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RESEARCH ARTICLE
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Photosynthetic Efficiency Affected by Different Isolates of Coffee Leaf Rust from Cajamarca, Peru

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ABSTRACT

Coffee leaf rust (*Hemileia vastatrix*) represents a significant threat to coffee production, making the study of host-pathogen interactions essential. This study investigated the aggressiveness of *H. vastatrix* and its impact on Coffea arabica cultivar Bourbon plants. Nine isolates of *H. vastatrix* (1RN-9RN), collected from San Ignacio, Cajamarca, were used to inoculate Bourbon coffee plants in San Ramón, Chanchamayo. The aggressiveness of the pathogen was assessed based on several parameters: incubation period (IP), latency period (LP), medium latency period (MLP), and frequency of infection (FI). Additionally, the plant response was evaluated by measuring chlorophyll content and using the OJIP test to assess fluorescence. The ranges for IP, LP,mLP, and FI were 17–17.9, 20.7–26.8, 22–32, and 5.8–8.7 days after inoculation (dai), respectively. No significant differences were observed in IP or FI. Chlorophyll content varied between 27.78 and 42.32 units at 37dai. Regarding the OJIP analysis, the majority of the *H. vastatrix* isolates caused a variation in the Fv/Fm values, which ranged from 0.43 to 0.73 at 37dai. The performance index (Pi) ranged from 0.49 to 3.41 at 37dai, showing a decrease in most isolates, except for isolate 7RN. A percentage variation was observed in the following variables: ABS/RC (4.38–120.93%), TRo/RC (6.33–45.03%), and ETo/RC (0.32–30.44%). Furthermore, a physiological response indicative of the photosynthetic defense mechanism was observed in the majority of isolates. This was reflected in the increased values of ABS/RC, ETo/RC, and TRo/RC, alongside the decrease in Fv/Fm and PI.

Keywords: Coffee rust, Aggressiveness, Bourbon coffee, Chlorophyll, Growth.

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INTRODUCTION

Coffee is a prominent commodity in global trade (Fromm, 2023), with significant economic, cultural and social impact (Bracken et al., 2023; Maspul, 2023). Grown in over 60 countries, it is primarily produced by an estimated 25 million farmers, most of whom are smallholders (less than 5 hectares) (Bracken et al., 2023; Fromm, 2023). Approximately 125 million people worldwide are directly engaged in various stages of the coffee value chain (Fromm, 2023). Furthermore, coffee cultivation plays a crucial role in supporting rural livelihoods, promoting biodiversity conservation, and advancing sustainable development (Harvey et al., 2021). In the field, plants are exposed to various types of biotic stress, primarily caused by a wide range of microorganisms, including fungi, bacteria and nematodes (Gull et al., 2019). In the case of coffee, a major limiting factor in production is the presence of *Hemileia vastatrix* Berk and Br, the causal agent of coffee leaf rust (CLR), which is regarded as the most significant disease affecting this crop (Silva et al., 2006; Oliveira et al., 2020).

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The incidence of CLR in Peru has been investigated by several researchers, who have reported an incidence of approximately 30% in susceptible varieties. However, this value may vary depending on climatic conditions and the genotype of the cultivar being studied (Alvarado-Huamán et al., 2020; Borjas-Ventura et al., 2020). As climate change alters environmental factors such as temperature and precipitation, the regions suitable for coffee cultivation are decreasing, impacting both yield and quality (Bracken et al., 2023). Additionally, the plant's response to pathogens is influenced not only by environmental conditions but also by the specific type of pathogen involved (Souza et al., 2017). In the case of coffee, the genetic diversity of *Hemileia vastatrix* is recognized, although it has been relatively understudied in the Peruvian context (Quispe-Apaza et al., 2017; Silva et al., 2022). Julca-Otiniano et al. (2024) reported the presence of four physiological races of *H. vastatrix* in Peru: XXXIV, XXXV and two new races. However, Silva et al. (2022) noted that globally, there are 55 known physiological races of this pathogen, each characterized by different combinations of virulence genes (v1-v9).

Each physiological race exhibits varying levels of pathogenic aggressiveness, as demonstrated in studies on Xanthomonas campestris (Távora et al., 2022), *Orobanche cumana* Wallr (Clapco, 2022) and *Phytophthora capsici* (Lee et al., 2021). Consequently, studies on aggressiveness in *H. vastatrix* could serve as indicators of the prevailing pathotypes in a given area. Aggressiveness can be assessed through factors such as incubation period, latency period, the number of sporulating lesions and the intensity of *H. vastatrix* infection. Chlorophyll is regarded as a key physiological indicator, with higher levels signifying optimal conditions for plant growth and development, while lower levels may indicate stress (Motyka et al., 2020).

The OJIP test provides a quantitative assessment of the kinetics of fluorescence production (Moreno et al., 2008) and could serve as a method to evaluate the impact of specific pathogens on plants. This analysis enables the examination of the health of photosystem II (PSII) and the components of the electron transport chain during photosynthesis (Toniutti et al., 2017). In the case of *H. vastatrix*, it has been observed that the Fv/Fm ratio (a parameter derived from the OJIP test) decreases following pathogen attack. However, this test has been underutilized (Honorato Junior et al., 2015; Salcedo-Sarmiento et al., 2021; Vitória et al., 2023). Several authors highlight the significance of the OJIP test, noting its utility in the early evaluation of plant responses to both biotic and abiotic stress (Ceacero et al., 2012; Mariño, 2014; Rodríguez et al., 2014; Moro et al., 2023). The existence of genetic diversity and physiological races of *H. vastatrix* are key factors driving this study, which aims to evaluate the aggressiveness and plant response induced by different *H. vastatrix* isolates. The goal of this research is to enhance the understanding of the plant's response to coffee leaf rust, with the ultimate aim of promoting crop sustainability in the face of climatic factors, agronomic practices and management strategies.

MATERIALS & METHODS

Study Site

Plots consisting of homogeneous coffee varieties were selected from fields cultivated by farmers in four districts (Chirinos, San José de Lourdes, Namballe and San Ignacio) within the province of San Ignacio, Cajamarca (Fig. 1). This region is characterized by an average temperature of 21.7°C (INGEMMET, 2020) and annual precipitation ranging from 1036 to 1451 mm (SENAMHI, 2021).

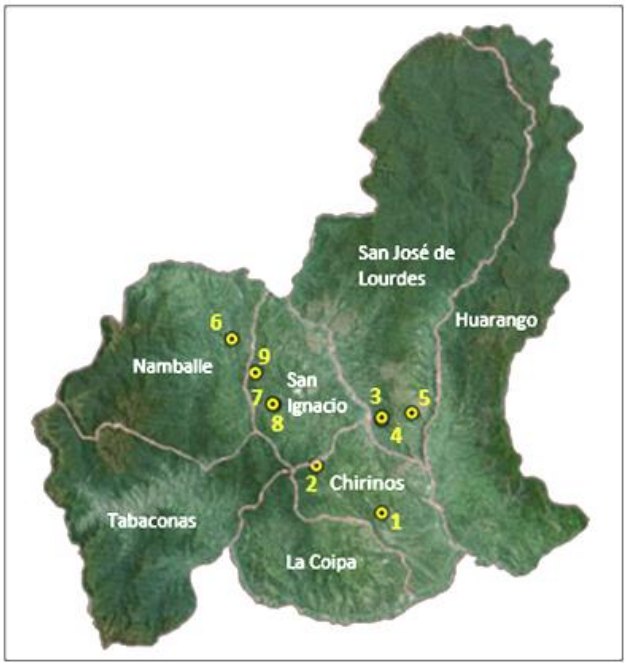

Fig. 1: Geographic location of selected *Hemileia vastatrix* isolates.

Urediniospores Collection

Urediniospores *of Hemileia vastatrix* were collected following the CIFC protocol. The isolates were placed in gelatin capsules, labeled, and accompanied by farm-specific information, as detailed in Table 1. The capsules were then transported to the Tropical Crops Laboratory at the Universidad Nacional Agraria La Molina (UNALM), located at the "La Génova" farm of the Instituto Regional de Desarrollo de Selva (IRD-Selva) in the district of San Ramón, province of Chanchamayo, department of Junín. The site is situated at an altitude of 965 m.a.s.l., with geographic coordinates of 11°05.790' S latitude and 75°20.969' W longitude.

Inoculation

Coffee plants of the Coffea arabica cv Bourbon were used for inoculation. The seeds were sown and cultivated in the Tropical Crops Nursery at UNALM, located in La Molina. The substrate employed was Plug Mix, consisting of compressed Sphagnum moss, perlite, and vermiculite. Fertilization occurred 6 weeks after transplanting, using hydroponic solutions with a macronutrient concentration ("A" solution) of 2mL/Land a micronutrient concentration ("B" solution) of 5mL/L. The combined solution (A+B) was applied at a rate of 300mL per plant, administered weekly.

Table 1: Collection data from coffee farms in San Ignacio, Cajamarca

Farm	Producer	Altitude	East (m)	North (m)	Locality	District	Province	Region	Cultivar	Plant age (years)
1RN	Elvenninga Yapaujo	1535	9411042	735414	Huarango Cazado	Chirinos	San Ignacio	Cajamarca	Caturra	20
2RN	Cotrina	1691	9418711	724720	El Sauce	Chirinos	San Ignacio	Cajamarca	Typica	20
3RN	-	1117	9426132	735443	Los Alpes	San José de Lourdes	San Ignacio	Cajamarca	Typica	20
4RN	Saúl Quiñonez	1283	9426615	739013	San Juan de Pacay	San José de Lourdes	San Ignacio	Cajamarca	Catimor	10
5RN	Miguel Camacho	1056	9427271	740383	San Juan de Pacay	San José de Lourdes	San Ignacio	Cajamarca	Catimor	3
6RN	-	828	9439411	710686	Vicente La Vega	Namballe	San Ignacio	Cajamarca	Typica	10
7RN	Juan Garcia	1403	9428717	717522	Alfonso Ugarte	San Ignacio	San Ignacio	Cajamarca	Pache	10
8RN	Mario Garcia	1376	9428612	717812	Alfonso Ugarte	San Ignacio	San Ignacio	Cajamarca	Catimor	10
9RN	-	1700	9433834	714684	Cruz de Chalpon	San Ignacio	San Ignacio	Cajamarca	Geisha	10

Urediniospores of *H. vastatrix* were inoculated onto the leaves of 6-month-old Coffea arabica cv. Bourbon Red. Young leaves were selected from each plant, and the urediniospores were evenly distributed on the underside of the leaves. Inoculation was performed at 5 pm, immediately followed by the application of distilled water using a sprayer to form a thin water film on the leaf surface, ensuring the urediniospores were not washed away. The plants were then covered with transparent plastic bags for 24 hours and further shaded with newspaper to maintain the required darkness (Várzea et al., 2023). After this incubation period, the newspaper was removed, allowing the germinated urediniospores to continue their development. During the evaluation period, temperatures ranged from 15.3 to 32.8°C, and relative humidity varied from 41.1% to 100%.

Aggressiveness Assessment

Aggressiveness was assessed by evaluating the incubation period (the time interval from inoculation to the initial appearance of disease symptoms, manifested as small chlorotic areas) (Santacreo, 1989); the latency period (the number of days between inoculation and the onset of sporulation in the observed lesions) (Leguizamón et al., 1998); the average latency period (the time in days from inoculation to sporulation of 50% of the pustules on inoculated leaves) (Leguizamón et al., 1998) and the frequency of infection (the number of lesions with spore production per leaf, evaluated using an arbitrary scale ranging from 0 to 9) (Eskes, 1983) (Fig. 2).

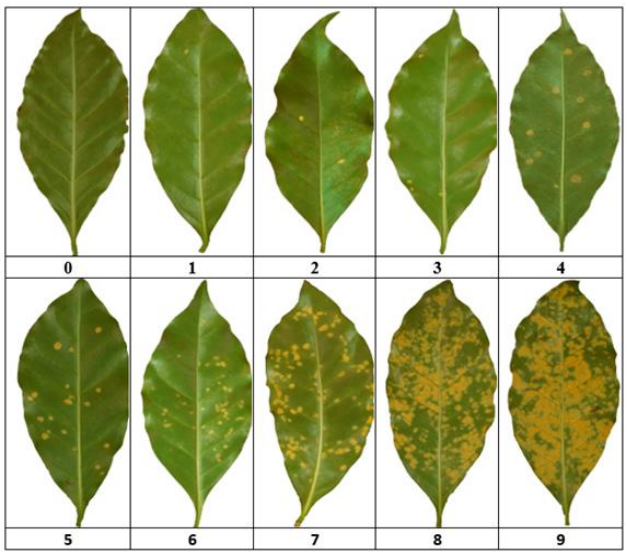

Fig. 2: Infection frequency scale of coffee leaf rust (0-9). Adapted from Eskes (1983).

Ecophysiological Assessment

Chlorophyll content was estimated using the KONICA MINOLTA SPAD-502 PLUS chlorophyll meter. Additionally, the OP-30p fluorometer from OPTI-SCIENCE was employed to measure OJIP test parameters, including Fv/Fm (maximum photochemical efficiency), Pi (Performance index), ABS/RC (average photon flux absorbed by the PSII reaction center), TRO/RC (maximum exciton trapping flux by PSII), and ETO/RC (electron transport flux from QA to QB by PSII).

Measurements of both chlorophyll content and fluorescence were taken at five distinct time points: one day before inoculation, two days after inoculation, 17 days after inoculation, 25 days after inoculation, and 37 days after inoculation.

Data Analysis

Nine treatments were conducted using different isolates of coffee leaf rust collected from four districts in the province of San Ignacio. Each treatment included 10 replicates, with one replicate corresponding to an individual plant. The data for each variable were analyzed using analysis of variance (ANOVA) and Tukey's mean comparison test (95% confidence level) in RStudio software.

RESULTS

Aggressiveness

Nine different isolates (1RN to 9RN) were analyzed, and the corresponding incubation periods, latency, mean latency, and frequency of infection were recorded (Fig. 3 and 4). No significant differences ($P \leq 0.05$) were observed among the isolates for the incubation period (IP), with values ranging from 17 to 17.9 days. However, both the latency period (LP) and mean latency period (MLP) exhibited significant variation, with values ranging from 20.7 to 26.8 days and from 22 to 32 days, respectively. Regarding the frequency of infection (FI), no statistical differences were found among the isolates, with values ranging from 5.80 to 8.70 (Fig. 3).

When analyzing the aggressiveness variables collectively, isolates 3RN, 5RN, 8RN, and 9RN were the most aggressive, with an incubation period (IP) ranging from 17 to 17.6 days, a latency period (LP) from 20.7 to 23.9 days, a mean latency period (MLP) from 22 to 26.8 days, and a frequency of infection (FI) ranging from 5.8 to 6.9, respectively. The remaining isolates were considered less aggressive, with an average IP of 17.32 days, LP of 25.36 days, MLP of 30.04 days, and FI of 6.84 (Fig. 3).

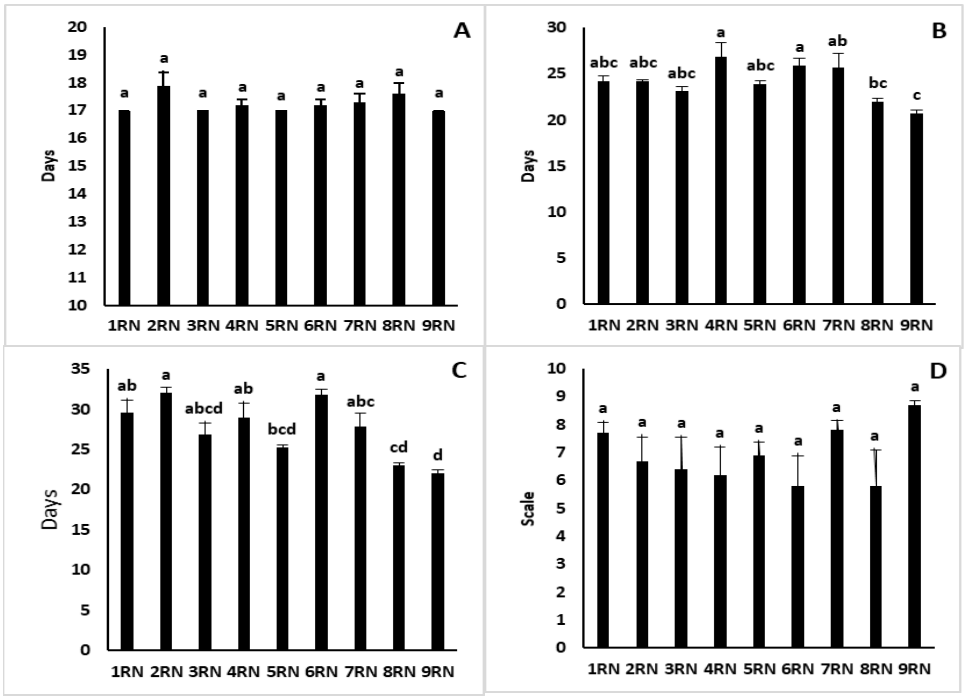

Fig. 3: Aggressiveness of different population of *H. vastatrix* collected in Cajamarca. A) Incubation period. B) Latency period. C) Mid-latency period. D) Frequency of infection; 1RN - 9RN: Different isolates. Different letters indicate statistical differences (Tukey 95%).

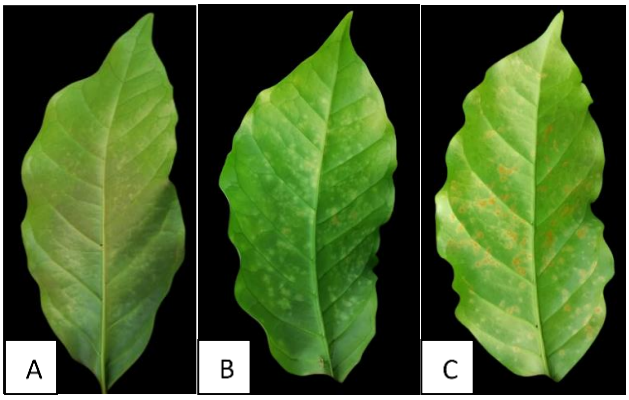

Fig. 4: Aggressiveness of *Hemileia vastatrix:* (A) Incubation period, (B) Latency period and (C) Mid-latency period.

Content of Chlorophylls

Chlorophyll content decreased as the dormancy and mid-dormancy periods progressed (Fig. 5). At the conclusion of the evaluations (37 days after inoculation), only isolates 3RN, 4RN, 8RN and 9RN showed significant reductions (P≤0.05) in chlorophyll content, with decreases ranging from 32.43% to 40.06% compared to the initial measurement (1 day after inoculation).

OJIP Test

In most isolates, a significant decrease in the Fv/Fm value was observed after the latency period, with values ranging from 0.43 to 0.74 at 37 days after inoculation (dai). Isolate 7RN was the only isolate that did not exhibit a significant decline in Fv/Fm, while the other isolates showed significant reductions ranging from 6.78% to 42.89% (Fig. 6). The performance index (Pi) ranged from 0.49 to 3.41 (Fig. 7). Isolates 3RN, 4RN, 5RN, 6RN, and 9RN caused a significant reduction in Pi values compared to the last evaluation (37 days after inoculation), with decreases ranging from 36.01% to 82.4%. Only isolate 7RN exhibited an increase of 36.01% between the first evaluation (1 day before inoculation) and the last evaluation (37dai) (Fig. 7). The ETo/RC ranged from 4.23 to 5.19 at 37 days after inoculation (dai). Only isolates 4RN, 5RN, and 6RN showed no significant differences (P≤0.05), with variation ranging from 0.66% to 10.97%. In contrast, the other isolates exhibited statistically significant differences (P≤0.05), with increases ranging from 17.05% to 29.21% compared to the first evaluation (1 day before inoculation) (Table 2). For the other parameters evaluated (ABS/RC and TRO/RC), significant increases (P≤0.05) were observed in nearly all isolates, except for isolate 7RN, which showed minimal percentage variations of 0.07% (ABS/RC) and 0.08% (TRO/RC) compared to the initial evaluation (Table 2).

DISCUSSION

Aggressiveness

Hemileia vastatrix is one of the most harmful microorganisms affecting global coffee production (Berihun & Alemu, 2023). The first outbreak occurred in Sri Lanka in the 1800s (Ramírez-Camejo et al., 2022) and the most recent outbreak occurred between 2011 and 2013. This fungus can cause yield losses of up to 75% (Koutouleas, 2023). Despite being a major threat to coffee production for over a century, growers have not yet effectively managed this pathogen. A crucial step in controlling *H. vastatrix* is understanding its genetic diversity and the aggressiveness of different isolates. With this knowledge, growers and researchers can develop more targeted and effective control strategies.

The short incubation period (IP) observed in this study (17-17.9 days) (Fig. 3) is likely due to the use of the Bourbon cultivar, which is considered susceptible to *Hemileia vastatrix* (WCR, 2024), leading to a reduced time until the appearance of the first visible symptoms (IP). Other studies have reported longer incubation periods, ranging from 25 to 30 days (Pires et al., 2020; Pozza et al., 2021).

Table 2: ETo/RC, ABS/RC Y TRo/RC in coffee plants inoculated with different populations of *H. vastatrix* of Cajamarca

	ETo/RC				ABS/RC				TRo/RC			
	1dbi	17dai	25dai	37dai	1dbi	17dai	25dai	37dai	1dbi	17dai	25dai	37dai
1RN	4.28±0.09b	4.63±0.15ab	4.46±0.16b	5.19±0.21a	1.76±0.05b	1.83±0.07b	1.94±0.08ab	2.25±0.13a	1.30±0.02b	1.38±0.06ab	1.38±0.04ab	1.51±0.05a
2RN	4.36±0.07b	4.45±0.13b	4.12±0.21b	5.10±0.12a	1.91±0.06b	1.84±0.06b	1.89±0.06b	2.36±0.16a	1.38±0.03b	1.34±0.04b	1.36±0.04b	1.57±0.05a
3RN	4.26±0.05b	4.37±0.12b	4.76±0.12ab	5.15±0.25a	2.09±0.04b	1.82±0.05b	2.25±0.10b	3.38±0.30a	1.48±0.02bc	1.32±0.03c	1.52±0.03b	1.72±0.07a
4RN	4.25±0.10a	4.51±0.10 a	4.46±0.13a	4.23±0.22a	1.78±0.05b	1.73±0.02b	1.84±0.04b	2.54±0.08a	1.34±0.03b	1.31±0.02b	1.38±0.03 b	1.58±0.03a
5RN	4.35±0.11ab	3.99±0.17b	4.89±0.26a	4.66±0.27ab	1.74±0.07b	1.79±0.07b	2.50±0.21a	3.03±0.26a	1.30±0.04b	1.28±0.04b	1.55±0.07ab	1.71±0.11a
6RN	4.30±0.14ab	4.14±0.09b	4.59±0.15ab	4.77±0.20a	1.75±0.06bc	1.59±0.03c	1.92±0.04b	2.24±0.11a	1.30±0.04bc	1.21±0.02c	1.41±0.03ab	1.50±0.03a
7RN	3.87±0.12c	4.88±0.14a	4.47±0.07b	4.99±0.05a	1.85±0.05a	1.81±0.05a	1.82±0.06a	1.91±0.05a	1.33±0.03a	1.36±0.04a	1.33±0.03a	1.41±0.03a
8RN	4.10±0.14c	4.67±0.20bc	5.53±0.17a	4.85±0.22ab	1.87±0.06b	1.80±0.03b	1.98±0.08b	3.17±0.58a	1.34±0.04b	1.34±0.03b	1.43±0.04ab	1.58±0.07a
9RN	3.92±0.11c	4.30±0.07bc	4.58±0.19ab	4.87±0.15a	1.87±0.05b	1.86±0.04b	2.48±0.15b	4.13±0.31a	1.34±0.02b	1.36±0.03b	1.56±0.05b	1.94±0.12a

Different letters indicate statistical differences (Tukey 95%) in the row. dbi: days before inoculation,dai: days after inoculation. 1RN - 9RN: Different isolates

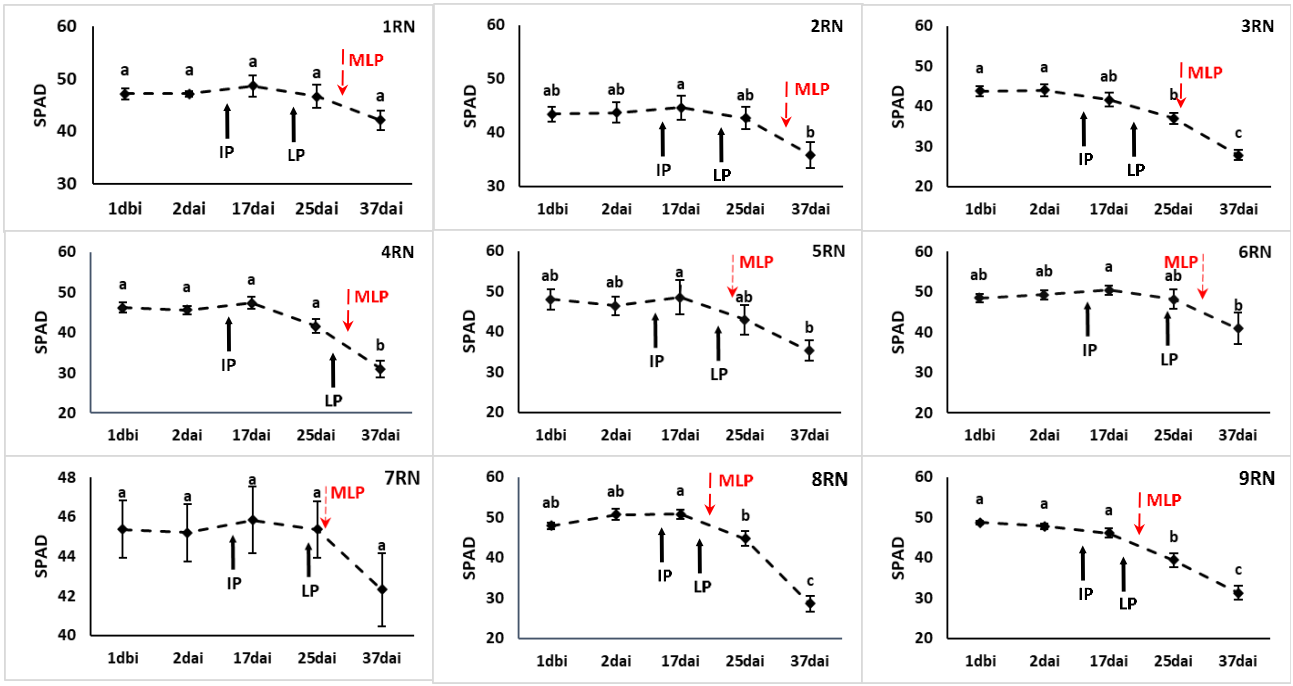

Fig. 5: Content of chlorophylls in different moments of disease coffee leaf rust on coffee cv Bourbon; Red line (MLP): Medium latent period. IP: Incubation period. LP: Latent period. Different letters indicate statistical differences (Tukey 95%). dbi: days before inoculation,dai: days after inoculation. 1RN - 9RN: Different isolates.

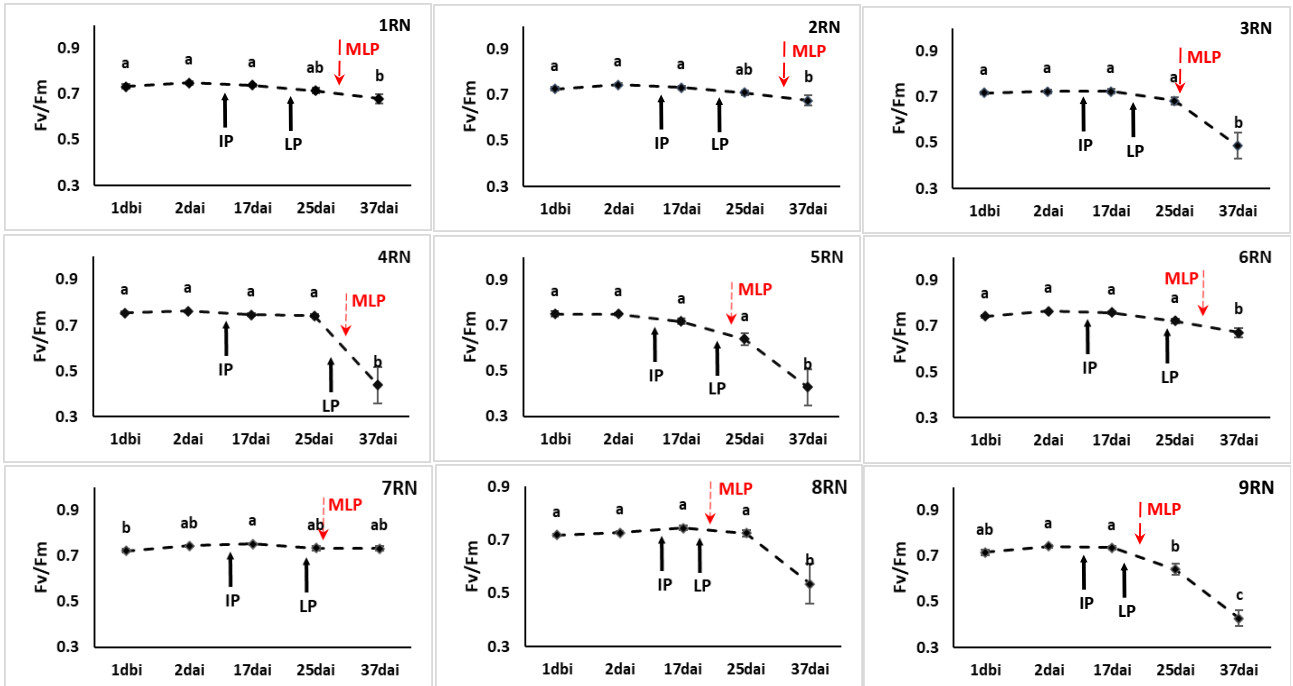

Fig. 6: Variation of Fv/Fm in coffee cv Bourbon in five different times; Red line (MLP): Medium latent period. IP: Incubation period. LP: Latent period. Different letters indicate statistical differences (Tukey 95%). dbi: days before inoculation,dai: days after inoculation. 1RN - 9RN: Different isolates.

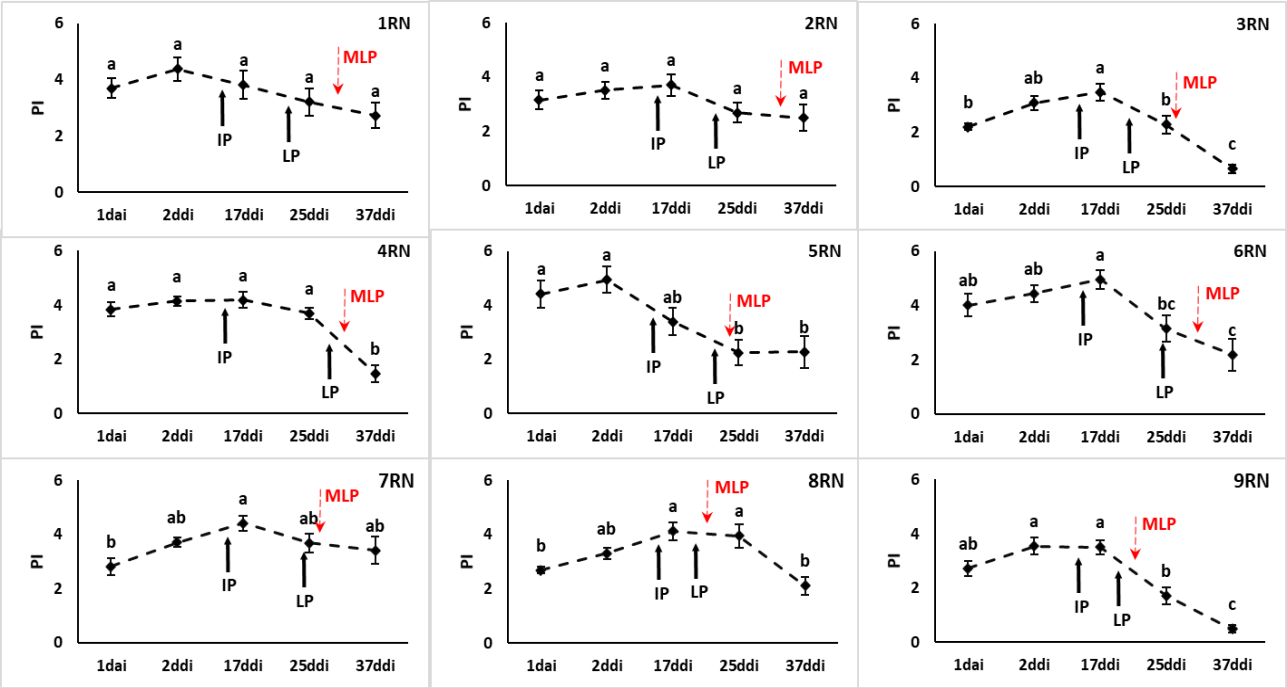

Fig. 7: Variation of PI in coffee cv Bourbon in five different times; Red line (MLP): Medium latent period. IP: Incubation period. LP: Latent period. Different letters indicate statistical differences (Tukey 95%). dbi: days before inoculation,dai: days after inoculation. 1RN - 9RN: Different isolates.

On the other hand, the latency period (LP) and mean latency period (MLP) were not necessarily influenced by the same factors as the incubation period (IP). The values obtained for LP (20.7 to 26.8 days) (Fig. 3) fall within the ranges reported by Toniutti et al. (2017) (21-37 days) and Maia et al. (2017) (17-50 days), whose evaluations were conducted on susceptible cultivars. All isolates exhibited the same incubation period (IP), but isolates 4RN and 6RN took longer to show signs of infection (LP). In contrast, isolate 9RN had the shortest latency period (LP) ($P \leq 0.05$) (Fig. 3). This result suggests that isolate 9RN likely possesses highly efficient biochemical mechanisms for colonizing plant cells.

According to Lovelace et al. (2023), pathogens can create favorable conditions for successful colonization through specific proteins (effectors), which enable the pathogen to access nutrients during the early stages of infection, leading to early sporulation (Walters et al., 2008). Subit et al. (2021) suggest that disease progression may vary once the pathogen enters the plant cell, where fungal maturation must proceed with differentiation into sori.

The frequency of infection is the most reliable method for quantifying the damage caused by coffee leaf rust, as it involves counting pustules, making it more precise and less subjective (Gallego-Sánchez et al., 2020). According to the scale used, no significant differences ($P \leq 0.05$) were found among the isolates. This can be explained by the fact that when inoculating a susceptible variety like red Bourbon, the frequency of infection is typically high and develops rapidly (Virginio & Astorga, 2015; Quiroga-Cardona, 2021). As noted by Campos (2015) and Quiroga-Cardona (2021), the variability in infection is influenced by the population under examination and is affected by factors such as leaf age, light intensity, and environmental conditions.

Content of Chlorophylls

Chlorophylls are a group of essential pigments in plants, responsible for absorbing light energy during photosynthesis, which is vital for their growth (Li et al., 2018; Zulkarnaini et al., 2019; Ebrahimi et al., 2023; Jin et al., 2023). Chlorophyll content in leaves serves as an indicator of photosynthetic capacity and plant health (Zulkarnaini et al., 2019). This variable is considered a physiological indicator, with high chlorophyll levels indicating optimal growth and development conditions, while lower levels reflect stress (Motyka et al., 2020).

In the case of *H. vastatrix*-induced chlorosis, which begins with the appearance of visible symptoms (IP), chlorophyll content declines starting from the latency period (LP), leading to a reduction in photosynthetic area and disruption of the photosynthetic process (Gortari et al., 2018; INIAP, 1993). This is particularly evident in isolates 3RN, 4RN, 8RN, and 9RN, where a significant decrease ($P \leq 0.05$) in chlorophyll content was observed between measurements taken at 37 days after inoculation (dai) and 1 day before inoculation (dbi) (Fig. 5). In contrast, the remaining isolates did not exhibit a statistically significant reduction ($P \leq 0.05$), which may be attributed to similarities in growth stage and leaf nitrogen status (Taha et al., 2024), despite the presence of *H. vastatrix*.

OJIP Test

The OJIP test has been underutilized in the analysis of *H. vastatrix* (Honorato Junior et al., 2015; Salcedo-Sarmiento et al., 2021; Vitória et al., 2023). This test provides valuable insights into the physiological condition of photosystem II (PSII) and the components involved in the electron transport chain during photosynthesis (Toniutti et al., 2017). A dark-adapted plant typically exhibits Fv/Fm values between 0.75 and 0.85, which is an

estimate of the PSII quantum yield; a decrease in this value indicates photoinhibition damage (Carrasco & Escobar, 2002). As noted by Mariño (2014), stressed plants show a reduction in photosynthesis, suggesting the presence of potential photoinhibitory damage in Photosystem II (PSII), which ultimately affects photosynthetic efficiency.

In our study, at 37 days after inoculation (dai) (the final evaluation), Fv/Fm values across all isolates (0.43 to 0.73) (Fig. 6) were lower than those typically observed in unstressed plants. This decrease can be attributed to the effects of *H. vastatrix* on the isolates. As reported by Salcedo-Sarmiento et al. (2021), *H. vastatrix* induces alterations in the photosynthetic performance of leaves as the disease progresses. However, isolate 7RN, despite having a Fv/Fm value of 0.73 at 37dai, did not show a significant decrease ($P \leq 0.05$) compared to the initial evaluation (Fig. 6). This could be due to its relatively low reduction in chlorophyll content (6.24%), which was not statistically significant ($P \leq 0.05$) and its higher chlorophyll content at 37dai (42.32%) compared to the other isolates (Fig. 5). Fv/Fm values are influenced by chlorophyll content, as indicated by León-Burgos et al. (2022). A decrease in Fv/Fm is associated with oxidative damage to photosynthetic pigments such as chlorophylls, resulting in physiological alterations in PSII in stressed plants. Therefore, it can be inferred that the greater chlorophyll content in isolate 7RN may help buffer the photoinhibitory damage caused by *H. vastatrix*.

The performance index (Pi) is related to the concentration of reaction centers, the quantum efficiency of primary photochemistry, and the conversion of excited energy into electron transport (Strasser et al., 2000). Its value decreases, similar to Fv/Fm, when the plant is stressed. As noted by Moro et al. (2023), stress can alter the Pi of photosystem II (PSII). Among the isolates evaluated, only 3RN, 4RN, 5RN, 6RN, and 9RN exhibited a significant reduction ($P \leq 0.05$), with a decrease ranging from 36.11% to 82.4%. According to Rodríguez et al. (2014), plants under stress conditions inhibit PSII, leading to damage in the D1 protein, which results in a decrease in Pi. The remaining isolates did not show a significant change ($P \leq 0.05$) (Fig. 7), similar to findings in *Fusarium* and *Colletotrichum graminicola* infections, where Pi did not significantly decrease in wheat (Spanic et al., 2017) and maize (Campos et al., 2021), respectively. It is important to note that Pi is more strongly associated with abiotic stress factors such as low temperatures (Rodríguez et al., 2014), waterlogging (Saravia-Castillo et al., 2022), salinity (Salim et al., 2021) and drought (Barboričová et al., 2022).

Regarding ETo/RC, it represents the specific flux of electron transport per reaction centers (Moreno et al., 2008). This parameter is useful for assessing species-specific responses under stress conditions (Ostrowska & Hura, 2022). In the present study, isolates 4RN, 5RN and 6RN did not exhibit significant variation ($P \leq 0.05$), with isolate 4RN showing a decrease in ETo/RC. This can be attributed to the presence of a higher number of active reaction centers (Kumar et al., 2020). In contrast, the other isolates showed a significant increase ($P \leq 0.05$) (Table 2). As noted by Ostrowska & Hura (2022), ETo/RC increases under stress conditions, exhibiting an inverse relationship with Pi.

ABS/RC represents the absorption flux (Moreno et al., 2016), indicating the amount of light energy captured by each reaction center (Khan et al., 2021). Therefore, the increase in the ABS/RC ratio observed in the isolates may be linked to an expansion of the antenna complex, which supplies excitation energy to the active reaction centers (RCs) (Moro et al., 2023). The findings of this study align with those of Baghbani et al. (2019), Liu et al. (2023) and Marques et al. (2024), who reported an increase in the ABS/RC ratio due to the presence of *Fusarium verticillioides*, *Bursaphelenchus xylophilus*, and *Fusarium equiseti*, pathogens that induce stress similar to that caused by H. vastatrix. Isolate 7RN was the only one that did not show a significant increase in ABS/RC (0.07%) (Table 2). This can be attributed to its high chlorophyll content at 37dai (Fig. 5) and its relatively lower Fv/Fm value (Fig. 6) compared to the other isolates. The TRo/RC ratio represents the maximum rate at which the reaction center traps an exciton, leading to the reduction of QA (Kumar et al., 2020). In this study, a significant increase in TRo/RC was observed in most isolates, except for isolate 7RN, in the presence of *H. vastatrix*. Li et al. (2022) reported a similar trend when investigating the impact of *Puccinia graminis* f. sp. avenae on oats. The patterns of ABS/RC and TRo/RC were consistent in this study, with isolate 7RN being the only one that did not exhibit significant variation (Table 2).

Conclusion

In this work, the isolates presented a rapid incubation period (IP) of 17–17.9 days and latency period (LP) of 20.7 to 26.8 days. No significant differences were observed in the PI and infection frequency across the isolates. Isolates 4RN and 6RN exhibited the longest latency period, while isolates 2RN and 6RN had the longest mean latency period (MLP) compared to isolate 9RN, which showed the lowest values for both LP and MLP. Based on PI, LP, MLP and infection frequency, isolates 3RN, 5RN, 8RN, and 9RN were considered the most aggressive. Some *H. vastatrix* populations affected chlorophyll levels and parameters associated with the OJIP test. Chlorophyll levels decreased after the LP and MLP, with the reduction being more pronounced in 9RN compared to 7RN, which had the highest chlorophyll content at 37 days after inoculation (42.32). A photosynthetic defense mechanism was observed in most isolates, as indicated by increases in ABS/RC, ETo/RC, and TRo/RC when Fv/Fm and PI levels decreased, except for isolate 4RN, where ETo/RC decreased by 0.32%, and isolate 7RN, where Fv/Fm and PI increased by 1.57 and 36.01%, respectively.

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