cultivar IPR 102 were weakly infected by IBSBF 249, or presented moderately resistant reaction, whereas accession E-233 was moderately resistant to IBSBF 3037(weakly colonization).

Pseudomonas syringae pv. *tabaci* strains caused similar symptoms to those observed for the bacterial halo blight in the inoculated plants. The IBSBF 2240 strain cause a weakly reaction in the E-268 accession, characterized by one point in the severity scale, while the IBSBF 2241, IBSBF 2249 and IBSBF 3272 strain were not pathogenic to the seven Ethiopian accessions of Arabica coffee. All strains caused disease in Mundo Novo IAC 376-4 cultivar and did not induce any symptoms in leaves of IPR 102 cultivar (Table 1).

The Ethiopian accessions evaluated in our study were selected by the previous resistance to *P. syringae* pv. *garcae* (Mohan; Cardoso; Paiva, 1978) and from artificial inoculations of isolates IBSBF 75 and IBSBF 1197 from *P. syringae* pv. *garcae* carried out by our research group. All wild accessions were evaluated for infection by 20 *P. syringae* pv. *garcae* strains from IBSBF Culture Collection, since previous studies conducted by IAC with the cultivars Bourbon Amarelo IAC J30 and Mundo Novo IAC 376-4 of *C. arabica* revealed pathogenic variability in all *P. syringae* pv. *garcae* strains from the same collection.

In our study, the seven wild accession were uniformly resistant to 18 Brazilian *P. syringae* pv. *garcae* strains, behaving as resistant to the bacterial strains from different origins. Thus, strains from different regions of São Paulo, such as Southwest (IBSBF 75), Midwest (IBSBF 248, IBSBF 3110 and IBSBF 3212), Northeast (IBSBF 3046 and IBSBF 3053) and Central East (IBSBF 1197 and IBSBF 3004) showed similar behavior to those from the South of Minas Gerais State (IBSBF 1293, IBSBF 3069, IBSBF 2998, IBSBF 3032 and IBSBF 3107), from Cerrado (IBSBF 2512, IBSBF 3024 and IBSBF 3114) or Paraná State (IBSBF 3241 and IBSBF 3269) strains.

Table 1: Severity reaction of Ethiopian accessions of *C. arabica* to strains of *Pseudomonas syringae* pv. *garcae* and *P. syringae* pv. *tabaci*.

IBSBF[1]	Country	State-City/Year	Host	Ethiopian accession[2]						Control		
				E-351	E-338	E-442	E-7	E-287	E-233	E-268	IPR 102[R]	IAC 376-4[S]
P. syringae pv. *garcae*												
75	Brazil	SP-Pirajú/1977	*C. arabica*	-	-	-	-	-	-	-	-	+++
248[P]	Brazil	SP-Garça/1958	*C. arabica*	-	-	-	-	-	-	-	-	++
3110	Brazil	SP-Garça/2012	*C. arabica*	-	-	-	-	-	-	-	-	++
3212	Brazil	SP-Getulina/2014	*C. canephora* cv. Ipiranga	-	-	-	-	-	-	-	-	+++
1197	Brazil	SP-Campinas/1995	*C. arabica*	-	-	-	-	-	-	-	-	+++
3004	Brazil	SP-Serra Negra/2008	*C. arabica* cv. Catucaí	-	-	-	-	-	-	-	-	+++
3046	Brazil	SP-Divinolândia/2009	*C. arabica*	-	-	-	-	-	-	-	-	+++
3053	Brazil	SP-Caconde/2009	*C. arabica*	-	-	-	-	-	-	-	-	+
1293	Brazil	MG-Guaxupé/1997	*C. arabica*	-	-	-	-	-	-	-	-	+++
3069	Brazil	MG-Guaxupé/2010	*C. arabica* cv. Catuaí	-	-	-	-	-	-	-	-	+
2998	Brazil	MG-Carmo de Minas/2008	*C. arabica* cv. Bourbon Amarelo	-	-	-	-	-	-	-	-	+
3032	Brazil	MG-Albertina/2009	*C. arabica*	-	-	-	-	-	-	-	-	+
3107	Brazil	MG-Monte Santo de Minas/2000	*C. arabica* cv. Mundo Novo	-	-	-	-	-	-	-	-	+++
2512	Brazil	MG-Patrocínio/2007	*C. arabica*	-	-	-	-	-	-	-	-	+++
3114	Brazil	MG-Coromandel/2012	*C. arabica*	-	-	-	-	-	-	-	-	+++
3024	Brazil	MG-Serra do Salitre/2011	*C. arabica*	-	-	-	-	-	-	-	-	++
3241	Brazil	PR-Assaí/2015	*C. arabica*	-	-	-	-	-	-	-	-	+++
3269	Brazil	PR-Congonhinhas/2015	*C. arabica* cv. IPR 100	-	-	-	-	-	-	-	-	+
249	Kenya	- /1962	*C. arabica*	++	+w	++	+	++	+	++	+w	++
3037	Kenya	- /1972	*C. arabica*	++	++	+	++	+++	+w	+	++	++
P. syringae pv. *tabaci*												
2240	Brazil	SP-Avaré/2005	*C. arabica* cv. Catuaí	-	-	-	-	-	-	+w	-	+
2241	Brazil	SP-Avaré/2005	*C. arabica* cv. Catuaí	-	-	-	-	-	-	-	-	+++
2249	Brazil	SP-Arandu/2005	*C. arabica* cv. Catuaí	-	-	-	-	-	-	-	-	++
3272	Brazil	PR-Londrina/2017	*C. arabica* E-1401	-	-	-	-	-	-	-	-	+

[1]Strain identity in Phytobacteria Culture Collection of Instituto Biológico (IBSBF); [2]Ethiopian accessions from FAO Coffee Mission to Ethiopia 1964-1965 (Meyer et al., 1968); *C. arabica* E-1401 = selection of arabica coffee by IAPAR. - = absence of symptoms or Hypersensitivity Reaction (0 points on scale); +w = weak reaction on some wounds (1 point on scale); + = low pathogenicity in total inoculated area (2 points on scale); ++ = moderate pathogenicity (3 points on scale); +++ = intense pathogenicity (4 or 5 points on scale); Points scale proposed by Rodrigues et al. (2017); [R] Resistant control; [S] Susceptible control. [P] Pathotype strain.

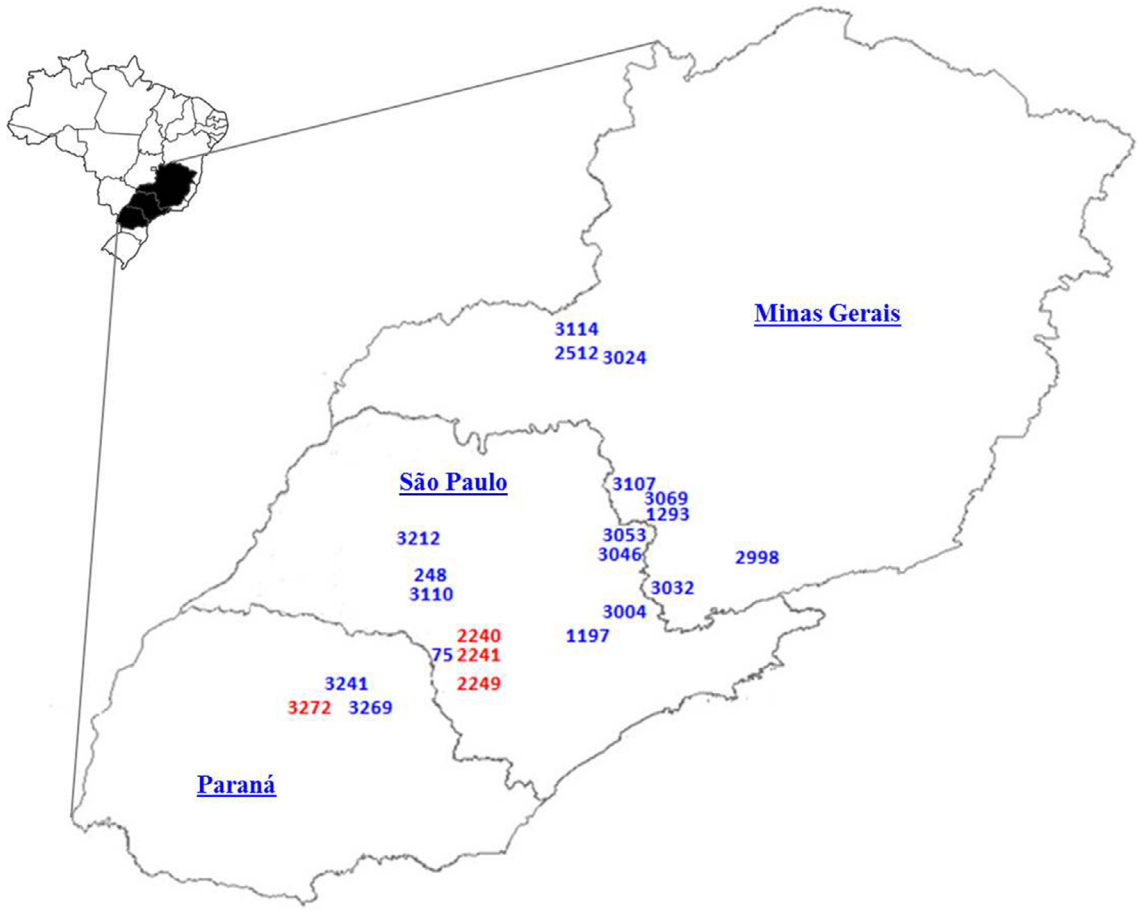

Figure 1: Origin of *Pseudomonas syringae* pv. *garcae* (blue) and *P. syringae* pv. *tabaci* (red) strains. The numbers correspond to the Phytobacteria Culture Collection of the Biological Institute (IBSBF), Campinas, SP, Brazil identification.

These results are of particular importance in coffee breeding since the broad spectrum of Ethiopian accessions germplasm resistance must certainly imply in the stability of the planting of new cultivars resistant to the bacterial halo blight in several Brazilian regions. Another important result was the confirmation that the Brazilian cultivar IPR 102 was simultaneously resistant to both pathovars. In previous studies, this cultivar was reported to be resistant to *P. syringae* pv. *garcae* (Ito et al., 2008; Sera; Sera; Fazuoli, 2017; Fernandes et al., 2020) and to *P. syringae* pv. *tabaci* (Fernandes et al., 2020), however in the latter case, it had not been confirmed, as it was a field study, without controlled inoculation. Rodrigues et al. (2019) also identified high resistance in the cultivar IPR 102 for both *P. syringae* pathovars, however for the latter, susceptible segregating plants were found, indicating that it is necessary to make selections within that cultivar to identify individual plants with homozygous resistance.

Regardless of the high resistance presented of accessions to Brazilian *P. syringae* pv. *garcae* strains, Ethiopian wild accessions of Arabica coffee proved to be susceptible in different levels to Kenyan strains, corroborating the results obtained by Kairu (1997) who also observed that Brazilian and Kenyan strains presented different behavior on pathogenicity to cultivar SL-28 of *C. arabica*. Cultivar SL-28 (Bourbon-related group) was selected on coffee population from Tanzania (past Tanganyika) (Van Der Vossen; Cook; Murakaru, 1976), indicating to be a native population resistant to bacterial halo blight from Brazil, as observed for the wild accessions.

In a recent study, Rodrigues et al. (2017a) observed that cultivar SL-28 showed resistance against a mixture of IBSBF 75 and IBSBF 1197, two highly aggressive *P. syringae* pv. *garcae* strains, tested separately in the present study, showing that the cultivar SL-28 is probably resistant to Brazilian *P. syringae* pv. *garcae* strains from different regions.

Responses of Ethiopian wild accessions of Arabica coffee to infection by Kenyan *P. syringae* pv. *garcae* strains corroborate several studies that demonstrated important differences in pathogenicity of strains according to the inoculated genotype (Kairu, 1997; Ithiru et al., 2013; Ithiru; Gichuru; Alakonya, 2015; Hinkosa et al., 2017; Mwangi et al., 2018). However, the resistance to *P. syringae* pv. *garcae* strains from African continent, as observed in the E-338 accession inoculated with the IBSBF 249 strain or the E-233 accession infected by the IBSBF 3037 strain, are scarce and, according to our data, the Kenyan strains were less agressive to the Mundo Novo IAC 376-4 cultivar than most of ten Brazilian *P. syringae* pv. *garcae* strains, suggesting the possible existence of intraspecific variability among the African strains.

Intraspecific diversity occurs in several phytobacterial genera, the occurrence of distinct races or pathovars of *P. syringae* is common and can be identified based on differences in bacterial pathogenicity on differentiating plants. This characteristic was already used to determine pathogenic races of *P. syringae* pv. *glycinea* (Prom; Venette, 1997), *P. syringae* pv. *pisi* (Bevan et al., 1995), *P. syringae* pv. *phaseolicola* (Duncan et al., 2014) and numerous other examples.

Differential reaction of *P. syringae* strains in coffee was reported by Paradela et al. (1974) apud Rodrigues et al. (2017a); however, without any additional information or deposit of isolates in an official Culture Collection becomes impossible to reproduce this study and confirm de results. Therefore, the presumption of race occurrence among Brazilian isolates of *P. syringae* pv. *garcae*, although small, should be maintained. In our study, we found clear differences in the reactions of resistance of wild accesses and cultivar IPR 102 inoculated with Brazilian and Kenyan strains, with strong indications that strains from these two countries may represent different physiological races or grups. On the other hand, among Brazilian strains it does not appear that there are different physiological races.

Previously studies showed that *P. syringae* pv. *garcae* strains from Kenya, diverge from Brazilian strains regarding epidemiological aspects (Kairu, 1997).

High resistance to *P. syringae* pv. *garcae* strains from Brazil were detected in *C. arabica* Ethiopian accessions, and probably such resistance was determined by the action of a dominant gene since highly resistant plants are immune to the pathogen (Dada not published). The results obtained herein, as well as those described by Ithiru et al. (2013) and Ithiru, Gichuru and Alakonya (2015) indicate that high resistance reaction does not occur when plants were tested against Kenyan strains and probably the resistance is like a quantitative inheritance or other major genes influence the resistance in this interaction.

Divergences between *P. syringae* obtained from coffee trees were reported by other methods of analysis. To distinguish bacterial strains of diverse geographical origin, Ovod et al. (2004) used serological tests of the structure of O-Polysaccharides of the bacterium. By the molecular techniques, studies on RAPD markers (Hinkosa et al., 2017) and/or via 16S ribosomal RNA sequence analysis (Mwangi et al., 2018) or rep-PCR (Maciel et al., 2018), made possible the evaluation of the dissimilarity between isolates of different origin, as well as the genetic diversity of strains from the same country.

Recently Dutta et al. (2018) proposed *Pseudomonas coronafaciens* as a new species within the genus reallocating the pathovar *garcae* to this new species. Results obtained by Kairu (1997) detect no biochemical divergence between coffee *P. syringae* pv. *garcae* Brazilian and Kenyan strains and *P. syringae* species.

Biochemical similarity of *P. syringae* pv. *garcae* from Brazil and Kenya with the *P. syringae* species (Kairu, 1997), the molecular low similarity (Maciel at al., 2018) and the divergence of *P. coronafaciens* and *P. syringae* species described by Dutta et al. (2018), take us to consider the hypothesis that Brazilian and Kenyan isolates could be a distinct races of *P. syringae* pv. *garcae* or different pathovars of *P. syringae*. However, the studies conducted by Dutta et al. (2018) not include the Kenyan strains, and those studies were based in only one *P. syringae* pv. *garcae* strain (NCPPB 588 = IBSBF 248). Would it be like Brazilian strains belonging to *P. coronafaciens* specie and Kenyan strains belonging to *P. syringae*? This is one of the phytopathological fields of this interaction, with several opportunities for studies, besides those mentioned by Badel and Zambolin (2019).

The aspect of variability on pathogenicity among *P. syringae* pv. *tabaci* strains should be better studied, but few strains of this pathovar, isolated from *Coffea* spp., were deposited in official Culture Collections. For this reason, the prospection of bacterial leaf spot strains in different

Brazilian producing regions are necessary to obtain more information about this pathosystem, poorly studied until the moment. The agronomic importance of *P. syringae* pv. *tabaci* on coffee crop is referred to the range of host plants as cited by Rodrigues et al., (2017b), an aspect that can impact positively for the introduction of the disease in new areas.

The presence of *P. syringae* pv. *tabaci* in the coffee crops occurs a long time ago. In the same way as previously reported, in two field studies they identified the mixed infection by both *P. syringae* pathovars in the same location from similar symptoms in *C. arabica* leaves (Rodrigues et al., 2017b; Fernandes et al., 2020). However, the correct diagnosis of the bacterial halo blight and bacterial leaf spot can only be made by isolation and characterization of the causal agent or agents by biochemical (Young; Triggs, 1994), serological (Beriam et al., 2017) and/or molecular techniques (Maciel, 2018). It is importance to mention that no study aiming to evaluate different methods of control against bacterial leaf spot, caused by *P. syringae* pv. *tabaci* on coffees was describe yet.

Finally, the pathogenic diversity of *P. syringae* pv. *tabaci*, the absence of resistant selections to Kenyan *P. syringae* pv. *garcae* strains, and the possibility of pathogen transmission via seeds (Belan et al., 2016), strongly indicate that breeding programs need to establish strategies for the transfer of pathogenic *P. syringae* resistance alleles to coffee trees, avoiding future losses associated with this bacterial species. To support the decision made in coffee breeding programs the resistance heritage must be discovered.

Although Brazilian legislation permits the introduction of coffee seeds only by official agencies, which in theory guarantees the health of the obtained seedlings, quarantine measures should be strict to exotic *P. syringae* pv. *garcae* strains in national territory, due to its pathogenic potential for the crop and considering the susceptibility of the national cultivars.

CONCLUSIONS

The wild accessions of *Coffea arabica* from Ethiopia are resistant to the wide genetic variability of Brazilian *Pseudomonas syringae* pv. *garcae* strains. Several accessions from Ethiopia and the cultivar IPR 102 were simultaneously resistant to the *Pseudomonas syringae* pvs. *garcae* and *tabaci*. Wild accesses from Ethiopia and IPR 102 proved to be resistant to Brazilian strains, but not to the Kenyan strains, strongly suggesting that the Brazilian and Kenyan *P. syringae* pv. *garcae* strains belong to different groups.

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