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S_H1 leaf rust and bacterial halo blight coffee resistances are genetically independent

Lucas Mateus Rivero Rodrigues*, Masako Toma Braghini, Oliveiro Guerreiro Filho

Instituto Agronômico - Centro de Café Alcides Carvalho - Campinas (SP), Brazil.

ABSTRACT: Coffee resistance to *Pseudomonas syringae* pv. *garcae* has been associated to pleiotropic effect of S_H1 allele, present in coffee plants resistant to certain races of *Hemileia vastatrix*, the causal agent of leaf rust, or genetic linkage between resistance alleles to both pathogens. To validate this hypothesis, 63 coffee plants in F_2 generation were evaluated for resistance to 2 isolates of *H. vastatrix* carriers of alleles, respectively, $_v2$, $_v5$ (isolate I/2015) and $_v1$; $_v2$; $_v5$ (isolate II/2015) with the objective to confirm presence of S_H1 allele in resistant plants to isolate I/2015. The same coffee plants were evaluated for resistance to a mixture of *P. syringae* pv. *garcae* strains highly pathogenic to coffee. Results showed that, among F_2 coffee allele S_H1 carriers, resistant to isolate I/2015, resistant and susceptible plants to bacterial halo blight were found; the same segregation occurs between F_2 homozygous for S_H1 allele, susceptible to the same isolate (I/2015) of *H. vastatrix*. Results also indicate that there is no pleiotropic effect of gene or allele S_H1 connection between genes conferring resistance to leaf rust caused by *H. vastatrix* and bacterial halo blight caused by *P. syringae* pv. *garcae*.

Key words: *Coffea arabica* L., resistant cultivars, *Pseudomonas syringae* pv. *garcae*, linkage, *Hemileia vastatrix*, pleiotropic effect.

*Correspondence author: lucasmriverorodrigues@gmail.com

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INTRODUCTION

Leaf rust is the most important disease of coffee plantations, and widespread in major *Coffee arabica* L. producing countries. Variability of the causal agent, fungus *Hemileia vastatrix* Berkeley & Broome, is quite wide, and there are currently 45 known races of this fungus in world (Várzea and Marques 2005). In Brazilian coffee plantations, 17 races have already been identified (Zambolim et al. 2005).

Races of *H. vastatrix* are characterized by variable combinations of resistance genes, in total of 9, denominated S_H1 to S_H9 (Várzea et al. 2005), with direct implications for pathogenicity and in determining amplitude and nature of their hosts.

Studies conducted by Moraes et al. (1975) with diverse *Coffea* spp. germplasm exhibiting resistance to different races of *H. vastatrix* resulted in the identification of sources of resistance to bacterial halo blight, caused by *Pseudomonas syringae* pv. *garcae*, in *C. arabica* exotic varieties of Ethiopian origin, known as Harar, Dilla and Alghe, S 12 Kaffa, and Geisha, all of which are carriers of S_H1 allele.

Multiple resistance to leaf rust and bacterial halo blight presented in these exotic varieties, could be explained according to Carvalho (1988), by the genetic linkage between resistance alleles to both pathogens or pleiotropic effect of S_H1 allele. Sera (2001) and Fazuoli et al. (2009) also attributed to S_H1 allele, present in the Ethiopian varieties (Bettencourt and Carvalho 1968), evaluated by Moraes et al. (1975), the simultaneous resistance to some races of *H. vastatrix* and bacterial halo blight caused by *P. syringae* pv. *garcae*.

According to Bettencourt and Carvalho (1968), S_H1 allele is widespread in major coffee growing areas of Ethiopia. Studies conducted by these authors, allowed S_H1 allele identification in various selections as Barbuk Sudan, BE-2 Ghembi, BE-4 Ennarea, BE-5 Wush-Wush, BE-6 Moderalo, BE-7 Boggia, BE-8 Era, BE-14 Loulo, Dilla & Alghe, Geisha, Lejeune's, S 6 Cioiccie, S 9 Arba Gougou, S 12 Kaffa, S 17 Yrgalem, U l Dalecho, occurring individually or in combination with other resistance genes, as S_H4 (S_H1 S_H4); S_H5 (S_H1 S_H5) and S_H4 and S_H5 (S_H1 S_H4 S_H5) and conferring resistance to 16 of 24 *H. vastatrix* races known at the period in which the research was conducted.

With S_H1 allele conferring resistance to both pathogens, the search for sources of coffee resistant plants to *P. syringae* pv. *garcae* will be facilitated and occur simultaneous with tests to evaluate resistance to *H. vastatrix*. Therefore, the present study aimed to investigate whether coffee simultaneous resistance to *H. vastatrix* and *P. syringae* pv. *garcae* is due to pleiotrophic effect of S_H1 allele or genetic linkage between resistance alleles to both pathogens.

MATERIAL AND METHODS

F_2 progenies of coffee, from H8089-2 and H8089-7, composed respectively of 34 and 29 plants, were evaluated to infection by bacterium *P. syringae* pv. *garcae* and fungus *H. vastatrix*. These plants were derived from crossing between Catuaí Vermelho IAC 24 cultivar and IAC 1137-5 of Geisha originally from Ethiopia, carrier of S_H1 and S_H5 alleles (Bettencourt and Carvalho 1968). The Geisha selection was introduced in the coffee collection of Agronomic Institute (IAC) in 1953, coming from the United States Department of Agriculture under registration PI 205.928.

Plants were inoculated with bacteria obtained from the IBSBF Culture Collection of the Biological Institute, Campinas, Brazil, strains IBSBF 75 and IBSBF 1197 of *P. syringae* pv. *garcae*, in mixture, with approximate concentration of 10^8 UFC·mL^{-1} (600 nm, absorbance = 0.25), by abrasion technique, which constitutes of friction against ab axial surface of leaves with medium grit sandpaper disposed in circular area (Ø1.5 cm) previously soaked in bacterial suspension causing limb injuries with no tissue drilling. Analysis of disease severity was carried out by using a rating scale of 0 to 5 points adapted from Paradela et al. (1974), as follows: 0 = no anasarca or chlorosis symptoms or hypersensitivity reaction around injured tissues; 1 = initial of bacterial colonization around lesions, with up to 10% of inoculated area showing disease symptom; 2 = 11 – 25% of inoculated area showing disease symptoms and with or without yellow halo; 3 = 26 – 50% of inoculated area showing disease development, yellowish halo pronounced around lesions; 4 = 51 – 75% of inoculated area with necrosis, yellowish halo throughout inoculated area; 5 = 100% necrosis of inoculated area. Plants were evaluated weekly up to 42 days after inoculation.

To confirm the presence of dominant S_H1 allele, 63 F_2 coffee plants were inoculated with 2 isolates of *H. vastatrix* obtained on differentiating plants grown in Centro de Café Alcides Carvalho from IAC.

The isolates used in this research were I/2015 carrying alleles $_V2$ and $_V5$ and isolate II/2015 the carrier of the alleles of virulence $_V1$; $_V2$; and $_V5$. These aspects were confirmed by inoculation of this isolates on known differentiating plants to *H. vastatrix* races.

Disks of coffee leaves were inoculated with a drop of uredospore suspension with approximate concentration of 1.5×10^5 uredospore·mL^{-1} of isolates I/2015 and II/2015, and subsequently kept in humid chambers in accordance with the methodology proposed by Eskes and Toma-Braghini (1981).

Two parameters were used to determine rust reaction: disease severity, evaluated with a 0 to 9 points scale, 0 being assigned to resistant plants without symptoms and 9, plants with high incidence of large lesions and regular and intense sporulation; and also type of reaction of lesions, evaluated by 0 to 4 point scale, in which 0 was assigned to immune plants without injuries and, four points to high susceptible plants with intense sporulation (Eskes and Toma-Braghini 1981).

Possibility of pleiotropic effect of S_H1 allele or any genetic connection between resistance alleles to the pathogens used in this was investigated through reactions of plants to infection by bacteria strains IBSBF 75 and IBSBF 1197 and isolate I/2015 of fungus, and concordance between evaluated methods through following parameters:

- Accuracy of method (AM) = (a + d)/n, calculated from sum of simultaneously resistant plants (a) and simultaneously susceptible (d) to rust and bacterial halo blight divided by total number of plants (n).
- False positive rate (FPR) = b/(b + d), calculated by dividing the number of resistant plants to leaf rust, but susceptible to bacterial halo blight (b) by total of susceptible plants to bacterial halo blight (b + d).
- False negative rate (FNR) = c/(a + c), calculated by division of number of susceptible plants to leaf rust, but resistant to bacterial halo blight (c) by total of resistant plants to bacterial halo blight (a + c).
- Total rate error (TRE) = (b + c)/n, calculated from sum of resistant plants to leaf rust, but susceptible to bacterial halo blight (b) and number of susceptible plants to leaf rust, but resistant to bacterial halo blight (c) and divided by total number of plants (n).
- McNemar chi-square with Yates continuity correction: $(\chi^2_{McNemar}) = (|b - c| - 0.5)^2/b + c$, used to estimate significance between 2 assessments.
- P value = quantitative measure of significance robustness of $\chi^2_{McNemar}$ test calculated.
- Yule association coefficient (Q): measure degree of association between determined classes on evaluated plants for resistance to leaf rust and bacterial halo blight.

RESULTS AND DISCUSSION

The results related to resistance expression in progenies F_2 from H8089-2 and H8089-7 to infection by *P. syringae* pv. *garcae* and isolates I/2015 and II/2015 of *H. vastatrix* are shown in Table 1.

According to the obtained results, F_2 plants are segregating for resistance to isolate I/2015 of *H. vastatrix*, but they proved to be susceptible to isolate II/2015. On the other hand, 34 plants of H8089-2 were susceptible to bacterial halo blight while 29 progenies of H8089-7 segregate in approximate 1R:1S ratio (Table 1).

From the total progeny analyzed regarding response to infection with isolate I/2015 of *H. vastatrix*, 16 plants presented resistance reaction and 47 were susceptible. On the other hand, regarding reaction to infection by *P. syringae* pv. *garcae*, 15 plants were identified with resistance reaction while remaining 48 showed to be susceptible (Table 1).

These results show heterozygous nature of F_1 matrices (S_H1 s_H1) and, as demonstrated by Bettencourt and →

Table 1. Reaction to infection by *Pseudomonas syringae* pv. *garcae* and isolates I/2015 and II/2015 of *Hemileia vastatrix* on F_2 progeny from H8089-2 and H8089-7, hybrids between Catuaí Vermelho IAC 24 cultivar and Geisha IAC 1137-5 selection.

Parameter	H8089-2	H8089-7	Total
Plants (nº)	34	29	63
$P_{HV}I_{I/2015}$[1] (R:S)	6:28	10:19	16:47
$P_{HV}I_{II/2015}$[2] (R:S)	0:34	0:29	0:63
P_{PSG}[3] (R:S)	0:34	15:14	15:48

[1]Proportion of resistant and susceptible plants to isolate I/2015 of *H. vastatrix*; [2]Proportion of resistant and susceptible plants to isolate II/2015 of *H. vastatrix*; [3]Proportion of resistant and susceptible plants to *P. syringae* pv. *garcae*. P = Proportion; R = Resistant; S = Susceptible.

Carvalho (1968), genitors, Catuaí Vermelho IAC 24 cultivar and Geisha IAC 1137-5 selection were respectively homozygous for s_H1 and S_H1 alleles. Furthermore, according to these results, it was possible to confirm the presence of dominant allele S_H1 in a third of plants, six coffee progenies of H8089-2 and ten coffee plants of H8089-7. In the gene-to-gene theory (Flor 1971), these coffee plants, carriers of at least one dominant allele S_H1, had broken down resistance when inoculated with isolate II/2015 that carried $_v1$ allele.

In the hypothesis of S_H1 allele present pleiotropic effect or occurrence of genetic linkage between alleles that confer resistance to *H. vastatrix* and *P. syringae* pv. *garcae* as reported by Carvalho (1988), coffee plants carrying S_H1 allele should be resistant to both pathogens.

Thus, F_2 progenies, segregating, when inoculated with isolate I/2015 of *H. vastatrix*, should have the same reaction when inoculated with bacterium *P. syringae* pv. *garcae*, that is, resistant and susceptible plants to isolate I/2015 of *H. vastatrix* should be, respectively, resistant and susceptible to *P. syringae* pv. *garcae*.

In Table 2, it is presented the comparative data analysis of F_2 progenies reaction with regarding to infections by *P. syringae* pv. *garcae* and by isolate I/2015 of *H. vastatrix*.

From 63 F_2 coffee plants evaluated, 44 revealed simultaneously resistance (6) and susceptibility (38) to the isolate I/2015 of *H. vastarix* and to bacterial halo blight. Nineteen plants showed an opposite reaction to biotic agents, 10 of them were resistant to leaf rust and susceptible to bacterial halo blight, and 9 were resistant to *P. syringae* pv. *garcae* and susceptible to *H. vastarix* (Table 2). The results of statistical analysis performed with experimental data are described in Table 3.

Accuracy of method (AM) calculated from comparative data analysis of coffee reaction to infection by *P. syringae* pv. *garcae* and isolate I/2015 of *H. vastatrix* denote that plants simultaneously resistant or susceptible to both pathogens was 69.84% considering total number of F_2 plants in the same group the total rate error (TRE) was equal to 30.16%.

The $\chi^2_{McNemar}$ test was significant in progenies from H8089-2 (4.17; p = 0.04), not significant in progenies from H8089-7 (1.23; p = 0.27) and also not significant in total group of plants (0.56; p = 0.45). However, significant value of $\chi^2_{McNemar}$ test calculated on H8089-2 progeny reveals inconsistency between the 2 classifications; the number of resistant plants to isolate I/2015 of *H. vastatrix* (6) is not the same number of resistant plants to *P. syringae* pv. *garcae* (0).

On the other hand, even though $\chi^2_{McNemar}$ value was not significant, in the analysis of all plants evaluated, resistance to biotic agents was not proven to be simultaneous, since 10 plants resistant to the isolate I/2015 of *H. vastatrix* carrying S_H1 allele proved to be susceptible to bacterial halo blight and 9 plants resistant to bacterial halo blight have shown susceptibility to the isolate I/2015 of *H. vastatrix*, and false positive rate, in same group of plants, was equal to 15.87% and the false negative in second was 14.29%; the total error rate was 30.16%.

Yule association coefficient (Q) was equal to 0.25 for H8089-7 progeny, 0.40 in total group of plants, and has not been calculated for H8089-2 progeny, since false negative rate, i.e. of susceptible plants to isolate I/2015 →

Table 2. Total number of F_2 coffee plants of 2 hybrids H8089 resistant and susceptible to *Pseudomonas syringae* pv. *garcae* and isolate I/2015 of *Hemileia vastatrix*.

H. vastatrix isolate I/2015	*P. syringae* pv. *garcae*		Total
	Resistant	Susceptible	
	n°		
Resistant	6^a	10^b	16^{a+b}
Susceptible	9^c	38^d	47^{c+d}
Total	15^{a+c}	48^{b+d}	63^n

Table 3. Estimation of parameters related to coffee F_2 progeny of 2 hybrids H8089 response to infection by the bacterium *Pseudomonas syringae* pv. *garcae* and fungus *Hemileia vastatrix*.

Parameters	H8089-2	H8089-7	Total
Accuracy of the method (%)	82.35	55.17	69.84
False positive rate (%)	17.64	13.79	15.87
False negative rate (%)	0	31.03	14.29
Total rate error (%)	17.64	44.82	30.16
$\chi^2_{McNemar}$	4.17^*	1.23^{ns}	0.56^{ns}
P-value	0.04	0.27	0.45
Yule association coefficient	-	0.25	0.43
Standard deviation of Yule association coefficient	-	0.37	0.32
Confidence interval of 95% of Yule association coefficient	-	0.47 – 0.98	0.40 – 1.06

*Significant at 5% probability; nsNon-significant; -Non-calculated values, since false negative rate is 0.

of *H. vastatrix*, but resistant to *P. syringae* pv. *garcae*, was equal to 0. The magnitude of Yule coefficient, which ranges between 0 and ±1, observed individually in progeny H8089-7, and on F_2 population as a whole (H8089-2 and H8089-7), confirms the lack of association between resistant and susceptible classes determined in the evaluation ratings of plants resistance to leaf rust and bacterial halo blight.

CONCLUSION

In accordance with the analyzed data, we can conclude that S_H1 gene has no pleiotropic effect or genetic linkage between genes which confer resistance to isolates homozygous for $_V1$ allele of leaf rust caused by *H. vastatrix* and to bacterial halo blight caused by *P. syringae* pv. *garcae*.

Search for resistance sources to bacterial halo blight for use in breeding programs aiming to develop cultivars with simultaneous resistance to both biotic agents should not be restricted to *Coffea* germplasm carrying S_H1 gene with resistance to races or isolates of *H. vastatrix*.

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