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ORIGINAL ARTICLE

Evaluating coffee genotypes for resistance to coffee leaf rust in Brazil

Andreisa Fabri Lima[1] · Deila Magna dos Santos Botelho[2] · Leonor Guerra-Guimarães[3,4] · Vinicius Teixeira Andrade[1] · Mário Lúcio Vilela de Resende[2] · Mariana de Lima Santos[2] · Juliana Costa de Rezende Abrahão[1]

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Abstract
This study integrates data from Brazilian coffee cultivars with different resistant genetic sources to coffee leaf rust (CLR), a foliar disease caused by the fungus *Hemileia vastatrix* Berk. & Broome. The aim was to characterize the performance of some of the main Brazilian cultivars regarding resistance to CLR. Seedlings of 29 genotypes, including 25 established cultivars and four coffee progenies in the final selection phase, were maintained in a greenhouse, and inoculated with spores of *H. vastatrix*. Evaluation of CLR disease was based on incidence (leaf with or without spores) and severity (sporulating leaf area) parameters, assessed using diagrammatic scale and digital image analysis. Twenty-one coffee genotypes, representing 72% of those studied, showed no disease symptoms despite their diverse genetic backgrounds. Eighteen cultivars and three progenies were classified as highly resistant (HR). However, cultivars Guará, Rouxinol, IPR 102, and Catucaí Amarelo 2SL displayed intermediate disease incidence and severity levels, categorized as moderately resistant (MR). The Catuaí Vermelho IAC 99 cultivar and H29-1–8-5 progeny were susceptible to CLR exhibiting 100% incidence and severity levels of 5 (10.1–25.0%) and 6 (> 25% of area infected by rust), respectively, while others such as Catucaí Amarelo and Azulão cultivars were slightly susceptible. The information obtained here is crucial for plant breeders to strategically incorporate diverse sources of resistance (complete and partial) into crop varieties to enhance their resilience against a wide array of pathogen races. Additionally, identifying genotypes whose resistance remains unaffected by *H. vastatrix* pathotypes from the southern region of Minas Gerais, as observed in this study, can provide a solid basis for recommending coffee cultivars for planting and defining disease management, ultimately contributing to the sustainability of coffee production.

Keywords Diagrammatic scale · Digital image analysis · Disease incidence · Severity · Resistance · *Hemileia vastatrix*

Introduction

Coffee production plays an integral role in the economic and social development of over 50 countries where coffee is grown, with Brazil leading as the largest producer (International Coffee Organization 2022). However, the sustainability of this production can be compromised by adverse impacts on productivity, such as, extreme climatic events or the occurrence of fungal diseases (Fones et al. 2020). These events can not only affect the global coffee supply but also pose significant challenges to the coffee industry's economy.

Coffee leaf rust (CLR), caused by the biotrophic fungus *Hemileia vastatrix* Berkeley & Broome, is the main disease that negatively affects the production of arabica coffee in Brazil and all the other coffee growing countries (Avelino et al. 2015; Talhinhas et al. 2017; Zambolim and Caixeta 2021; Li et al. 2021; Ramírez-Camejo et al. 2022). The characteristic symptom of the disease are orange spores on the lower surface of the leaves, which can cause intense defoliation and, consequently, reduce the photosynthetic leaf area, culminating in yield loss (Rhiney et al. 2021). At high infection levels, branches dieback, depletion and deformation of the plants occurs (Zambolim 2016; Talhinhas et al.

✉ Juliana Costa de Rezende Abrahão
julianacosta@epamig.br

[1] Empresa de Pesquisa Agropecuária de Minas Gerais, Universidade Federal de Lavras, Lavras, Minas Gerais 37200-900, Brazil

[2] Departamento de Fitopatologia, Universidade Federal de Lavras, Lavras, Minas Gerais 37200-900, Brazil

[3] Centro de Investigação das Ferrugens Do Cafeeiro, Instituto Superior de Agronomia, Universidade de Lisboa, 2784-505 Oeiras, Lisbon, Portugal

[4] LEAF Research Unit & Associated Laboratory TERRA, Instituto Superior de Agronomia, Universidade de Lisboa, Tapada da Ajuda, 1349-017 Lisbon, Portugal

2017). Depending on climatic conditions and if no disease management practices are implemented, coffee production losses can reach up to 50% (Zambolim and Caixeta 2021).

The primary approach for managing CLR involves scheduled applications of fungicides (Zambolim 2016). However, these treatments may be delayed due to factors like prolonged rain, which can fail to control the disease effectively. Limited registered chemical molecules may also show low efficacy in highly susceptible cultivars. Additionally, regulatory standards are becoming more stringent, with substances like epoxiconazole banned in the European Union (EU) and United Kingdom (UK) due to human health concerns (Friedrich et al. 2021). This scenario reinforces the importance of using resistant cultivars, such as derivatives of the Timor Hybrid, Icatu, or BA10, which tend to require fewer field management practices, thereby promoting sustainable and eco-friendly agricultural methods.

Disease resistance is a crucial aspect of crop breeding, requiring constant updates due to pathogen adaptation to plant genotypes. Developing varieties with effective, stable, and broad-spectrum resistance poses a challenge. Plant disease resistance can be classified based on various factors, including genetic background (monogenic/polygenic), phenotypic response (complete/partial resistance), and the nature of resistance against pathogens (race-specific and race nonspecific) (Martins et al. 2020). Historically, selection for resistance against *H. vastatrix* has relied on specific complete resistance from major genes/factors (S_H1- S_H9), following the recognition of the "gene-to-gene" concept in coffee-rust interactions (Noronha-Wagner and Bettencourt 1967; Bettencourt and Noronha-Wagner 1971; Bettencourt and Rodrigues 1988). Resistance factors S_H1, 2, 4, and 5 derived from *C. arabica*, S_H6, 7, 8, and 9 from *C. canephora* ancestors, and S_H3 comes from an introgression of *C. liberica* (Noronha-Wagner and Bettencourt 1967; Bettencourt and Noronha-Wagner 1971; Bettencourt and Rodrigues 1988). Other resistance genes, in addition to S_H1 to S_H9, have been identified but require further characterization; collectively confer resistance against over 55 physiological races of *H. vastatrix* (Talhinhas et al. 2017; Barka et al. 2020; Almeida et al. 2020).

According to the recent review on the breeding programs against CLR in Brazil (Zambolim et al. 2024), several accessions of Timor hybrid (HDT) sent by CIFC (Centro de Investigação das Ferrugens do Cafeeiro, Portugal) over the years, including CIFC 832/1, CIFC 832/2 and CIFC 2570 have become the primary sources of resistance against CLR. HDT, a natural hybrid (*C. arabica* × *C. canephora*) found in East-Timor Island, was used in controlled crosses alongside traditional *C. arabica* varieties, (e.g., Villa Sarchi, Caturra and Catuaí) originated promising progenies of the various populations generically called Catimor, Sarchimor, Cachimor, Cavimor (Zambolim et al. 2024). Other source of resistance used by Brazilian breeding programs was Icatu and its derivatives (e.g., Icatu x Catuaí). Icatu populations resulted from the hybridization of a controlled cross between *C. canephora* var. Robusta × *C. arabica* var. Bourbon Vermelho crossbred with *C. arabica* var. Mundo Novo developed at Instituto Agronômico de Campinas (IAC, Brazil). The BA accessions are tetraploid coffee plants resulting from the cross between *C. arabica* and *C. liberica* var. *liberica* (developed at the Balehonnur Experimental Station in India) and their derivatives (e.g., Catuaí x BA-10) are known to carry the resistance factor SH3. In a less extent, wild Arabica Ethiopian accessions, including landraces such as Geisha, have also been used as rust-resistant genetic resource (Sera et al. 2022; Zambolim et al. 2024).

However, the pathogen's ability to change and adapt, along with frequent shifts in pathotypes, has led to the gradual weakening of resistance provided by major S_H genes/factors. The pathogen evolves new virulence genes through consecutive mutations (Rodrigues Junior et al. 2000), or potentially through introgression or recombination events (Silva et al. 2018) Nowadays, 15 out of the 55 *H. vastatrix* races (with different combinations of virulent genes) were found in Brazil, where race II is the most prevalent (Zambolim 2016). Indeed, the resistance of some coffee cultivars has been broken in many coffee regions in Brazil, showing incomplete/partial resistance (due to minor genes), which slows down the spread of disease and reduces leaf loss (Zambolim et al. 2024). Coffee breeding programs are working on ways to make Arabica cultivars more resistant to CLR by combining specific (complete) and nonspecific (partial) resistance strategies (Silva et al. 2022; Sera et al. 2022; Zambolim et al. 2024).

This study aimed to characterize the main commercial coffee cultivars with different genetic background of rust resistance under controlled conditions (greenhouse), evaluating the disease incidence and severity. For this purpose, digital image analysis software, ImageJ, was employed. ImageJ allows for lesion quantification via scanner, enabling precise measurement of disease severity (Schindelin et al. 2015). These results were then compared with disease severity evaluations using a diagrammatic scale (Figueiredo et al. 2022). The evolution of disease expression assessment methods plays a crucial role in the effective development of new cultivars and in characterization of resistance of existing ones.

Material and methods

Plant material

The experiments were carried out in a greenhouse located in the Department of Phytopathology of the Universidade

Federal de Lavras (UFLA, Brazil), with 29 genotypes of *C. arabica*, 25 of which were commercial cultivars, including the susceptible cultivar Catuaí Vermelho IAC 99 (Sera et al. 2022), and four progenies in the final stage of improvement (F $_{6:7}$) (Table 1). The studied coffee genotypes were developed by the breeding programs of IAC (Instituto Agronômico de Campinas), EPAMIG (Empresa de Pesquisa Agropecuária de Minas Gerais), UFV (Universidade Federal de Viçosa), UFLA (Universidade Federal de Lavras), Fundação Procafé, IDR-Paraná (Instituto de Desenvolvimento Rural do Paraná), and EMBRAPA Café (Empresa Brasileira de Pesquisa Agropecuária).

Seeds from all genotypes were sown in germinators built with 5-L plastic trays containing autoclaved sand. The germinating chamber was adjusted to 30 ºC temperature and 80% relative humidity. The seedlings were transplanted into a tube containing a commercial substrate based on pine bark, peat, expanded vermiculite and ground charcoal (recommended for coffee) (Vallone et al. 2010), in the cotyledon leaf phase. Sprinkler irrigation was carried out on all plants, during the morning and afternoon, to maintain the substrate at field capacity. The seedlings were inoculated with urediniospores of *H. vastatrix* when they reached the stage of three pairs of fully expanded leaves.

Inoculation with *H. vastatrix*

H. vastatrix inoculum was collected from coffee leaves with rust symptoms from field plants of the Catuai cultivar in the southern region of Minas Gerais. The pustules from

Table 1 Coffee genotypes/cultivars studied, development institution and genetic background

Coffee genotypes/cultivars	Instituition	Genetic background
H29-1–8–51[1]	EPAMIG/UFV/UFLA	Icatu IAC H3851-2 × Catimor UFV 1603–215
Acauãnovo	Fundação Procafé	Mundo Novo IAC 388–17 × Sarchimor IAC 1668
Arara	Fundação Procafé	Catuaí Amarelo x Obatã IAC 1669–20
Asa Branca	Fundação Procafé	Mundo Novo IAC 388–17 × Sarchimor IAC 1668
Azulão	Fundação Procafé	Catuaí Vermelho x Icatu Vermelho 785
Beija-flôr	Fundação Procafé	Catuaí Vermelho x Icatu Vermelho 785
Catiguá Amarelo (6IP1FBS)[1]	EPAMIG/UFV/UFLA	Catuaí Amarelo IAC 86 × HDT UFV 440–110
Catiguá Amarelo (FOV)[1]	EPAMIG/UFV/UFLA	Catuaí Amarelo IAC 86 × HDT UFV 440–110
IAC Catuaí SH3	IAC	Catuaí Vermelho IAC 46 × IAC 1110–8 (BA10)
Catuaí Vermelho IAC 99[2]	IAC	Mundo Novo IAC 374–19 × Caturra Amarelo IAC 476–11
Catucaí Amarelo 2SL	Fundação Procafé	Catuaí x Icatu (natural hybrid)
Catucaí Amarelo 24/137	Fundação Procafé	Catuaí Vermelho x Icatu Vermelho 785
Graúna	Fundação Procafé	Mundo Novo IAC 388–17 × Sarchimor IAC 1668
Guará	Fundação Procafé	Catuaí Vermelho x Icatu Vermelho 785
IAC 125 RN	Fundação Procafé	Villa Sarchi CIFC 971/10 × HDT CIFC 832/2
IPR 102	IDR Paraná	Catuaí Vermelho IAC 99 × Dwarf Icatu
IPR 103	IDR Paraná	Catuaí Vermelho IAC 99 × Dwarf Icatu
IPR 105	IDR Paraná	Catuaí Vermelho IAC 81 x (Catuaí Vermelho IAC 81 × IAC 1110–8—BA-10)
IPR 107	IDR Paraná	Iapar 59 × Mundo Novo IAC 376–4
Japy	Fundação Procafé	Catuaí Vermelho x Icatu Vermelho 785
MGS Ametista	EPAMIG/UFV/UFLA	Catuaí Amarelo IAC 86 × HDT UFV 446–08
MGS Aranãs	EPAMIG/UFV/UFLA	Icatu IAC H3851-2 × Catimor UFV 1603–215
MGS Paraiso 2	EPAMIG/UFV/UFLA	Catuaí Amarelo IAC 30 × HDT UFV 445–46
MGS Catucaí Pioneira	EPAMIG/UFV/UFLA	Catuaí Vermelho x Icatu Vermelho 785
MGS Turmalina	EPAMIG/UFV/UFLA	Catuaí Amarelo IAC 30 × HDT UFV 445–46
IAC Obatã 4739	IAC	IAC Obatã1669-20–1 × Catuaí Amarelo IAC 62
Paraíso MG H419-1	EPAMIG/UFV/UFLA	Catuaí Amarelo IAC 30 × HDT UFV 445–46
Rouxinol	Fundação Procafé	Catuaí Vermelho x Icatu Vermelho 785
Sagarana 19[1]	EPAMIG/UFV/UFLA	Catuaí Amarelo IAC 30 × HDT UFV 445–46

[1]Progenies in F $_{6:7}$ generation from the EPAMIG breeding program; [2] Cultivar used as a susceptible control; HDT Timor Hybrid

IAC—Instituto Agronômico de Campinas, EPAMIG—Empresa de Pesquisa Agropecuária de Minas Gerais, UFV—Universidade Federal de Viçosa, UFLA—Universidade Federal de Lavras, IDR-Paraná—Instituto de Desenvolvimento Rural do Paraná, EMBRAPA Café—Empresa Brasileira de Pesquisa Agropecuária, CIFC – Centro de Investigação das Ferrugens do Cafeeiro (Portugal).

those leaves were collected and placed in microtubes protected from light and stored at 8 ºC. The inoculum viability was verified by germinating the spores in 2% agar medium (Zambolim and Chaves 1974). Urediniospores were added to distilled water and the suspension was calibrated to a concentration of 7×10^5 urediniospores/mL. The suspension was sprayed on the abaxial surface of the leaf. Two pairs of leaves were inoculated per plant. After inoculation, the plants were kept in the dark in a growth chamber for 72 h at 22 °C ± 2 °C temperature and 90% relative humidity (Salustiano et al. 2008; Monteiro et al. 2016).

Evaluation of CLR incidence and severity

The CLR disease incidence and severity parameters were quantified 45 days after inoculation with *H. vastatrix*, evaluating four leaves per plant. Incidence was defined as the percentage of leaves with spores (Vale et al. 2001). Severity was defined as the percentage of sporulating leaf area at the end of the experiment, which was at 45 days. The disease severity was obtained visually and through image analysis. The visual assessment of the disease severity was carried out using the diagrammatic scale of Figueiredo et al. (2022), which covers six classes, related to the proportion of area infected by rust: Class 0: 0%; Class 1: 0.1–1%; Class 2: 1.1–2.0%; Class 3: 2.1–5.0%; Class 4: 5.1–10.0%; Class 5: 10.1–25.0%; and Class 6: > 25.1%. Leaves with the presence of spores were highlighted and scanned for image analysis of the sporulating leaf area, as described by Gomes et al. (2023), using ImageJ software version 1.53e (Schindelin et al. 2015).

Based on the disease incidence results, we categorized the genotypes into different levels of rust resistance: Highly resistant (HR), also known as immune or complete resistance (no disease symptoms); moderately resistant (MR) (low proportion of leaves with disease symptoms on a plant); slightly susceptible (SS), also called moderately susceptible (medium proportion of leaves with disease symptoms), and susceptible (S) (high proportion of leaves with disease symptoms).

Experimental design and data analysis

The experiment was conducted in randomized blocks, with three replications and each experimental plot consisting of one plant. The experiment was repeated three times, to ensure greater reliability of the results. Disease incidence obtained at 45 days after inoculation was analyzed according to a generalized linear model (GLM) with quasi-binomial distribution (Nelder and Wedderburn 2000). Data fit to the GLM model quality checking was carried out using the half-normal probability graph with simulation envelope from the hnp package (Moral et al. 2017). The difference between genotypes was verified through multiple comparisons (Tukey's post hoc test, $p < 0.05$) using the "glht" function of the multicomp package. The sporulating leaf area (%) was compared to the confidence interval overlap (95% CI). All parameters were analyzed in the statistical program R, version 4.3.0 (R CoreTeam 2023).

Results

When evaluating the disease incidence, 21 genotypes [Acauãnovo, Arara, Asa Branca, Beija-flôr, Catiguá Amarelo (6IP1FBS), Catiguá Amarelo (FOV), Catuaí SH3, Graúna, IAC 125 RN, IPR 103, IPR 105, IPR 107, Japy, MGS Ametista, MGS Aranãs, MGS Paraíso 2, MGS Pioneira, MGS Turmalina, Obatã IAC 4739, Paraíso MG H419-1, and Sagarana 19 – Table 2] representing approximately 72% of the total genotypes studied, showed no symptoms, despite their diverse genetic background. Among these genotypes, fifteen had HDT (Catimor or Sarchimor) in their genetic background. Two genotypes, IAC Catuaí SH3 and IPR 105, originated from BA accessions, which carry the S_H3 gene introgressed from *C. liberica*. Four genotypes, Beija-flôr, IPR 103, Japy, and MGS Pioneira, derived from Icatu hybrid (details on Table 1). All the 21 genotypes (18 cultivars and three progenies) were classified as highly resistant (HR) (Table 2). The eight remining coffee genotypes presented different percentages of disease incidence and severity levels ($p < 0.0001$; $F_{5, 184} = 7.417$). Cultivar Catuaí Vermelho IAC 99 and the progeny H29-1–8-5 showed 100% CLR incidence, with a severity level of 6 and 5, respectively. Both genotypes were classified as susceptible (S). The CLR incidence was also high for the genotypes Catucaí Amarelo 24/137 (64%) and Azulão (60%) with a severity level of 4 and 3, respectively. Both genotypes were classified as slightly susceptible (SS). Cultivars Guará, Rouxinol, IPR 102 and Catucaí Amarelo 2SL, presented intermediate values of disease incidence and severity level (3) and were classified as moderately resistant (MR). For these last cultivars, the mean CLR incidence ranged from 9.38 to 23.33% (Table 2).

The disease severity was also performed by image analysis. The eight genotypes that showed sporulation were divided into three groups by overlapping confidence intervals. (Fig. 1). The first group, comprising only the highly susceptible cultivar Catuaí Vermelho IAC 99, had the largest area, ranging from 32.66% to 40.86% with a mean value of 36.76%. This group was classified at level 6 (> 25,1%). The second group, composed of the progeny H29-1–8-5, showing a mean value of 16.63%, ranging from 13.76% to 19.5%, was classified at level 5 (10.1% to 25%). The last two groups, level 3 and 4 showed an overlapping of the confidence interval values. The cultivars Rouxinol (0% to 3.53%), IPR 102

Table 2 Assessment of CLR incidence and severity and coffee resistance classification, at 45 days after inoculation with *Hemileia vastatrix*

Genotype	CLR Severity Level[2]	CLR incidence (%)[3]	Coffee Resistance level[4]
Acauãnovo	0	0 *	HR
Arara	0	0 *	HR
Asa Branca	0	0 *	HR
Beija-flôr	0	0 *	HR
Catiguá Amarelo (6IP1FBS)[1]	0	0 *	HR
Catiguá Amarelo (FOV)[1]	0	0 *	HR
IAC Catuaí SH3	0	0 *	HR
Graúna	0	0 *	HR
IAC 125 RN	0	0 *	HR
IPR 103	0	0 *	HR
IPR 105	0	0 *	HR
IPR 107	0	0 *	HR
Japy	0	0 *	HR
MGS Ametista	0	0 *	HR
MGS Aranãs	0	0 *	HR
MGS Paraíso 2	0	0 *	HR
MGS Pioneira	0	0 *	HR
MGS Turmalina	0	0 *	HR
IAC Obatã 4739	0	0 *	HR
Paraíso MG H419-1	0	0 *	HR
Sagarana 19[1]	0	0 *	HR
Rouxinol	3	9.38 ± 5,0.23 c	MR
IPR 102	3	14.71 ± 6.17 c	MR
Catucaí Amarelo 2SL	3	20.59 ± 7.04 c	MR
Guará	3	23.33 ± 7.85 bc	MR
Azulão	3	60.00 ± 9.10 ab	SS
Catucaí Amarelo 24/137	4	64.00 ± 9.80 a	SS
H29-1–8-5[1]	5	100 ± 0.00 *	S
Catuaí Vermelho IAC 99	6	100 ± 0.00 *	S

[1] Progenies in the $F_{6:7}$ from EPAMIG breeding program

[2] Severity level according to the diagrammatic scale of Figueiredo et al. (2022)

[3] Means followed by different letters in the column indicate a significant difference between genotypes (GLM with quasi-binomial distribution followed by Tukey's post hoc test; $p < 0.05$)

[4] Resistance classification according to the leaf incidence percentage: HR—Highly Resistant; MR—Moderately Resistant; SS – Slightly Susceptible. S – Susceptible

*Treatment removed from statistical analysis due to the lack of variability

(0% to 4.75%), Catucaí Amarelo 2SL (0% to 4.42%), Guará (0% to 4.46%), and Azulão (0% to 4.44%), with respective mean values of 0.18%; 0.05%; 1.64%; 1.62%; 1.59% felt into level 3 (2.1 to 5.0%) not differing statistically from the Catucaí Amarelo 24/137 genotype, classified as CLR severity level 4 (5.1% to 10%), since the sporulating leaf area ranged from 1.05% to 6.81%.

Discussion

The characterization of the 25 commercial cultivars studied revealed that under controlled conditions, 18 were classified as highly resistant (HR) against CLR. The sources of resistance of these cultivars come from HDT (carrying the *C. canephora* resistant factors), BA series (carrying the S_H3 locus) and, in a smaller number, from Icatu (Rodrigues-Junior et al. 1975; Várzea and Marques 2005; Ribas et al. 2011; Angelo et al. 2023). In addition to the susceptible Catuaí Vermelho IAC 99, other six cultivars exhibited also disease symptoms. Although with different levels of susceptibility/moderately resistant (SS and MR) these cultivars: Rouxinol, IPR102, Catucaí Amarelo 2SL, Azulão, Guará and Catucaí Amarelo 24/137 were all originated from Icatu hybrid. The Icatu selections, launched in 1992, were already recognized for their partial/incomplete resistance to *H. vastatrix* at that time. Nowadays, they are generally classified by research institutes as having low partial/incomplete resistance (also known as horizontal or quantitative resistance) or being moderately susceptible (Zambolim et al. 2024). The intermediate levels of resistance appear to be potentially influenced by minor genes (Vale et al. 2001; Sera et al. 2022; Santos et al. 2022). Our results further suggest that under field conditions, it would be advisable to implement some chemical control strategies, particularly for the Azulão and Catucaí Amarelo 24/137 cultivars, which exhibited 60% CLR incidence.

Our study's results, obtained from inoculations under controlled greenhouse inoculations, showed minor discrepancies compared to the field results reported by Sera et al. (2022). These discrepancies were obtained for a few cultivars with partial/incomplete resistance. For instance, the cultivar Azulão was classified as slightly susceptible in our study, but under field conditions showed a moderate level of resistance (Sera et al. 2022). Another highlight is Catucaí Amarelo 2SL, which, in our study, showed susceptibility to rust, comparing with the slight resistance classification attributed by Sera et al. (2022). It has been observed that cultivar resistance varies according to the geographic region. This variability has been associated with the complex interaction among the environment, host, and pathogen, which is influenced by the prevalence of specific strains in different regions (Talhinhas et al. 2017). Although, the field results (Sera et al. 2022) offer a broader and more challenging perspective on the natural environment, our study provides valuable information on cultivar-specific responses under controlled conditions. This contrast highlights the continuous

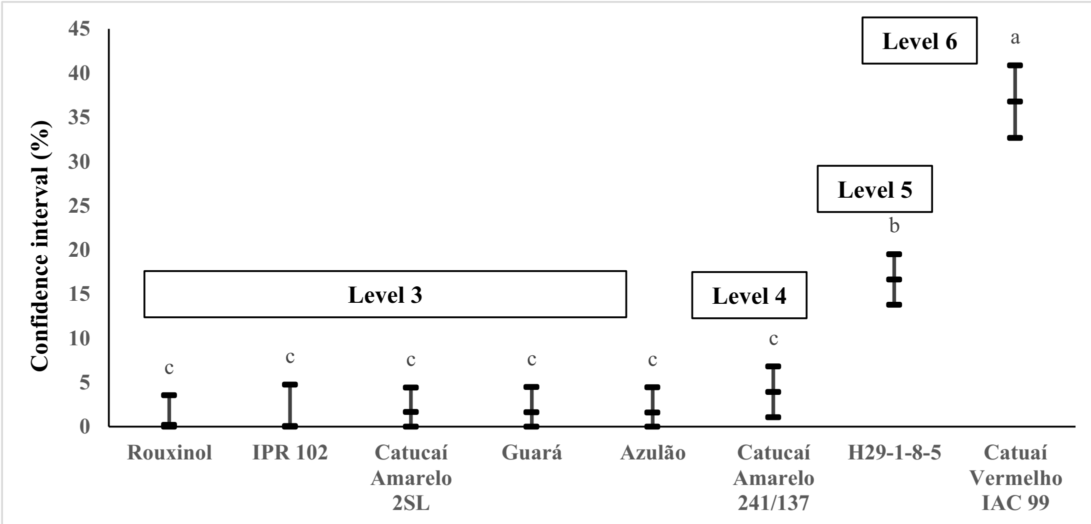

Fig. 1 Confidence interval of disease severity assessed by the sporulating leaf area obtained by digital image analysis (ImageJ software) in susceptible genotypes of *Coffea arabica* 45 days after inoculation with *Hemileia vastatrix*. Means followed by different letters differ from each other due to overlapping confidence intervals

need for research to understand the complexity of resistance to coffee rust, emphasizing the importance of integrating data obtained in different contexts for a more comprehensive view.

It is worth noting that in order to carry out this study, cultivars developed by Brazilian institutions that practice the genetic improvement of coffee plants (IAC, EPAMIG/UFV/UFLA, Fundação Procafé, IDR-Paraná, and EMBRAPA Café) were considered. In the strategies of these institutions, breeders prioritize obtaining cultivars with levels of resistance combined through gene pyramiding. The main purpose is to ensure resistance durability, reduce dependence on chemical control for rust, and prevent productivity losses (Silva et al. 2006). Considering the four progenies studied, three were classified as highly resistant and one as susceptible, the latter showing 100% CLR incidence. The susceptible progeny, H29-1–8–51, originated from the cross of Icatu IAC H3851-2 × Catimor UFV 1603–215, the same one that originated the cultivar MGS Aranãs (classified as highly resistant) (Table 1—source of resistance information). Although, with the same genetic background and inoculum pressure, the resistance response of those genotypes was completely different. This result suggests that during the genetic selection performed on the progeny H29-1–8–51, the potential S_H resistance genes were probably lost. The evaluation of CLR incidence and severity along the different progeny generations, under controlled greenhouse inoculations, is of utmost importance for enhancing the genetic resistance of coffee cultivars. By identifying plants with high resistance to CLR across multiple generations, breeders can selectively breed for these desirable traits, leading to the development of improved coffee cultivars with enhanced resistance to CLR and potentially other diseases as well. Moreover, research institutions continued to conduct experiments in different geographic locations to identify additional or enhanced sources of resistance, which involved the assessment of disease incidence and severity in newly developed cultivars (Carvalho et al. 2017; Moreira et al. 2022).

To ensure a comprehensive and reliable analysis, we employed two distinct approaches to assess rust severity: visual assessment and image analysis. Using ImageJ processing software, as suggested by Schindelin et al. (2015), we achieved precise lesion quantification via scanner, enabling detailed measurement of disease severity. Additionally, the visual assessment, based on a diagrammatic scale (Figueiredo et al. 2022), proved to be a practical, easy, and non-destructive method. The results obtained from both techniques showed a notable overlap, enhancing the consistency and validity of the conclusions drawn in this study.

From the examination of 25 cultivars and four progenies, it was observed that eight, including the control, had their resistance surpassed by pathotypes from the southern region of Minas Gerais. This underscores the importance of evaluating coffee resistance to diseases under greenhouse conditions for obtaining precise data, thus minimizing the risk of oversight and false positives regarding resistance. Such precision aids farmers in making informed decisions about cultivar selection, thereby reducing the probability of crop losses and the overuse of pesticides. Furthermore, the identification of genotypes that have not yet had their resistance supplanted by *H. vastatrix*, as observed in this study, provides a solid basis for coffee genetic improvement programs and the sustainability of coffee production. This information is crucial for plant breeders to strategically incorporate diverse sources of resistance (complete and partial) into crop varieties. Such strategic integration aims to enhance the resilience of coffee plants against a wide array of pathogens.

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Authors contributions Conceptualization, performed the methodology, validated the data, carried out the data curation, writing – original draft, writing – review & editing: Andreisa Fabri Lima; performed the methodology, writing – review & editing: Deila Magna dos Santos Botelho; Interpretation of data and writing – review & editing: Leonor Guerra-Guimarães, Vinicius Teixeira Andrade and Mariana de Lima Santos; Resources, Supervision, writing – review & editing: Mário Lúcio Vilela de Resende; conceptualization, performed the methodology, Resources, Supervision, writing – original draft, writing – review & editing: Juliana Costa de Rezende Abrahão.

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Data availability All data generated or analysed during this study are included in this article.

Declarations

Conflict of interest The authors declare no competing interests.

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