Australasian Plant Pathology
https://doi.org/10.1007/s13313-024-00988-2

ORIGINAL RESEARCH ARTICLE

Characterization and functional validation of a genomic region involved in resistance to rust race II in *Coffea arabica*

Caroline Ariyoshi[1] · Suzana Tiemi Ivamoto-Suzuki[2] · Viviane Yumi Baba[2] · Masako Toma-Braghini[3] · Gustavo Hiroshi Sera[1] · Adrian Powell[4] · Sandra Maria Bellodi Cação[5] · Eveline Teixeira Caixeta[6,7] · Luiz Filipe Protasio Pereira[1,7]

Received: 11 December 2023 / Accepted: 8 August 2024

Abstract

Coffee leaf rust (CLR) is one of the most economically important diseases affecting *Coffea arabica* production, having a significant economic impact. Among the main goals of coffee breeding programs is the development of cultivars resistant to this disease. A source of resistance genes is Híbrido de Timor (HdT), a spontaneous hybrid originated from the cross between *C. arabica* and *C. canephora*. Previously, in a transcriptome study, the *Ca TDF77 NBS-LRR* gene from HdT involved in resistance to CLR was identified. Hence, our aim was to characterize the genomic region surrounding the *Ca TDF77 NBS-LRR* gene in *Coffea* spp. Furthermore, we aimed to analyze the transcriptional profile of this gene, in the *C. arabica* cultivar IAPAR 59, which is originated from HdT introgression and is resistant to CLR race II. The outcome delineated the gene's localization on chromosome 11 (canephora subgenome) of *C. arabica*, spotlighting intragenic polymorphisms between HdT and Arabica coffee susceptible to CLR race II. The genomic region surrounding the gene in *Coffea* spp. revealed a tandem structure and transposable elements. Notably, within IAPAR 59, the gene exhibited significant upregulation at 24 and 72 h post CLR infection, contrasting starkly with the susceptible genotype. This observation validates its role in fortifying the defense mechanism of this particular cultivar. This study enriches our understanding of the evolutionary dynamics of *Coffea* spp. genomes and also provides genomic resources instrumental in devising biotechnological strategies for resistance to CLR.

Keywords Híbrido de Timor · TIR-NBS-LRR · IAPAR 59 · *Hemileia vastatrix* · Plant-pathogen molecular interaction

Introduction

Coffee is one of the most widely traded tropical commodities, with 80% of global production attributed to farming households (FAO 2022). Among the coffee species (*Coffea* spp.), *C. arabica* L. and *C. canephora* P., account for 56% and 44% of worldwide production, respectively (ICO 2023). *C. arabica*, which originated from natural interspecific hybridization involving *C. canephora* and *C. eugenioides*, yields a higher quality beverage that carries greater economic value (Clarindo and Carvalho 2008; Bertrand et al. 2006).

The majority of cultivated *C. arabica* are susceptible to the main coffee diseases and pests. Coffee leaf rust (CLR), caused by the biotrophic fungus *Hemileia vastatrix* Berk. and Broome (Basidiomycota, Pucciniales), is the most important disease affecting Arabica coffee (Talhinhas et al. 2017). In Brazil, production losses caused by CLR can reach more than 50%, depending on favorable conditions for the disease development,

✉ Luiz Filipe Protasio Pereira
filipe.pereira@embrapa.br

1 Área de Melhoramento Genético e Propagação Vegetal, Instituto de Desenvolvimento Rural do Paraná, Londrina, Paraná 86047-902, Brazil

2 Departamento de Agronomia, Universidade Estadual de Londrina, Londrina, Paraná 86057-970, Brazil

3 Centro de Café Alcides Carvalho, Instituto Agronômico, Campinas, São Paulo 13001-970, Brazil

4 Boyce Thompson Institute, Cornell University, Ithaca, NY 14853, USA

5 Tropical Melhoramento & Genética, Cambé, Paraná 86188-000, Brazil

6 Instituto de Biotecnologia Aplicada à Agricultura, Universidade Federal de Viçosa, Viçosa, Minas Gerais 36570-900, Brazil

7 Empresa Brasileira de Pesquisa Agropecuária (Embrapa Café), Brasília, Distrito Federal 70770-901, Brazil

Published online: 19 August 2024

prolonged periods of drought, and resistance level of cultivars (Zambolim and Caixeta 2021; Sera et al. 2022). This disease has also become a problem for other *C. arabica* producing regions such as Colombia, Central America, Mexico, Peru and Ecuador (Cristancho et al. 2012; Avelino et al. 2015), and nowadays in Hawaii (Keith et al. 2022). Early defoliation and desiccation of branches due to CLR result in reduced fruit production in the following year (Zambolim 2016).

The coffee plant's defense against CLR is contingent upon at least nine major genes with dominant effects (S_H1 - S_H9). The S_H1, S_H2, S_H4 and S_H5 genes have been identified in Ethiopian accessions of *C. arabica*. The S_H3 gene is derived from *C. liberica*, while S_H6, S_H7, S_H8 and S_H9, and others not yet identified biologically, come from *C. canephora* (Rodrigues et al. 1975; Bettencourt 1981; Várzea and Marques 2005). Flor's hypothesis (1971) is applicable to the interaction between the coffee and CLR, postulating a complementary virulence gene (*vr* gene) in the pathogen for each host-resistance gene (Noronha-Wagner and Bettencourt 1967). More than 50 physiological races of CLR have already been identified and spread throughout all coffee growing areas. CLR race II is the most common and widespread in Brazilian coffee plantations (Talhinhas et al. 2017; Zambolim and Caixeta 2021).

A spontaneous hybrid between *C. canephora* and *C. arabica*, designated as Híbrido de Timor (HdT), became a valuable material for breeding programs, facilitating the integration of CLR resistance genes from *C. canephora* into *C. arabica* (Avelino et al. 2015). The main sources of resistance used by Brazilian and other American countries in genetic breeding programs are HdT CIFC 832/1 and HdT CIFC 832/2. The first was crossed with Caturra Vermelho CIFC 19/1 giving rise to the cultivars of the Catimor group, while the second was crossed with Villa Sarchi CIFC 971/10 giving rise to the cultivars of the Sarchimor group. Those crossing were performed by the Coffee Rust Research Center (Centro de Investigação das Ferrugens do Cafeeiro, CIFC) and selected resistant plants were provided to all coffee-growing countries (Munõz-Pajares et al. 2023). HdT derivative is the main source of rust resistance in coffee breeding programs worldwide. Among the Sarchimor cultivars developed in Brazil, IAPAR 59 stands out for remaining highly resistant to CLR for more than three decades, since its commercial launch (Del Grossi et al. 2013; Sera et al. 2022).

Substantial efforts have been dedicated to unraveling the molecular mechanisms governing genetic resistance to CLR and to applying this knowledge in breeding (Diola 2009; Diola et al. 2011, 2013; Pestana et al. 2015; Florez et al. 2017; Barka et al. 2020; Almeida et al. 2021; Angelo et al. 2023; Silva et al. 2023). Sequence Characterized Amplified Region (SCAR) markers were used to order eight clones from a Bacterial Artificial Chromosome (BAC) library of HdT CIFC 832/2, located near a CLR race II resistance locus (Diola 2009; Cação et al. 2013). Two of these clones were pinpointed as overlapping the resistance gene in the physical map. Employing a transcriptomic approach, genes exhibiting increased expression during the incompatible interaction between HdT UFV 427−15 and CLR race II were identified (Diola et al., 2009; Diola et al. 2013). Notably, among these upregulated genes, one was predicted to encode an NBS-LRR protein and was referred to as *Ca TDF77 NBS-LRR* in the studies.

The genes belonging to the NBS-LRR class are the most representative among those that encode resistance proteins (Jones et al. 2016). NBS-LRR proteins are polymorphic intracellular receptors that intercept pathogen effectors (avirulence proteins – *avr*) and induce a robust resistance called effector-triggered immunity (ETI). ETI inhibits pathogen growth and is often associated with localized plant cell death, referred to as the hypersensitivity response (HR) (Cui et al. 2015). According to their N-terminal domain, NBS-LRR proteins can be classified as coiled-coil type (CC), TOLL/ INTERLEUKIN-1 RECEPTOR/RESISTANCE PROTEIN (TIR) type, and RPW8 type (CCR) (Jones et al. 2016).

The detailed knowledge of a gene linked to a trait of interest allows the implementation of biotechnological tools for the development of elite genotypes. Characterization of genes can enable the definition of targets for gene editing and/or genetic transformation (Jiang et al. 2019; Nagy et al. 2021). Moreover, from genomic characterization, functional polymorphisms between contrasting genotypes for the trait can be identified and used for the development of molecular markers (Salgotra and Stewart 2020). Another approach involves the genomic characterization of orthologs, which facilitates studies on evolutionary relationships (Ratnaparkhe et al. 2011; Santos et al. 2022).

Therefore, the objectives of this study encompassed: (1) characterizing a genomic locus implicated in CLR resistance in *Coffea* spp.; (2) identifying allelic divergence between the *Ca TDF77 NBS-LRR* gene from HdT and from *C. arabica* cultivar susceptible to CLR race II; and (3) validating the activity of the *Ca TDF77 NBS-LRR* gene in the defense response to CLR in *C. arabica* cv. IAPAR 59.

Materials and methods

Characterization of genomic regions

To identify the specific genomic locus hosting the *Ca TDF77 NBS-LRR* gene within *C. arabica*, a homology search was conducted. The gene's unique primers, as described by Diola (2009), were employed to scan the *C. arabica* cv. Caturra Vermelho genome available on the NCBI platform for public access (GCA_003713225.1). In order to identify the orthologous genomic region in *C. canephora* and *C. eugenioides*, ancestral species of *C. arabica*, the search for

homology using the specific primers for *Ca TDF77 NBS-LRR* gene was also carried out in *C. canephora* genome accessed on the Coffee Genome Hub (http://coffee-genome.org) (Denoeud et al. 2014), and in *C. eugenioides* genome available on the NCBI (GCA_003713205.1). Homology searches were conducted directly within the genome databases, utilizing the BLASTn tool.

Genomic regions of approximately 21 kilobase pairs (kbp) of *C. arabica* cv. Caturra Vermelho, *C. canephora*, and *C. eugenioides*, surrounding genomic positions homologous to the pair of primers targeting *Ca TDF77 NBS-LRR*, were submitted to the NCBI domain platform (Lu et al. 2020). This methodological approach facilitated the precise delineation of the *Ca TDF77 NBS-LRR* gene location within the *C. arabica* genome and its corresponding orthologs in *C. canephora* and *C. eugenioides*.

Predictive analyses of the secondary structures of Ca TDF77 NBS-LRR protein and its orthologous proteins were conducted using the web-based program SMART - Simple Modular Architecture Research Tool (http://smart.embl.de) (Letunic and Bork 2018).

Clone BAC HdT library sequencing

The clone BAC HdT CIFC 832/2, namely 14F3, identified to overlap the locus of resistance to CLR race II (Diola 2009), was sequenced employing Illumina HiSeq 2000 technology (100 base pairs, paired-end sequences), at the Carolina Center for Genome Sciences, University of North Carolina.

Illumina raw reads from clone BAC HdT CIFC 832/2–14F3 were trimmed using Trimmomatic v0.36 (Bolger et al. 2014) with parameters: sliding window: 4:25; leading: 25; trailing: 25; minlen: 50. The FastQC v0.11.5 software (www.bioinformatics.babraham.ac.uk/projects/fastqc/) was used to evaluate the quality of reads before and after trimming. Trimmed results were mapped to the *C. canephora* v1.0 reference genome using HISAT2 (Denoeud et al. 2014; Kim et al. 2019). Insert sizes were analyzed using the Picard InsertSizeMetrics function (http://broadinstitute.github.io/picard/javadoc/picard/analysis/InsertSizeMetrics.html). The reads with average insert size of 250 base pairs (bp) were assembled using SOAPdenovo2 (Luo et al. 2012). Sequence statistics of the assembly were calculated using a Perl script FastaSeqStats (https://github.com/aubombarely/GenoToolBox/blob/master/SeqTools/FastaSeqStats). Contigs more than 500 bp and with 61 K-mer were used to assemble scaffolds.

Using the sequence of primers specific to the *Ca TDF77 NBS-LRR* gene, a homology search was conducted within the scaffolds assembly of HdT CIFC 832/2. For this, we used BioEdit software (http://www.mbio.ncsu.edu/BioEdit/bioedit.html) (Hall 1999) with BLASTn tool local alignment.

Table 1 *Coffea arabica* cultivars used in gene expression assays, its respective S_H genes and resistance responses to *Hemileia vastatrix* race II

C. arabica cultivar	Resistance genes	Interaction with *H. vastatrix* race II
IAPAR 59	S_H5, S_H6, S_H7, S_H8, S_H9, $S_H?$	resistant
Catuaí Vermelho IAC 99	S_H5	susceptible

Polymorphism identification

Aiming to identify potential polymorphisms linked to the *Ca TDF77 NBS-LRR* gene functionality, an alignment was performed between *C. arabica* cv. Caturra Vermelho (S_H5, susceptible to CLR race II) and HdT CIFC 832/2 (S_H5, S_H6, S_H7, S_H8, S_H9, $S_H?$, resistant to CLR race II). For this, the *C. arabica* cv. Caturra Vermelho genome and HdT CIFC 832/2 scaffolds database were used. Alignments were performed using the BioEdit software and BLASTn tool for local alignment. BLASTn parameters were calibrated with an expected cutoff value of 0.1 and 250 alignment descriptions.

Gene expression assays

To validate the activity of *Ca TDF77 NBS-LRR* gene in *C. arabica* cv. IAPAR 59, infection assays were carried out using CLR race II (*vr*5), following the methodology described by Eskes and Toma-Braghini (1982). For comparative analysis, *C. arabica* cv. Catuaí Vermelho IAC 99, known for its susceptibility, was utilized as control. The S_H genes identified in these cultivars were detailed in Table 1.

Following inoculation, the leaf discs were transferred to Gerbox® acrylic boxes, and kept in the absence of light for 24 h. These boxes contained a foam layer (0.8 cm) with 30 mL of water, maintained at 22 ± 3 ºC. To assess spore viability, three drops of the uredospore solution were dispensed onto a microscope slide and left for 5 h without exposure to light. The resulting solution was then examined under a light microscope to verify the germination of *H. vastatrix* uredospores with the presence of haustorium.

Biological triplicates of leaf disc samples were collected at 0, 24, 48, and 72 h after inoculation (hai). RNA extraction followed the protocol described by Chang et al. (1993). Subsequently, cDNAs were synthesized using 2.5 µg of RNA and the reverse transcriptase enzyme (SuperScript® III First-Strand Synthesis SuperMix kit -Invitrogen), following the manufacturer's instructions.

The pair of primers specific to the *Ca TDF77 NBS-LRR* gene was employed to generate amplicons of 80 bp at an annealing temperature of 60 °C (Table 2). For data

normalization, *GAPDH* gene transcripts were used (Table 2) (Barsalobres-Cavallari et al. 2009).

The transcriptional profile of the genes was assessed using qPCR (7500 Fast Real-Time PCR System, Applied Biosystems) with the following components: 12.5 µL of 2x SYBR Green/ROX qPCR Master Mix - Applied Biosystems, 0.5 µL of each primer (10 µM), 10.5 µL of water, and 1 µL of cDNA (20 ng).

Reactions were prepared in technical triplicates, employing the following thermocycling parameters: an initial denaturation at 95 ºC for 10 min, followed by 40 amplification cycles at 95 ºC for 30 s and 60 ºC for 60 s. To ensure the specificity of the amplification products, dissociation curves were analyzed.

Relative gene expression was evaluated through ΔΔCt = ΔCt (sample) – ΔCt (normalizer), using GenEx software (MultiD Analyzes AB, Göteborg, Sweden). The transcriptional pattern at 0 h for each cultivar was used as the calibrator to compare the transcriptional patterns at different hai. Standard deviation calculations were also performed using the GenEX software. Statistical analysis was carried out employing analysis of variance (ANOVA) and the Tukey test, both at a significance level of 5%.

Samples of inoculated leaf discs were monitored for up to 21 days after inoculation (dai) to assess phenotypes and inoculum efficiency.

Results

Clone BAC HdT library sequencing

The sequencing of reads from clone BAC HdT CIFC 832/2–14F3 yielded an assembly of 493 scaffolds, varying in size from 501 bp to 92,164 bp. One specific scaffold (2902) from clone BAC HdT CIFC 832/2–14F3 exhibited complementarity with the *Ca TDF77 NBS-LRR* primers (Supplementary Fig. 1). This outcome establishes that the locus associated with resistance to CLR, initially identified through SCAR markers on a physical map by Diola (2009), indeed corresponds to the *Ca TDF77 NBS-LRR* gene.

Genomic characterization and polymorphism identification

The *Ca TDF77 NBS-LRR* primers exhibited complementarity with chromosome (chr) 11 of the canephora subgenome (sg) and chr 5 of the eugenioides sg within *C. arabica* cv. Caturra Vermelho. In the *C. canephora* genome, complementarity was observed on chr 11, whereas in the *C. eugenioides* genome, it was detected on chr 5 and chr 11. The specific base pair positions of the genomic regions complementary to the *Ca TDF77 NBS-LRR* primers are illustrated in Supplementary Fig. 1.

Upon subjecting the genomic sequences surrounding the region complementary to the *Ca TDF77 NBS-LRR* primers to domain identification analysis, intriguing findings surfaced. For instance, on chr 11 of *C. canephora*, *C. eugenioides*, and on chr 11 (canephora sg) of *C. arabica* cv. Caturra, tandem TIR-NBS-LRR genes were observed (Fig. 1). Additionally, on chr 5 of *C. eugenioides* and chr 5 (eugenioides sg) of *C. arabica* cv. Caturra, the analysis revealed the presence of the NB-ARC domain alongside other domains associated with nucleic acid binding functions, such as zinc-binding in reverse transcriptase (zf-RTV) and DUF4283. Transposable elements (TEs), including retrotransposon gag protein (RT-gag), retropepsin_like, and BED zinc finger (zf-BED) were also identified on chr 5 of *C. eugenioides* and chr 5 (eugenioides sg) of *C. arabica* cv. Caturra.

The precise positions of these genomic regions within each chromosome investigated in this study were detailed in Fig. 1. Specifically, the *Ca TDF77 NBS-LRR* gene was located between 33,482,226 bp and 33,491,120 bp on chr 11 (canephora sg) of *C. arabica* cv. Caturra. Orthologs to the *Ca TDF77 NBS-LRR* gene were identified between 30,628,644 bp and 30,637,615 bp on chr 11 of *C. canephora*, and between 44,304,326 bp and 44,313,218 bp on chr 11 of *C. eugenioides*.

Upon alignment the Caturra genome and the HdT scaffolds database, 12 scaffolds from clone BAC HdT − 14F3 displayed significant similarity (Table 3). Among these scaffolds, only three (2902, 2612, and 2552) showed polymorphisms compared to Caturra (Table 3; Fig. 1).

HdT scaffolds 2902, and 2552 were complementary to Caturra genomic regions that completely cover the NB-ARC and TIR domains of the rust resistance gene, respectively. Furthermore, HdT scaffold 2902 was complementary to a small part of the LRR domain. HdT scaffold 2612 was complementary to a genomic region between the NB-ARC and TIR domains (Fig. 1).

The alignment between the HdT scaffold 2902 and Caturra showed 98% nucleotide identity, revealing 24 single-nucleotide polymorphisms (SNPs) and six insertions and deletions (InDels). HdT scaffold 2612 exhibited 94% nucleotide identity, involving 30 SNPs and 25 InDels. HdT

Table 2 Primer sequences used in qPCR

Target	Forward/Reverse (5'- 3')	Reference
Ca TDF77 NBS-LRR	ATCAGTTGGTAAAACTGCCG/CTAGCTGGCTCGAGAGAATG	Diola (2009)
GAPDH	GGCTGGAAACCCCTTCATTT/TGAAAGCAATATGCACAGTTGGA	Barsalobres-Cavallari et al. (2009)

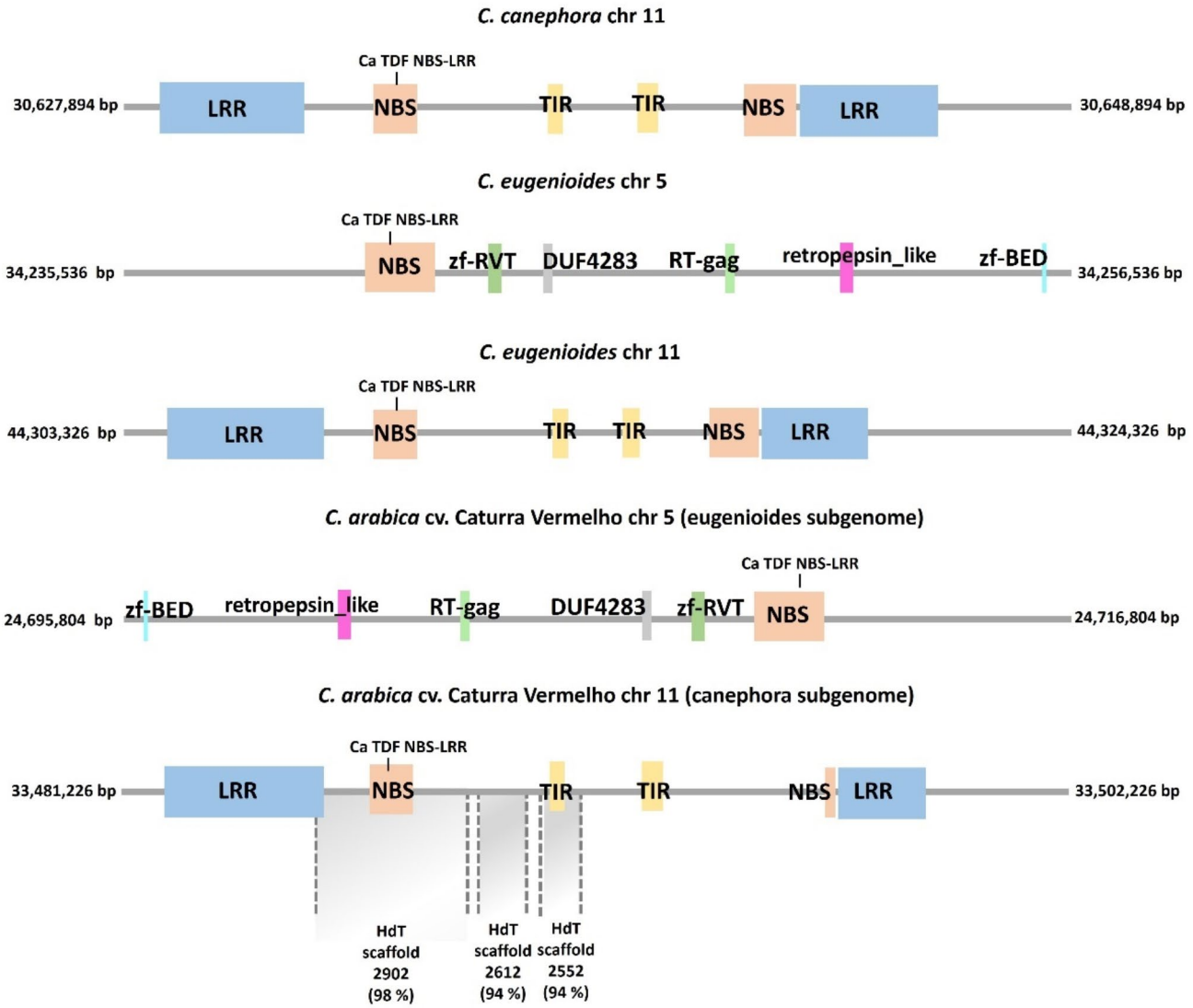

Fig. 1 Schematic representation of genomic regions surrounding the sequences complementary to *Ca TDF77 NBS-LRR* primers in *C. canephora*, *C. eugenioides*, and *C. arabica* cv. Caturra Vermelho. The designation 'Ca TDF77 NBS-LRR' in this figure denotes the homologous position with primers specific to a *Hemileia vastatrix* race II resistance gene, identified in a transcriptome study (Diola 2009; Diola et al. 2013). Dotted lines in the figure delineate polymorphic genomic regions between *C. arabica* cv. Caturra Vermelho and HdT CIFC 832/2. Each polymorphic genomic region between Caturra and HdT CIFC 832/2 is detailed, showing the corresponding HdT scaffold and the percentage of nucleotide identity

scaffold 2552 displayed 94% nucleotide identity, with 21 SNPs and 27 InDels.

Proteins structure

The *Ca TDF77 NBS-LRR* gene, identified on chr 11 (canephora sg) of *C. arabica* cv. Caturra, and its ortholog discovered on chr 11 of *C. canephora*, were found to have predicted protein sequences comprising 1,301 amino acids (aa) and 1,225 aa, respectively. Both *C. arabica* and *C. canephora* exhibited proteins with well-defined TIR-NBS-LRR domains, as depicted in Fig. 2. Similarly, the orthologous protein structure identified on chr 11 of *C. eugenioides* displayed TIR-NBS-LRR domains. However, in *C. eugenioides*, an observed premature peptide truncation disrupted the structure into two segments: a sequence consisting of 755 aa containing the TIR and NB-ARC domains, and another segment of 627 aa containing the LRR domain (Fig. 2).

Ca TDF77 NBS-LRR gene activity in response to CLR

During the absence of the pathogen (0 hai), the *Ca TDF77 NBS-LRR* gene exhibited similar transcript levels in both

Table 3 Scaffolds of clone BAC HdT CIFC 832/2–14F3 that showed significant similarity compared to the *Coffea arabica* cv., Caturra Vermelho genome

HdT CIFC 832/2	lenght (pb)	Bits score	aligment e-value	identities	genomic region (bp) on chr 11 (canephora subgenome) of *C. arabica* Caturra
scaffold 3000	6,721	1,198	0	100%	33,481,226–33,482,424
scaffold 2712	1,428	1,428	0	100%	33,482,415–33,483,842
scaffold 2758	1,700	1,700	0	100%	33,483,830–33,485,529
scaffold 2902	3,593	3,414	0	98%	33,485,469–33,489,065
scaffold 2612	1,084	833	0	94%	33,489,151–33,490,209
scaffold 2552	923	727	0	94%	33,490,522–33,491,442
scaffold 2678	1,307	1,306	0	100%	33,492,005–33,493,310
scaffold 2512	856	856	0	100%	33,493,370–33,494,225
scaffold 2316	520	520	0	100%	33,494,445–33,494,964
scaffold 2978	5,528	5,528	0	100%	33,494,959–33,500,486
scaffold 2664	1,246	1,246	0	100%	33,500,479–33,501,724
scaffold 2404	626	428	0	100%	33,501,800–33,502,227

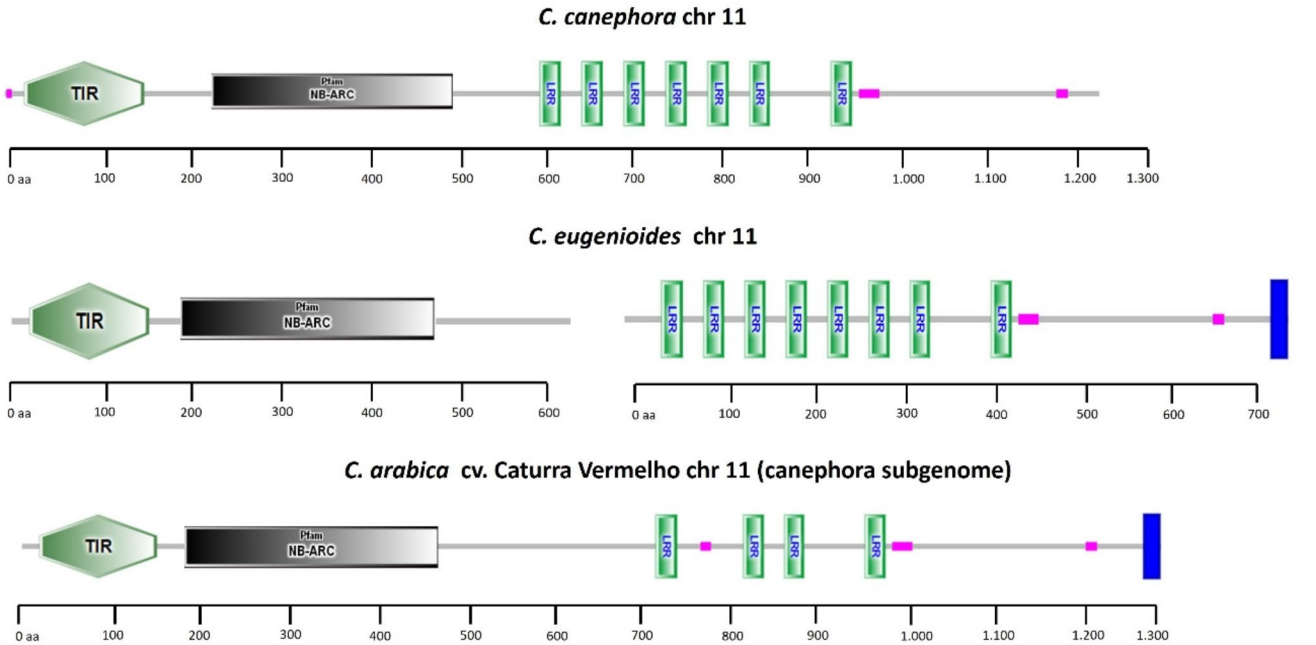

Fig. 2 TIR-NBS-LRR proteins encoded by the *Ca TDF77 NBS-LRR* gene in *Coffea arabica* cv. Caturra Vermelho and its orthologs in *C. canephora* and *C. eugenioides*. Peptide domain prediction showed the presence of TIR, NB-ARC, and LRR domains in all three species. A premature peptide truncation was identified in *C. eugenioides*. In the visual representation, purple regions denote low-complexity proteins, while blue regions represent transmembrane structures, both identified using the web-based program SMART - Simple Modular Architecture Research Tool (http://smart.embl.de)

IAPAR 59 and Catuaí Vermelho (Fig. 3a). However, following inoculation, the pathogen triggered distinct responses in these cultivars. At 24 hai, IAPAR 59 maintained transcriptional levels similar to those observed at 0 h, whereas Catuaí Vermelho displayed down-regulation. Both cultivars showed up-regulation at 48 hai, although the up-regulated transcriptional profile persisted only in IAPAR 59 at 72 hai (Fig. 3a).

Catuaí Vermelho samples showed the presence of spores at 21 dai, indicating the complete reproductive cycle and compatible interaction with CLR race II. IAPAR 59 samples showed no symptoms (Fig. 3b).

Discussion

Comparative genomic study

The findings of this work indicate that the TIR-NBS- LRR tandem structure were present in the common ancestor of *C. canephora* and *C. eugenioides.* This tandem structure

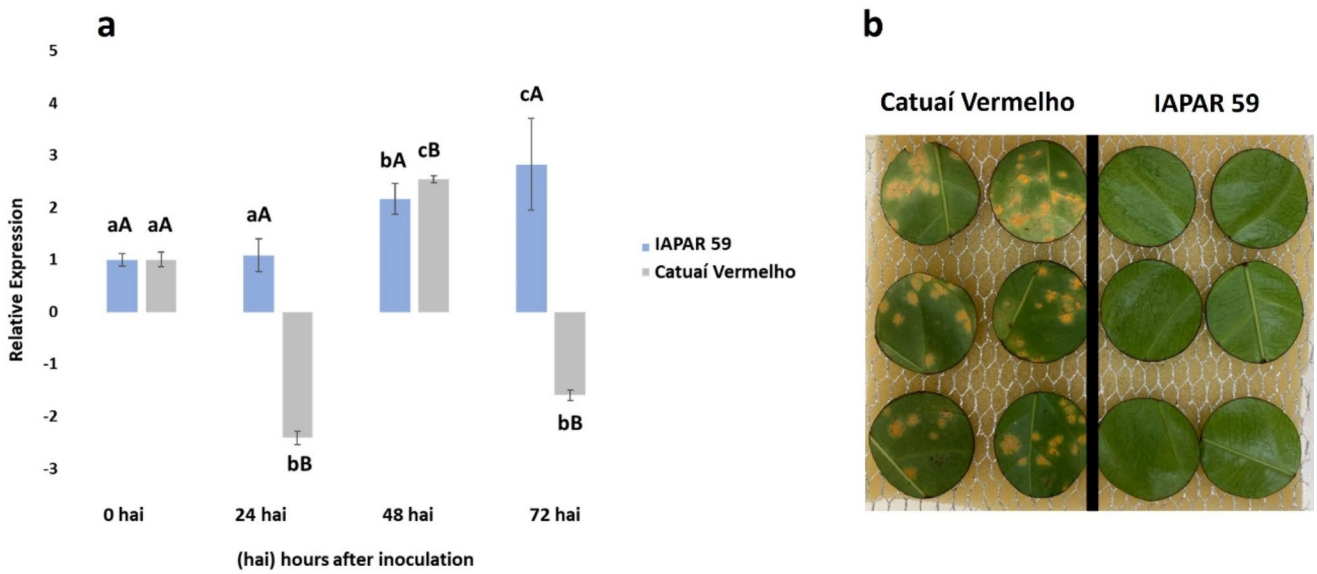

Fig. 3 **(a)** Transcriptional profile of the *Ca TDF77 NBS-LRR* gene during the interaction between coffee plants and *Hemileia vastatrix* race II at 0, 24, 48, and 72 h after inoculation (hai). The values represent the mean ± standard deviation ($n = 3$). Lowercase letters denote comparisons within the same cultivar at different times post-inoculation, while uppercase letters compare different cultivars at the same time post-inoculation. Different letters indicate statistical significance between groups, determined by Tukey's test at a 5% probability level. **(b)** shows the phenotypic response to CLR race II in coffee cultivars observed 21 days after inoculation

has been maintained in *C. arabica*, an interspecific hybrid of *C. canephora* and *C. eugenioides* (Fig. 1). In order to detect hundreds of pathogens and pests, NBS-LRRs receptors must be capable of responding to various elicitors. This is possible due to the diversity of resistance genes in plants (Shao et al. 2016). The majority of NBS-LRRs are grouped in tandem duplicates. This repetitive genomic structure allows new paralog generation through rearrangements between the duplicates (Andersen et al. 2020). These rearrangements are recognized as primary sources of new resistance genes that can recognize new pathogen effectors (Ratnaparkhe et al. 2011).

Results for the Ca TDF77 NBS-LRR protein secondary structure prediction in *C. arabica* cv. Caturra, *C. canephora*, and *C. eugenioides* indicated extreme allelic divergence between orthologs, mainly within the LRR domain (Fig. 2). This suggests an evolutionary trajectory for this genomic region that may contribute to functional innovation in NBS-LRR proteins. Another aspect to consider regarding the evolutionary dynamics of the *Ca TDF77 NBS-LRR* gene is the presence of transposable elements (TEs) identified on chr 5 of *C. eugenioides* and chr 5 (eugenioides sg) of *C. arabica* cv. Caturra. TEs can influence regulatory networks and produce genetic variation, potentially promoting mechanisms for genome evolution and adaptation through rapid phenotypic variation (Zhang et al. 2021; Springer et al. 2018; Niu et al. 2019). Furthermore, recombination events can occur between highly homologous regions scattered by related TEs at distant genomic positions, resulting in large-scale deletions, duplications, and inversions (Bennetzen and Wang 2014).

The homeologous counterpart to the *Ca TDF77 NBS-LRR* gene on chr 11 of the eugenioides subgenome in *C. arabica* cv. Caturra Vermelho was not identified. Correspondence was also not identified for the other NBS-LRR tandemly aligned with the *Ca TDF77 NBS-LRR* gene. However, the absence of these homeologous counterparts may be related to the quality of the genome assembly. Therefore, further investigations are necessary to confirm this loss of homeologous regions.

Genomic characterization and potential biotechnological tools

Polymorphisms identified between the *Ca TDF77 NBS-LRR* gene of Caturra Vermelho and HdT CIFC 832/2, particularly those inserted in the NB-ARC and TIR domains, might impact gene functionality and efficiency in pathogen detection and activation of the defense mechanism. The NBS central domain consists of strictly ordered conserved motifs crucial for ATP and GTP binding and hydrolysis, leading to conformational modification and consequently shifting the protein state from 'OFF' to 'ON' (Bernoux et al. 2016).

Alterations within specific residues in NB-ARC domain motifs can compromise protein function, triggering self-activation or disrupting the regulation/initiation of defense

mechanisms. The TIR and CC domains might also contribute to pathogen recognition and subsequent signaling for plant defense response (Maekawa et al. 2011; Williams et al. 2014). Therefore, these polymorphisms hold potential for developing functional molecular markers (FMs) in coffee. FMs play a crucial role in gene pyramiding programs and marker-assisted selection (MAS), enhancing accuracy in identifying target plants while reducing time and costs.

Another biotechnological approach involving the characterization of the *Ca TDF77 NBS-LRR* gene position in the *C. arabica* genome is its cloning for genetic transformation events. In Ma et al. (2015), Agrobacterium-mediated transformation introduced the *Pi64* gene, an NBS-LRR, into rice blast-susceptible *Oryza sativa* cultivars. The authors demonstrated that introgressing the *Pi64* gene into susceptible cultivars conferred a high level of resistance to the disease, showcasing its potential in breeding programs.

Additionally, the genomic region responsible for resistance to CLR in *C. arabica*, identified in this study, consists of two TIR-NBS-LRR genes in tandem, presenting the potential for approaches aimed at creating new rearrangements through gene editing. Using CRISPR/Cas9 target-specific endonucleases, Nagy et al. (2021) cleaved disease resistance locus composed of tandem clusters in soy. Subsequently, natural DNA repair mechanisms facilitated new rearrangements, leading to new NBS-LRR variants.

Ca TDF77 NBS-LRR gene activity in response to CLR

One of the most remarkable adaptations of rust fungi is the haustorium, a specialized infection structure facilitating biotrophic association with hosts. This structure develops after penetrating a living host cell wall, enabling the pathogen to extract nutrients and secrete effector proteins that manipulate the physiological and immune responses of host cells (Garnica et al. 2014; Lorrain et al. 2019). Cytological studies on the interaction between hosts and rust fungi have consistently shown that plant resistance, controlled by resistance genes, is typically expressed after the initial haustorium formation, often triggering a hypersensitive response (HR) (Mellersh and Heath 2003). For *C. arabica* and CLR interaction, both pre-haustorial and post-haustorial resistance have been identified. Pre-haustorial resistance is probably associated with PAMP-triggered immunity (PTI) (Silva et al. 2002, 2008; Florez et al. 2017; Castro et al. 2022).

In a cytological study of incompatible interaction between HdT CIFC 832/2 and CLR race II, haustorium formation was observed starting at 48 hai (Diniz et al. 2012). Therefore, the up-regulation of the *Ca TDF77 NBS-LRR* gene at 48 and 72 hai in *C. arabica* cv. IAPAR 59, which carries HdT CIFC 832/2 introgression, corroborates with a post-haustorial defense mechanism. Another cytological study of the incompatible interaction between *C. arabica* S4 Agaro (S_H4, S_H5) and CLR race II showed the death of subsidiary and mesophilic cells invaded by haustorium from 72 hai (Silva et al. 2008). Consequently, the highest transcript levels of the *Ca TDF77 NBS-LRR* gene in the resistant cultivar IAPAR 59 at 72 hai might correlate with the gene's involvement in an effective plant defense response and control of pathogen proliferation. In susceptible coffee plants, the death of guard cells and adjacent cells was observed from the third day after inoculation, but only in a small percentage of infection sites where the fungus ceased its growth early (Silva et al. 2002). The decrease in *Ca TDF77 NBS-LRR* expression in Catuaí Vermelho at 72 hai might be linked to a deficiency in controlling infection in adjacent cells.

The formation of haustorial mother cells in the incompatible interaction between *C. arabica* and CLR was observed 24 h after it was seen in the compatible interaction (Silva et al. 2002). This demonstrates that the timing of haustorium formation depended on the plant genotype. Therefore, based on the hypothesis that haustorium formation in IAPAR 59 occurs more slowly than in Catuaí Vermelho, the repression of the *Ca TDF77 NBS-LRR* gene in Catuaí Vermelho at 24 hai might also indicate plant susceptibility in post-haustorial responses.

Overall, these results highlight the role of the *Ca TDF77 NBS-LRR* gene in the defense mechanism against CLR in *C. arabica* cultivar with HdT CIFC 832/2 introgression.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s13313-024-00988-2.

Acknowledgements We acknowledge the Consórcio Pesquisa Café, the Instituto Nacional de Ciência e Tecnologia do Café (INCT Café), the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), the Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG), and the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for financial support and fellowships.

Funding This research was funded by Consórcio Pesquisa Café, grant number 10.18.20.027.00, INCT Café and FAPEMIG. C.A received a PhD fellowship from CAPES (Finance Code 001). L.F.P.P. received a Research Fellowship from CNPq (311452/2020-5).

Data availability Illumina raw reads and scaffolds assembled from clone BAC HdT CIFC 832/2–14F3 are available on request from the authors.

Declarations

Ethical approval Not applicable. This article does not contain any studies with human participants or animals performed by any of the authors.

Conflict of interest The authors have no conflict of interest to declare that are relevant to this article.

References

Andersen EJ, Nepal MP, Purintun JM, Nelson D, Mermigka G, Sarris PF (2020) Wheat disease resistance genes and their diversification through integrated domain fusions. Front Genet 11. https://doi.org/10.3389/fgene.2020.00898

Angelo PCS, Sera GH, Shigueoka LH, Caixeta ET (2023) Rust resistance *SH3* loci in *Coffea* spp. Physiol Mol Plant Pathol 127:102111. https://doi.org/10.1016/j.pmpp.2023.102111

Avelino J, Cristancho M, Georgiou S, Imbach P, Aguilar L, Bornemann G, Läderach P, Anzueto F, Hruska AJ, Morales C (2015) The coffee rust crises in Colombia and Central America (2008–2013): impacts, plausible causes and proposed solutions. Food Secur 7:303–321. https://doi.org/10.1007/s12571-015-0446-9

Barka GD, Caixeta ET, Ferreira SS, Zambolim L (2020) *In silico* guided structural and functional analysis of genes with potential involvement in resistance to coffee leaf rust: a functional marker based approach. PLoS ONE 15(7):e0222747. https://doi.org/10.1371/journal.pone.0222747

Barsalobres-Cavallari CF, Severino FE, Maluf MP, Maia IG (2009) Identification of suitable internal control genes for expression studies in *Coffea arabica* under different experimental conditions. BMC Mol Biol 10:1. https://doi.org/10.1186/1471-2199-10-1

Bennetzen JL, Wang H (2014) The contributions of transposable elements to the structure, function, and evolution of plant genomes. Annu Rev Plant Biol 65:505–530. https://doi.org/10.1146/annurev-arplant-050213-035811

Bernoux M, Burdett H, Williams SJ, Zhang X, Chen C, Newell K, Lawrence GJ, Kobe B, Ellis JG, Anderson PA, Dodds PN (2016) Comparative analysis of the flax immune receptors L6 and L7 suggests an equilibrium-based switch activation model. Plant Cell 28(1):146–159. https://doi.org/10.1105/tpc.15.00303

Bertrand B, Vaast P, Alpizar E, Etienne H, Davrieux F, Charmetant P (2006) Comparison of bean biochemical composition and beverage quality of arabica hybrids involving sudanese-ethiopian origins with traditional varieties at various elevations in central America. Tree Physiol 26:1239–1248. https://doi.org/10.1093/treephys/26.9.1239

Bettencourt AJ (1981) Melhoramento genético do cafeeiro: transferência de factores de resistência à *Hemileia vastatrix* Berk. Br. para as principais cultivares de *Coffea arabica* L. Ultramar/CIFC, Lisbon

Bolger AM, Lohse M, Usadel B (2014) Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 30:2114–2120. https://doi.org/10.1093/bioinformatics/btu170

Cação SM, Silva NV, Domingues DS, Vieira LG, Diniz LE, Vinecky F, Alves GS, Andrade AC, Carpentieri-Pipolo V, Pereira LF (2013) Construction and characterization of a BAC library from the *Coffea arabica* genotype Timor Hybrid CIFC 832/2. Genetica 141(4–6):217–226. https://doi.org/10.1007/s10709-013-9720-y

Castro ISL, Freitas-Lopes RL, Ferreira SS, Maciel TEF, Florez JC, Zambolim EM, Zambolim L, Caixeta ET (2022) Transcriptome analysis uncovers the gene expression profile of *Hemileia vastatrix* (race XXXIII) during the interactions with resistant and susceptible coffee. *Agronomy* 12(2):444. https://doi.org/10.3390/agronomy12020444

Chang S, Puryear J, Cairney J (1993) A simple and efficient method for isolating RNA from pine trees. Plant Mol Biol 11:113–116. https://doi.org/10.1007/BF02670468

Clarindo WR, Carvalho CR (2008) First *Coffea arabica* karyogram showing that this species is a true allotetraploid. Plant Syst Evol 274:237–241. https://doi.org/10.1007/s00606-008-0050-y

Cristancho MA, Rozo Y, Escobar C, Rivillas CA, Gaitán AL (2012) Outbreak of coffee leaf rust (*Hemileia vastatrix*) in Colombia. New Dis Rep 25:19–19. https://doi.org/10.5197/j.2044-0588.2012.025.019

Cui H, Tsuda K, Parker JE (2015) Effector-triggered immunity: from pathogen perception to robust defense. Annu Rev Plant Biol 66:487–511. https://doi.org/10.1146/annurev-arplant-050213-040012

De Almeida DP, Castro ISL, Mendes TAO, Alves DR, Barka GD, Barreiros PRRM, Zambolim L, Sakiyama NS, Caixeta ET (2021) Receptor-like Kinase (RLK) as a candidate gene conferring resistance to *Hemileia vastatrix* in coffee. Sci Agric 78(6). https://doi.org/10.1590/1678-992X-2020-0023

Del Grossi L, Sera T, Sera GH, Fonseca ICDB, Ito DS, Shigueoka LH, Carvalho FG (2013) Rust resistance in arabic coffee cultivars in northern Paraná. Braz Arch Biol Technol 56:27–33. https://doi.org/10.1590/S1516-89132013000100004

Denoeud F, Carretero-Paulet L, Dereeper A, Droc G, Guyot R, Pietrella M, Zheng C, Alberti A, Anthony F, Aprea G et al (2014) The coffee genome provides insight into the convergent evolution of caffeine biosynthesis. Science 345:1181–1184. https://doi.org/10.1126/science.1255274

Diniz I, Talhinhas P, Azinheira HG, Várzea V, Medeira C, Maia I, Petitot AS, Nicole M, Fernandez D, Silva MC (2012) Cellular and molecular analyses of coffee resistance to *Hemileia vastatrix* and nonhost resistance to *Uromyces vignae* in the resistance-donor genotype HDT832/2. Eur J Plant Pathol 133:141–157. https://doi.org/10.1007/s10658-011-9925-9

Diola V (2009) Resistência à ferrugem do cafeeiro: mapeamento genético, físico e análise da expressão gênica em resposta a infecção de *H. vastatrix*. Ph.D. Thesis, Universidade Federal de Viçosa

Diola V, de Brito GG, Caixeta ET, Maciel-Zambolim E, Sakiyama NS, Loureiro ME (2011) High-density genetic mapping for coffee leaf rust resistance. Tree Genet Genomes 7:1199–1208. https://doi.org/10.1007/s11295-011-0406-2

Diola V, Brito GG, Caixeta ET, Pereira LF, Loureiro ME (2013) A new set of differentially expressed signaling genes is early expressed in coffee leaf rust race II incompatible interaction. Funct Integr Genomics 13(3):379–389. https://doi.org/10.1007/s10142-013-0330-7

Eskes AB, Toma-Braghini M (1982) Assessment methods for resistance to coffee leaf rust (*Hemileia vastatrix* Berk. and Br). Plant Protoc Bull FAO (Rome) 29:56–66

FAO - Food and Agriculture Organization of the United Nations (2022) Markets and trade/coffee. Commodity in focus. https://www.fao.org/markets-and-trade/commodities/coffee. Accessed 21 November 2022

Flor HH (1971) Current status of the gene-for-gene concept. Annu Rev Phytopathol 9:275–296

Florez JC, Mofatto LS, Freitas-Lopes doL, Ferreira R, Zambolim SS, Carazzolle EM, Zambolim MF, Caixeta L ET (2017) High throughput transcriptome analysis of coffee reveals prehaustorial resistance in response to *Hemileia vastatrix* infection. Plant Mol Biol 95(6):607–623. https://doi.org/10.1007/s11103-017-0676-7

Garnica DP, Nemri A, Upadhyaya NM, Rathjen JP, Dodds PN (2014) The ins and outs of rust haustoria. PLoS Pathog 10(9):e1004329. https://doi.org/10.1371/journal.ppat.1004329

Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp Ser 41:95–98

ICO - International Coffee Organization (2023) Coffee market report. www.ico.org/documents/cy2022-23/cmr-0123-e.pdf. Accessed 08 February 2023

Jiang H, Li K, Gai J (2019) *Agrobacterium rhizogenes*-induced soybean hairy roots versus *soybean mosaic virus* (ARISHR-SMV) is an efficient pathosystem for studying soybean–virus interactions. Plant Methods 15:56. https://doi.org/10.1186/s13007-019-0442-8

Jones JDG, Vance RE, Dangl JL (2016) Intracellular innate immune surveillance devices in plants and animals. Science 354:1117–1126. https://doi.org/10.1126/science.aaf6395

Keith LM, Sugiyama LS, Brill E, Adams BL, Fukada M, Hoffman KM, Ocenar J, Kawabata A, Kong AT, McKemy JM, Olmedo-Velarde A, Melzer MJ (2022) First report of coffee leaf rust caused by *Hemileia vastatrix* on coffee (*Coffea arabica*) in Hawaii. Plant Dis 106:2761. https://doi.org/10.1094/PDIS-05-21-1072-PDN

Kim D, Paggi JM, Park C, Bennett C, Salzberg SL (2019) Graph-based genome alignment and genotyping with HISAT2 and HISAT-genotype. Nat Biotechnol 37:907–915. https://doi.org/10.1038/s41587-019-0201-4

Letunic I, Bork P (2018) 20 years of the SMART protein domain annotation resource. Nucleic Acids Res 46:493–449. https://doi.org/10.1093/nar/gkx922

Lorrain C, Gonçalves dos Santos KC, Germain H, Hecker A, Duplessis S (2019) Advances in understanding obligate biotrophy in rust fungi. New Phytol 222:1190–1206. https://doi.org/10.1111/nph.15641

Lu S, Wang J, Chitsaz F, Derbyshire MK, Geer RC, Gonzales NR, Gwadz M, Hurwitz DI, Marchler GH, Song JS et al (2020) CDD/SPARCLE: the conserved domain database in 2020. Nucleic Acids Res 48(D1):265–268. https://doi.org/10.1093/nar/gkz991

Luo R, Liu B, Xie Y, Li Z, Huang W, Yuan J, He G, Chen Y, Pan Q, Liu Y et al (2012) SOAPdenovo2: an empirically improved memory-efficient short-read de novo assembler. Gigascience 1(1):18. https://doi.org/10.1186/2047-217X-1-18

Ma J, Lei C, Xu X, Hao K, Wang J, Cheng Z, Ma X, Ma J, Zhou K, Zhang X et al (2015) *Pi64*, encoding a novel CC-NBS-LRR protein, confers resistance to leaf and neck blast in rice. Mol Plant Microbe Interact 28(5):558–568. https://doi.org/10.1094/MPMI-11-14-0367-R

Maekawa T, Cheng W, Spiridon LN, Töller A, Lukasik E, Saijo Y, Liu P, Shen QH, Micluta MA et al (2011) Coiled-coil domain-dependent homodimerization of intracellular barley immune receptors defines a minimal functional module for triggering cell death. Cell Host Microbe 9(3):187–199. https://doi.org/10.1016/j.chom.2011.02.008

Mellersh DG, Heath MC (2003) An investigation into the involvement of defense signaling pathways in components of the non-host resistance of *Arabidopsis thaliana* to rust fungi also reveals a model system for studying rust fungal compatibility. Mol Plant Microbe Interact 16(5):398–404. https://doi.org/10.1094/MPMI.2003.16.5.398

Muñoz-Pajares AJ, Várzea V, Silva MC (2023) The story of coffee: legend and truth. Trends Plant Sci 28(5):501–504. https://doi.org/10.1016/j.tplants.2023.02.012

Nagy ED, Stevens JL, Yu N, Hubmeier CS, Lafaver N, Gillespie M, Gardunia B, Cheng Q, Johnson S, Vaughn AL, Vega ME, Deng M, Rymarquis L, Lawrence RJ, Garvey GS, Gaeta RT (2021) Novel disease resistance gene paralogs created by CRISPR/Cas9 in soy. Plant Cell Rep 40(6):1047–1058. https://doi.org/10.1007/s00299-021-02678-5

Niu XM, Xu YC, Li ZW, Bian YT, Hou XH, Chen JF, Zou YP, Jiang J, Wu Q, Ge S et al (2019) Transposable elements drive rapid phenotypic variation in *Capsella rubella*. PNAS USA 116:6908–6913. https://doi.org/10.1073/pnas.1811498116

Noronha-Wagner M, Bettencourt AJ (1967) Genetic study of resistance of *Coffea* spp. to leaf rust: I. Identification and behaviour of four factors conditioning disease reaction in *Coffea arabica* to twelve physiologic races of *Hemileia vastatrix*. Canad J Bot 45:2021–2031

Pestana KN, Capucho AS, Caixeta ET, de Almeida DP, Zambolim EM, Cruz CD, Zambolim L, Pereira AA, de Oliveira ACB, Sakiyama NS (2015) Inheritance study and linkage mapping of resistance loci to *Hemileia vastatrix* in Híbrido De Timor UFV 443-03. Tree Genet Genomes 11:72. https://doi.org/10.1007/s11295-015-0903-9

Ratnaparkhe MB, Wang X, Li J, Compton RO, Rainville LK, Lemke C, Kim C, Tang H, Paterson AH (2011) Comparative analysis of peanut NBS-LRR gene clusters suggests evolutionary innovation among duplicated domains and erosion of gene microsynteny. New Phytol 192(1):164–178. https://doi.org/10.1111/j.1469-8137.2011.03800.x

Rodrigues-Junior CJ, Bettencourt AJ, Rijo L (1975) Races of the pathogen and resistance to coffee rust. Annu Rev Phytopathol 13:49–70

Salgotra RK, Stewart CN Jr (2020) Functional markers for precision plant breeding. Int J Mol Sci 21(13):4792. https://doi.org/10.3390/ijms21134792

Santos ML, Resende MLV, Alves GSC, Huguet-Tapia JC, Resende MFRJr, Brawner JT (2022) Genome-wide identification, characterization, and comparative analysis of NLR resistance genes in *Coffea* spp. Front Plant Sci 13:868581. https://doi.org/10.3389/fpls.2022.868581

Sera GH, de Carvalho CHS, de Rezende Abrahão JC, Pozza EA, Matiello JB, de Almeida SR, Bartelega L, dos Botelho S, DM (2022) Coffee leaf rust in Brazil: historical events, current situation, and control measures. Agronomy 12:496. https://doi.org/10.3390/agronomy12020496

Shao ZQ, Xue JY, Wu P, Zhang YM, Wu Y, Hang YY et al (2016) Large-scale analyses of angiosperm nucleotide-binding site-leucine-rich repeat genes reveal three anciently diverged classes with distinct evolutionary patterns. Plant Physiol 170:2095–2109. https://doi.org/10.1104/pp.15.01487

Silva MC, Nicole M, Guerra-Guimarães L, Rodrigues CJ (2002) Hypersensitive cell death and post-haustorial defence responses arrest the orange rust (*Hemileia vastatrix*) growth in resistant coffee leaves. Physiol Mol Plant Pathol 60:169–183. https://doi.org/10.1006/pmpp.2002.0389

Silva MC, Guerra-Guimarães L, Loureiro A, Nicole MR (2008) Involvement of peroxidases in the coffee resistance to orange rust (*Hemileia vastatrix*). Physiol Mol Plant Pathol 72:29–38. https://doi.org/10.1016/j.pmpp.2008.04.004

Silva AG, Ariyoshi C, Shigueoka LH, Pereira LFP, Sera GH (2023) Assisted selection using molecular markers linked to rust resistance *SH3* gene in *Coffea arabica*. Crop Breed Appl Biotechnol 23(4):e445323413. https://doi.org/10.1590/1984-70332023v23n4a48

Springer NM, Anderson SN, Andorf CM, Ahern KR, Bai F, Barad O, Barbazuk WB, Bass HW, Baruch K (2018) The maize W22 genome provides a foundation for functional genomics and transposon biology. Nat Genet 50:1282–1288. https://doi.org/10.1038/s41588-018-0158-0

Talhinhas P, Batista D, Diniz I, Vieira A, Silva DN, Loureiro A, Tavares S, Pereira AP, Azinheira HG, Guerra-Guimarães L, Várzea V, Silva MDC (2017) The coffee leaf rust pathogen *Hemileia vastatrix*: one and a half centuries around the tropics. Mol Plant Pathol 18(8):1039–1051. https://doi.org/10.1111/mpp.12512

Várzea VMP, Marques DV (2005) Population variability of *Hemileia vastatrix* vs. coffee durable resistance. In: Zambolim L, Zambolim EM, Várzea VMP (eds) Durable resistance to coffee leaf rust. Eds. UFV, Visoça, pp 53–74

Williams SJ, Sohn KH, Wan L, Bernoux M, Sarris PF, Segonzac C, Ve T, Ma Y, Saucet SB, Ericsson DJ et al (2014) Structural basis for assembly and function of a heterodimeric plant immune receptor. Science 344(6181):299–304. https://doi.org/10.1126/science.1247357

Zambolim L (2016) Current status and management of coffee leaf rust in Brazil. Trop Plant Pathol 41:1–8. https://doi.org/10.1007/s40858-016-0065-9

Zambolim L, Caixeta ET (2021) An overview of physiological specialization of coffee leaf rust – new designation of pathotypes. Int J Curr Res 13:15564–15575. https://doi.org/10.24941/ijcr.40600.01.2021

Zhang Y, Li Z, Zhang Y, Lin K, Peng Y, Ye L, Zhuang Y, Wang M, Xie Y, Guo J, Teng W et al (2021) Evolutionary rewiring of the wheat transcriptional regulatory network by lineage-specific transposable elements. Genome Res 31(12):2276–2289. https://doi.org/10.1101/gr.275658.121