Chapter 4
Current Challenges and Genomic Advances Toward the Development of Coffee Genotypes Resistant to Biotic Stress

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Abstract Coffee (*Coffea* spp.) is an important agricultural world commodity. Biotic stresses caused by pests or phytopathogens can affect not only the coffee production, but also the grain quality. They interfere with physiological processes affecting plant growth and development, and damaging different plant organs and tissues, such as leaves, roots, and fruits. The use of chemicals to control diseases and pests directly

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affects coffee cultivation sustainability and are often inefficient. This scenario could worsen with the rapid insurgence of new and/or more aggressive pathogens and pests as a result of the world climate change. Thus, coffee breeding with a focus on the development of resistant cultivars is the best strategy to control these biotic stresses. In this context, biotechnological tools can help the coffee breeding in a persuasive way. Advances in genomic editing techniques, such as CRISPR, are capable of introducing punctual modifications in the plant genome. As an alternative to the use of chemicals, the sequence-specific gene silencing via RNA interference (RNAi) holds a great promise for effective management of agricultural pests. The emergence of high-throughput sequencing technology has allowed unprecedented advances in genomic and transcriptomic data. The genomic and transcriptomic coffee data can now be used to identify a large number of genes and molecular markers that determine coffee resistance to pathogens and pests. The identification of these genes helps to elucidate plant pathogen interactions, as well as can be targets for genome editing. The implementation of molecular markers, through assisted selection, can help accelerate breeding programs and pyrimidize resistance genes. In addition, the genomic and transcriptomic data of pathogens and pests are useful to identified targets for RNAi approaches. In this review, we address the research in modern genetics and molecular biology related to the main biotic stresses of coffee plants and its implications for coffee breeding.

Keywords *Coffea canephora* · *Coffea arabica* · Pathogens · Transcriptome · Molecular marker · Transgenic · Genome editing · RNA interference

4.1 Introduction

Coffee (*Coffea* spp.) is an important tropical agricultural commodity for the economy of several tropical countries, with a total revenue in 2014 of US$173 million in more than 80 countries (ICO 2019). Coffee also has an important socioeconomic role because it is related to the livelihood of more than 25 million farmers (Dumont 2019), in addition to the participation of more than 125 million workers in its laborious

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production chain (Dumont et al. 2019). Brazil, Vietnam, and Colombia are the world's largest producers and exporters, responsible for more than 60% of both.

The genus *Coffea* belongs to the Rubiaceae family, with more than 140 described species (Davis et al. 2011; Guyot et al. 2020); however, only *Coffea arabica* L. (arabica) and *Coffea canephora* Pierre ex A. Froehner (robusta) are considered commercially important because they represent approximately 60% and 40% of the global market, respectively (USDA 2020). In addition to these species, two other species are cultivated on a small scale to meet the local market demand: *Coffea liberica* and *Coffea racemosa* (Krishnan et al. 2015). Coffee is one of the most consumed beverages in the world, and its consumption has increased by 160% in the last 30 years. Therefore, it is necessary to develop new strategies and technologies to promote productivity increases of sustainable coffee plantations (FAO 2015; ICO 2019).

It is estimated that the reduction in agricultural productivity associated with damage caused by pests and phytopathogens (fungi, bacteria, viruses, and nematodes) is 20–30% in the main crops (Savary et al. 2019). Furthermore, global climatic changes can also increase disease or insect pest outbreaks, as well as expand these problems to areas where they were not previously prevalent (Dubberstein et al. 2018; Ziska et al. 2018). In coffee trees, infestation by pests and the presence of diseases are among the main factors that affect productivity and grain quality (Ventura et al. 2017). Biotic stress impairs physiological processes (photosynthesis, absorption, and translocation of water and nutrients), plant development, and damages different plant organs and tissues, such as leaves, roots, and fruits (Esgario et al. 2020).

Several pathogens that affect coffee production have been identified in different regions worldwide (Maghuly et al. 2020). The most important are coffee leaf rust (CLR), coffee berry disease (CBD), bacterial halo blight (BHB), coffee leaf miner (CLM), coffee berry borer (CBB), and nematodes. Farmer's control of these biotic stresses relies mainly on chemicals that can increase production costs, adversely affect human health, and cause environmental problems (Zambolim 2016; Talhinhas et al. 2017). Furthermore, the use of chemicals in some cases may have low effectiveness, as evidenced mainly in the management of nematodes and CBB (Avelino et al. 2018). In this context, the development and use of coffee cultivars resistant to pests and diseases is the best alternative.

Coffee breeding programs frequently focus on the development and selection of resistant genotypes as protagonists of an integrated management program for the main biotic stresses (Silva et al. 2018). However, coffee breeding programs are laborious, with long-term results (25–30 years). To meet the growing producer, consumer, and market demand, a complex, continuous, and dynamic breeding process is required, resulting in costly long projects for the development of superior cultivars. Therefore, the implementation of statistical and biotechnology tools can help to reduce the time and effort required to produce new coffee cultivars and consequently assist in the urgent need for chemical reduction (Hindorf and Omondi 2011; Kumar et al. 2016; Oliveira et al. 2021).

From this perspective, researchers have been seeking molecular markers linked to genes or lociassociated with coffee resistance (Pestana et al. 2015; Ariyoshi et al.

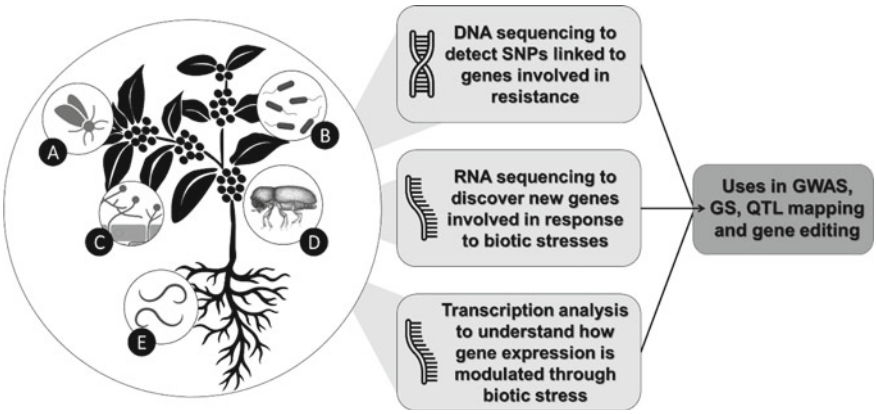

Fig. 4.1 Main biotic stresses that affect coffee trees and major research approaches for genetic improvement. (A) Coffee leaf miner (CLM); (B) bacterial halo blight (BHB); (C) coffee leaf rust (CLR) and coffee berry disease (CBD); (D) coffee berry borer (CBB); and (E) nematodes. GWAS: genome wide association studies; GS: genome selection; QTL: quantitative trait locus

2019; Gimase et al. 2020), elucidating the genes involved in plant defense mechanisms (Florez et al. 2017; Castro-Moretti et al. 2020), and determining the genes related to pathogen and pest effectiveness (Porto et al. 2019). These markers and genes can be incorporated in breeding programs that help breeders select resistant coffee plants, or these traits can be incorporated by genetic engineering and/or genome editing in elite coffee cultivars (Fig. 4.1).

In this review, we have focused on research that has used modern genetics and molecular biology related to the main biotic stresses of coffee plants and their implications for coffee breeding. We have discussed the problems encountered and the prospects for using genomic technologies, with the goal of developing new strategies to combat the main pests and pathogens in coffee farming.

4.2 Genomic Analyses for Major Biotic Stresses in Coffee

The implementation of resistant cultivars is considered the most effective and sustainable methodology for disease and pest management (Naidoo et al. 2019). Thus, genomic resource availability is a key factor in achieving this goal. Genomic DNA data allow researchers to access important information to design coffee breeding program strategies, such as the use of molecular markers for plant selection (Sant'ana et al. 2018; Gimase et al. 2020).

For successful breeding programs, screening for disease-resistant genotypes must be reliable and efficient. Molecular markers linked to resistance loci in coffee genotypes are advantageous for morphological evaluations (Alkimim et al. 2017). Molecular DNA markers are not affected by environmental effects, allow the selection of

resistant individuals in the absence of the pathogen, and can be applied at any stage of plant development. Additionally, molecular markers can effectively aid in the identification of genotypes with multiple resistance factors when there is a dominant or epistatic effect. This is useful for assisting the pyramidization of resistance genes, which is a promising strategy in breeding programs (Alkimim et al. 2017).

In the last few decades, remarkable advances in DNA sequencing technologies have emerged with the advent of next-generation sequencing (NGS) (de Filippis 2017). The cost of sequencing 1 million base pairs (1 Mb) of DNA was reduced from US$1000 in 2004 to US$0.008 in 2020 (NIH 2020). In addition to the evolution of sequencing technology, the equipment to store this volume of data and bioinformatics tools to conduct analyses have also evolved during the same period (de Filippis 2017). Currently, there are data available in several public databases for coffee genomes, pathogens, and pests (Table 4.1).

Table 4.1 High-throughput genome sequencing studies with coffee species and pathogens-pest related

Specie	Study	Bioproject (NCBI)	Reference—Institute
C. arabica Caturra	Genome	PRJNA506972	John Hopkins
C. arabica Red Bourbon	Genome	PRJNA554647	Scalabrin et al. (2020)
C. arabica Geisha	Genome	NA*	UC Davis—Phytozome
C. arabica Et39	Genome	PRJNA698600	ACGC (2014)
C. eugenioides CCC68	Genome	PRJNA508372	John Hopkins
C. eugenioides	Genome	PRJNA698600	ACGC (2014)
C. canephora DH 200-94	Genome	PRJEB4211	Denoeud et al. (2014)
C. canephora BUD15	Genome	PRJNA698600	ACGC (2014)
C. Arabica	Diversity	PRJEB9368	Lashermes et al. (2016)
C. Arabica	Diversity	PRJEB26929	Gimase et al. (2020)
C. Arabica	Diversity	PRJNA401643	Texas A&M
Coffea spp.	Diversity	PRJNA698600	ACGC (2014)
Coffea spp.	Diversity	PRJNA612193	Bawin et al. (2020)
Coffea spp.	Diversity	PRJNA401643	Texas A&M
Coffea spp.	Diversity	PRJNA352624	CRIN
Coffea spp.	Diversity	PRJNA242989	IRD
Coffea spp.	Diversity	PRJNA505204	Huang et al. (2020)
C. canephora	Metagenome	PRJNA526486	UNICAMP
Hypothenemus hampei	Genome	PRJNA279497	Vega et al. (2015)
Hypothenemus hampei	Genome	PRJNA626647	Navarro-Escalante et al. (2021)
Hemileia vastatrix	Genome	PRJNA419278	Porto et al. (2019)
Colletotrichum kahawae	Diversity	PRJEB26929	CICF

* NA: Not available

Genome sequencing provides not only a handful of genes, transposons, and noncoding RNA information (Lemos et al. 2020) but is also the starting point for modern breeding approaches, such as genomewide association studies (GWAS) and genome selection (GS) (Gimase et al. 2020). GWAS have emerged together with NGS technology as powerful tools for identifying molecular markers associated with agronomic traits of interest. GWAS can overcome the limitations of traditional genetic linkage mapping, including maps with little refinement and limited diversity to parents (Bartoli and Roux 2017). In addition to identifying molecular markers to be implemented in molecular marker-assisted selection (MAS), GWAS also allow the identification of gene linkages to the associated markers, enabling the elucidation of the molecular mechanisms of plant defense against pathogens (Chagné et al. 2019; Zhang et al. 2020).

GWAS can explore the genetic diversity found in wild crop relatives. This is extremely important in *C. arabica.* The recent origin of a single hybridization event and its predominantly autogamous reproduction, associated with the limited dispersion of plants worldwide, created a huge bottleneck in cultivar development (Setotaw et al. 2013; Merot-L'anthoense et al. 2019; Scalabrin et al. 2020). The *C. arabica* collections of wild crop relatives, such as from the survey organized by the Food and Agriculture Organization (FAO) in 1964–1965 in Ethiopia (FAO 1968), are essential for the exploration of their genetic variability and for application of modern breeding approaches. Based on the wild germplasm of *C. arabica*, several characterization and evaluation studies have been conducted to identify sources of resistance, such as against the nematodes *Meloidogyne paranaensis*, *M. incognita*, and *M. exigua* (Anzueto et al. 2001; Fatobene et al. 2017), BHB (Mohan et al. 1978), and CBD (van der Vossen and Walyaro 1980). All these agronomic traits have the potential to be used in GWAS to identify markers and genes related to plant defense.

4.3 Transcriptome Studies for Major Biotic Stresses in Coffee

Transcriptomic resource availability and genomic resources are key factors in the design of coffee breeding strategies (Noriega et al. 2019). The study of the transcriptome profile of genes during the plant-pathogen interaction is one of the main molecular approaches to elucidating the biological processes during the infection process.

Once pathogens are able to overcome mechanical defense barriers, the plant has receptors capable of recognizing the pathogen and activating signaling pathways that drive the expression of defense response genes (Andersen et al. 2018). At the first level of recognition, non-mutable molecules of the pathogen, known as pathogen-associated molecular patterns (PAMPs), can be detected by host proteins of the

pattern recognition receptors (PRRs). This first level of pathogen recognition activates PAMP-triggered immunity (PTI), which can be suppressed by pathogen effectors. The second level of recognition involves resistance proteins (R) capable of detecting effectors and activating effector-triggered immunity (ETI). Generally, ETI is accompanied by cell death, known as the hypersensitive response (HR) (Zipfel and Rathjen 2008). Through the loss or diversification of effectors, pathogens can successfully suppress ETI and colonize the host. The coevolution of pathogens and plants, and notably their repertoire of effectors and R proteins, led to the so-called zigzag model (Jones and Dangl 2006). Among the PRRs and R proteins, the classes with the greatest representativeness are the receptor-like kinases leucine-rich repeats (RLKs-LRRs), and the nucleotide binding sites leucine-rich repeats (NBSs-LRRs), respectively (Coll et al. 2011). Although ETI has greater magnitude and duration, defense signaling through PTI and ETI can activate similar downstream molecular events, such as mitogen-activated protein kinase (MAPK) activation, oxidative bursts, ion influxes, increased biosynthesis of plant defense hormones, and transcriptional regulation of defense genes (Tao et al. 2003; Navarro et al. 2004).

Transcriptome data can provide specific information about the class of plant genes (pathogen recognition, signaling, or defense response) and the time course that is being activated or suppressed, an important issue in plant pathogen interactions. Moreover, transcriptome studies have identified pathogen genes, such as effectors, that are active in the infection process. Therefore, expression profiling of hosts and pathogens can provide a new understanding of their interactions and allow the identification of virulence genes in the pathogen and defense pathways in host cells (Boyd et al. 2013; Florez et al. 2017). Despite the research on transcriptome of plant-pathogen interaction in coffee plants that had been developed (Table 4.2), the reduction in the cost of sequencing technologies will allow for a greater number of studies in this area, with a major advance in understanding the mechanisms of resistance to the main pests and pathogens of coffee trees.

Table 4.2 Transcriptomic sequencing data of *Coffea* spp.—pathogens interaction

Specie	Pathogen	Technique	Bioproject (NCBI)	Reference
Ck	CBD	Illumina (HiSeq2000)	PRJNA271934	NP
Hh	CBB	EST	NA	Idarraga et al. (2011)
Hh	CBB	Illumina (HiSeq2500)	SUB4491034	Noriega et al. (2019)
Hv	CLR	454 pyrosequencing	PRJNA188788	Cristancho et al. (2014)
Hv	CLR	454 pyrosequencing	NA	Fernandez et al. (2012)
Hv	CLR	Illumina (MiSeq)	NA	Florez et al. (2017)
Lc	CLM	EST	NA	Cardoso et al. (2014)

CBB: Coffee berry borer; CBD: coffee berry disease; Ck: *Colletotrichum kahawae*; CLM: coffee leaf miner; CLR: coffee leaf rust; Hh: *Hypothenemus hampei*; Hv: *Hemileia vastatrix*; Lc: *Leucoptera coffeella*; NA: not available; NP: not published.

4.4 Genetic Transformation, RNAi, and Genome Editing for Biotic Stress in Coffee

Despite the tremendous success in producing new varieties, the improvement in coffee and acquisition of a new cultivar based on traditional methodologies is a process that requires approximately 20–30 years (Melese 2016). It includes several steps, such as the selection of species or varieties to be used, their subsequent hybridization, and evaluation of the resulting progeny. In some cases, backcrosses and interspecific crosses are conducted (Orozco and Schieder 1982).

Although classical breeding is one of the main and most widely used strategies for obtaining improved cultivars, there are limitations in regard to the characteristics that can be added because most species of the genus *Coffea* are self-incompatible (Davis et al. 2006). Additionally, different ploidy of the allotetraploid *C. arabica* in relation to other *Coffea* species hinders the introduction of agronomic characteristics present in the *Coffea* genetic pool. Therefore, it is important to use techniques that can break the barriers between species, complement traditional genetic breeding programs, and the available genetic base.

Since the first commercial use of transgenic plants in 1996, genetic transformation has become an essential tool for the genetic improvement of crops (James 2018). Genetic transformation technology is considered an extension of conventional breeding technologies (Zhong 2001) and offers unique opportunities to overcome compatibility barriers between species and thus can develop phenotypes with desired traits that are not available in the germplasm of crop plants (Mishra and Slater 2012).

The main objectives of genetic improvement through the use of genetic engineering techniques in coffee are to introduce new traits into elite genotypes, develop new cultivars with desirable characteristics, such as resistance to pests and diseases, resistance to herbicides, tolerance to drought and frost, and increased cup quality (Mishra and Slater 2012). Additionally, genetic transformation can also be used as a tool for the functional validation of coffee genes and promoters (Brandalise et al. 2009; Cacas et al. 2011; Mishra and Slater 2012; Girotto et al. 2019).

The first report of genomic transformation in coffee was made by Spiral and Petiard (1991), wherein genomic transformation was mediated by the bacterium *Agrobacterium tumefaciens*. Later, Barton et al. (1991) used the indirect genomic editing methodology of electroporation, and van Boxtel et al. (1995) used particle bombardment, with successful regeneration of transformed explants with both methodologies. Transformation techniques are more efficient in *C. canephora* than in *C. arabica* and are associated with the difficulty in inducing embryogenic tissues and regeneration of explants in the latter (Kumar et al. 2006). Despite the constraints of *C. arabica* genetic transformation, successful transformation rates of up to 90% have been achieved with specific tissue culture conditions, such as a high auxin-to-cytokinin ratio associated with the use of specific callus phenotypes (Ribas et al. 2011).

Gene silencing using RNA interference (RNAi) strategies is an important tool for the control of biotic stresses (Cagliari et al. 2019). RNAi or post-transcriptional gene silencing (PTGS) is a mechanism in which small double-stranded RNA (dsRNA) regulate gene expression. Small pieces of RNA, called micro-RNA (miRNA) or small interfering RNA (siRNA), can shut down protein translation by binding to the messenger RNAs that code for these proteins. This was first described in *Caenorhabditis elegans,* but it is present in all eukaryotic organisms and has become a popular tool in functional genomics. RNAi technology can be used to control biotic stress with genetically modified plants with target mi or siRNA. Plants controlling western corn rootworm (*Diabrotica virgifera*) with RNAi have been commercially approved for use in Canada and USA. RNAi technology can also be used with non-transgenic methods, such as dsRNA sprayed products and could substitute chemical pesticides in the future.

Genomic editing by clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein 9 (Cas9) has been successfully used as a tool for the elimination of genes of agronomic interest, paving the way for improvement of coffee cultivars (Breitler et al. 2018). This technique uses guide RNA (gRNA), which indicates that the Cas9 enzyme is a target region in the genome. This target region suffers a double break in the DNA strands, which can lead to non-homologous recombination, thereby causing gene silencing or homologous recombination. Therefore, it is possible to change or cleave a specific gene and/or insert a new gene (Ran et al. 2013; Sander and Joung 2014). With the availability of genome sequences of *C. canephora* and *C. arabica*, transformation and genome editing via CRISPR/Cas9 became possible.

A web program called "CRIP" (Coffee gRNA Identification Program) was developed (Breitler et al. 2018), which allows the identification of all target sequences of single guide RNA (sgRNA) in the genome of *C. canephora*, with the advantage of identifying non-target sequences for each leader sequence. This program was tested by choosing regions of three exons for genome editing of a phytoene desaturase (CcPDS), which produces an easily visible albino phenotype when mutated. For one exon, genome editing was observed in 30.2% of the plants regenerated, with a total of nine different mutations, whereas no mutants were obtained for the other exons. None of the plants showed a complete albino phenotype, but a range of phenotypic mutants were attained that included plants with small, lanceolate leaves with abnormal pigmentation (yellow, chlorotic), shorter internodes, and missing leaves, or in some cases, in greater numbers than normal (Breitler et al. 2018). Thus, genome editing using CRISPR-Cas9 has been shown to be an efficient and reliable way to inactivate genes of agronomic interest in *C. canephora.*

Despite the high potential of genetic engineering in the development of resistant *Coffea* cultivars, molecular information regarding the defense mechanisms against biotic stresses is still limited. Additionally, one of the obstacles encountered in the development of genetically modified organisms (GMOs) in coffee growth is consumer approval and acceptance, which could represent an obstacle for coffee commercialization. Despite the use of commercial GMOs for more than 25 years in other species, marketing strategies should be taken into consideration to ensure the

public understanding of genetically modified (GM) coffee. With proper information regarding the benefits and possible risks of GMOs, these improved crops could be progressively incorporated into the market. However, CRISPR and other genome editing technologies, called new plant breeding technologies (NPBTs) (Wolt et al. 2016), have been classified as non-GMOs in several coffee-producing countries, as well as in the main importing and consuming countries. Plants produced by NBTs can bypass the legal regulations imposed on GMOs, saving time and costs related to regulatory approval. Hence, genome editing could become an alternative for the development of novel coffee varieties that are resistant to different biotic and abiotic factors, thereby increasing production and cup quality.

4.5 Breeding for Biotic Stress Resistance in Coffee: Some Case Studies

4.5.1 Coffee Leaf Rust (CLR)

Hemileia vastatrix Berk. et Br, the causative agent of CLR, is one of the major threats to coffee production in almost every coffee-producing region. The pathogen is a biotrophic fungus that attacks only plants in the genus *Coffea.* When not controlled through application of fungicides, the disease can reduce productivity by more than 50%, both in *C. arabica* and *C. canephora* (Zambolim 2016; Zambolim and Caixeta 2021). The disease can be managed by integrating several measures, but the use of resistant cultivars is the best alternative because of farmer sustainability and environmental concerns (Sanders 2019).

To assist in cultivar development, some resistant sources for CLR have already been identified. At least nine dominant genes (S_H1 to S_H9) present in different coffee species have been characterized to date. S_H1, S_H2, S_H4, and S_H5 genes were identified in *C. arabica,* S_H3 in *C. liberica*, and S_H6, S_H7, S_H8, and S_H9 in *C. canephora* (Noronha-Wagner and Bettencourt 1967; Rodrigues et al. 1975). Other major and minor genes have been reported in interspecific hybrids (Varzea and Marques 2005).

Although resistance sources are available, obtaining a cultivar with durable resistance is a challenge for breeders because of the variability in *H. vastatrix*. More than 50 physiological races of *H. vastatrix* have been identified worldwide, which makes it difficult to manage disease resistance (Zambolim 2016; Zambolim and Caixeta 2021). The high adaptive potential of the pathogen, the emergence of new physiological races, and a corresponding breakdown of resistance has been observed in many coffee cultivars (Varzea and Marques 2005; Capucho et al. 2012). Therefore, breeding programs are an important strategy for disease control.

Among the resistance sources, the Timor Hybrid (HdT) germplasm stands out because it is composed of genotypes with substantial genetic variability, with different genes for resistance to CLR and other diseases, such as CBD, root knot nematode (*Meloidgyne exigua*), and bacteriosis (*Pseudomonas syringae* pv. *garcae*). HdT is a

natural hybrid between the species *C. canephora* and *C. arabica* (Bettencourt 1973), presenting the resistance provided by *C. canephora* and the sensory characteristics of arabica, which are widely used in breeding programs (van der Vossen 2009; Setotaw et al. 2020).

The CLR-resistant cultivars derived from HdT germplasm have been planted in Latin America and East Africa and were first developed by the Coffee Rusts Research Center (CIFC, Centro de Investigação das Ferrugens do Cafeeiro) in Portugal. Since 1955, CIFC has received and characterized coffee and rust germplasm and supplied breeding programs (Talhinhas et al. 2017). CIFC developed the hybrids HW26 (Caturra Vermelho × HdT CIFC 832/1 -Catimor), H46 (Caturra Vermelho × HdT CIFC 832/2-Catimor), H361 (Villa Sarchi × HdT CIFC 832/2-Sarchimor), H528 (CatuaíAmarelo × HW26/13), and H529 (CaturraAmarelo × H361/3) (Silva et al. 2006). Some selected F_1 and F_2 plants with resistance to all known races were spread to many institutions in coffee-growing countries and were incorporated into their breeding programs.

Other natural interspecific hybrids used as CLR resistance sources are Indian selections (*C. arabica* × *C. liberica)* S.288, S.333, S.353 4/5, and Series BA. This germplasm carries the resistance gene S_H3 (Alkimim et al. 2017; Silva et al. 2019). HdT and Indian selections, which are tetraploid materials, are used to facilitate introgression resistance genes from diploid species (*C. canephora* and *C. liberica*) in the tetraploid species *C. arabica.* The accumulation of different genes in a cultivar allows the production of coffee with more durable resistance compared to that of cultivars with a single race-specific resistance gene.

Molecular markers associated with these genes have been identified to assist in the introgression of CLR resistance genes. Molecular markers are an important tool for improving the efficiency of selection in coffee breeding programs (Sousa et al. 2019), especially when different genes are accumulated. In breeding programs, a segregating coffee population inoculated with *H. vastatrix* races can be easily screened for resistance. However, it is difficult to distinguish between plants with one or more resistance genes because of epistatic effects. Because molecular markers show no such epistatic effects, MAS can be an efficient alternative (Pestana et al. 2015). MAS also allows the identification of resistance in the absence of pathogens.

Markers associated with CLR resistance genes in *C. liberica* and HdT have been identified. Prakash et al. (2004), using a segregating population with susceptible (Matari) and resistant (S. 288) parents for *H. vastatrix* race VIII (*Vr* 2,3,5), identified 21 amplified fragment length polymorphism (AFLP) markers linked to the S_H3 gene. Later, Mahé et al. (2008) developed sequence-characterized amplified region (SCAR) markers based on AFLP and a library of bacterial artificial chromosome (BAC) clones. The developed SCAR and three simple sequence repeat (SSR) markers were used to construct a linkage map containing the S_H3 resistance gene. The markers closest to the S_H3 gene (BA-48-21-f, Sat244, BA124-12 K-f, and Sp-M16-SH3) have been used in MAS (Alkimim et al. 2017).

Furthermore, molecular markers linked to HdT resistance genes were obtained from an F_2 population (HdT UFV 427-15 × CatuaíAmarelo) segregating to a gene that confers resistance to *H. vastatrix* race II (*Vr5*) (Diola et al. 2011). In this study, a

genetic map with 25 AFLP markers was obtained, which enabled the development of a high-density map with six SCAR markers closely linked to the CRL-resistant gene. SCAR markers delimited a chromosomal region of 9.45 cM, flanking the resistance gene by 0.7 and 0.9 cM.

Also, markers associated with HdT resistance genes were identified in other accessions. Four SSR markers were linked to the CRL-resistant quantitative trait locus (QTL) in the HdT-derived genotype DI.200 (Herrera et al. 2009; Romero et al. 2014). Phenotypic CLR resistance data were obtained in the field and from controlled inoculation using a mixture of uredospores collected in Caturra. The markers flanked the QTL in a region of 2.5 cM. This locus was aligned to chromosome 4 of the *C. canephora* reference genome.

Pestana et al. (2015) identified markers flanking the quantitative trait loci (QTLs) present in HdT UFV 443-03. A linkage map was constructed and indicated that this HdT has at least two independent dominant loci conferring resistance to race II of *H. vastatrix* and four independent dominant loci for resistance to race I and pathotype 001. Markers flanking these QTLs were identified based on the genetic map and used an artificial neural network statistical approach (Silva et al. 2017). Based on these studies, molecular markers flanking seven different loci of resistance to CLR have been identified. These markers have great potential to increase selection efficiency and allow the pyramidation of different resistance genes in new cultivars.

Coffee breeding programs with the goal of coffee resistance to CLR rely not only on molecular marker technology but also on structural and functional genomics to increase their efficiency and competitiveness. Studies of the transcriptional profile of the interaction between *H. vastatrix* and coffee allow the identification of pathogen virulence genes and host genes involved in the defense mechanism (specific and non-specific pathogens). The information obtained in these studies is useful in the identification of new genes to be incorporated into the cultivars, development of new markers, and incorporation of new directions into breeding programs. Additionally, they can be targets for genetic manipulation, such as for the constitutive activation of disease resistance signaling routes. Information regarding genes involved in pathogen infection and its interaction with coffee can also be used to identify new methods of disease control.

To identify *C. arabica* genes involved in a hypersensitive reaction (HR), Fernandez et al. (2004) analyzed expressed sequence tags (ESTs) obtained by suppression subtractive hybridization (SSH). HR is the most common expression of incompatibility interactions (resistance) and has been previously identified in the early stages of *H. vastatrix* infection (Silva et al. 2002). To differentiate the transcripts strictly involved in the defense response, two cDNA libraries from incompatible and compatible interactions were used in the SSH approach. Incompatible interactions were observed in the *C. arabica* Caturra (S_H5) inoculated with race VI and S4 Agaro (S_H4; S_H5) inoculated with *H. vastatrix* race II. Compatible interactions were performed with Caturra inoculated with race II and S4 Agaro with race XIV. The transcripts used to build a catalog of non-redundant ESTs represent genes with expression of early resistance mechanisms, because they were identified in coffee samples at 12, 24, and 48 h after inoculation (hai) with the fungus. Silva et al. (2002;

2008) used a microscopic approach to demonstrate that spore germination and development of the appressorium occurs 12–17 hai, hypha penetrates through the stomata at approximately 24 hai, and haustorial mother cells develop at 36–48 hai. Of the obtained ESTs, 13% may represent genes involved in plant defense reactions and 13% in signaling processes. The predicted proteins encoded by the ESTs had homologies with disease resistance proteins, stress- and defense-proteins, and components of cell signaling pathways. Using SSH, 28 genes were found to be involved in resistance mechanisms. Expression analysis of a subset of 13 candidate genes revealed some HR-upregulation with homology to proteins, such as chitinases, cytochrome P450, heat shock proteins (HSP), non-race-specific disease resistance (NDR1) protein, ionic channel (CNGC2/DND1) involved in the resistance to *Pseudomonas syringae*, kinases, and transcription factors.

In another transcriptome study using a differential analysis performed by combined cDNA-AFLP and bulk segregant analysis (BSA), coffee resistance gene candidates were identified in incompatible interactions (Diola et al. 2013). F_2 population plants (HdT UFV 427-15 × Catuaí Amarelo IAC 30) were inoculated with *H. vastatrix* race II, and leaves were collected at 48 and 72 hai. From these samples, 108 transcript-derived fragments (TDFs) were differentially expressed in resistant plants and sequenced. Among the sequenced TDFs, 20 and 22% were related to signaling and defense genes, respectively. Twenty-one genes were selected for validation using Quantitative reverse transcription PCR (RT-qPCR) at 0, 12, 24, 48, and 72 hai. The highest levels of transcription of the signaling and defense genes were observed at 24 and 72 hai, respectively. The *NBS-LRR*, *RGH1A*, *MEK*, *MAPK2*, *CDPK*, and *β-Zip* genes had the highest levels of transcript increase in the resistant genotypes in relation to susceptibility. Among the defense genes, a pathogen-related, *PR5*, was found. The results of this study reinforced the hypothesis that the transcription of signaling and defense genes have different time profiles and a greater transcription of signaling genes is capable of upregulating coffee defense genes (Diola et al. 2013).

The first transcriptome study using NGS technology was performed by Fernandez et al. (2012). In this study, data were collected 21 days after-inoculation (dai) with *H. vastatrix*, a stage when a large number of haustoria and hyphae were observed in infected *C. arabica* leaves. This allowed the identification of an exhaustive repertoire of genes expressed during the infection of both the plant (61% of the contigs) and the fungus (30%). Two proteins were identified as potential effectors active in the infection process: a flax rust haustorial expressed secreted protein (HESP) and bean rust transferred protein 1 (RTP1). Regarding the plant, 13 new WRKY transcription factors in coffee where identified. Additionally, contigs responsible for encoding pathogenesis-related (PR) proteins (1,3-b-glucanases, PR1b, PR-5 of the thaumatin-like protein family, and chitinases) were most abundant. The identification of effectors and polymorphisms in these regions has applicability in studies of genetic diversity, which can reveal the mechanisms underlying the dynamics of adaptation of the populations of the pathogen against the resistance of the plant. Additionally, the polymorphisms identified in the effectors could be used as molecular tools for the characterization and diagnosis of *H. vastatrix*.

Florez et al. (2017) performed RNA sequencing (RNAseq) to compare gene expression profiles and obtain a global overview of the transcriptome in both compatible and incompatible interactions of *C. arabica* and *H. vastatrix*. The genotypes Caturra CIFC 19\1 (susceptible) and HdT CIFC 832/1 (resistant) were inoculated with *H. vastatrix* race XXXIII. A database of 43,159 transcripts was obtained using bioinformatics tools. HdT responded to *H. vastatrix* infection with a larger number of upregulated genes than Caturra during the early infection response (12 and 24 hai). These data suggest that the infection of *H. vastatrix* to HdT, one of the most important CLR resistance sources, is related to pre-haustorial resistance. The identified genes were involved in receptor-like kinases, response ion fluxes, production of reactive oxygen species, protein phosphorylation, ethylene biosynthesis, and callose deposition. These genes are closely involved in the recognition of PAMPs and induction of PTI (Boller and Felix 2009). The resistant and susceptible genotypes also showed upregulation of genes associated with programmed cell death at 12 and 24 hai, respectively. According to the authors, this may infer that programmed cell death during the early response is efficient in containing the pathogen. A subset of candidate genes upregulated HdT-exclusive was analyzed by RT-qPCR, and most confirmed the higher expression in HdT at the early stage of infection. These genes include the putative basic helix-loop-helix bHLH DNA-binding superfamily protein, ethylene-responsive transcription factor 1 B, putative disease resistance protein RGA1, putative disease resistance response (dirigent-like protein) family protein, and premnaspiridione oxygenase genes. Interestingly, Capucho et al. (2012) reported a decrease in the resistance of cultivars with introgression of HdT (CIFC 832/1) infected with rust XXXIII. Thus, the results obtained in this work, based on the dynamics between the plant-pathogen interaction, could aid in the elucidation of the supplantation of the plant's resistance to specific races of the fungus and development of new strategies to control CLR.

Also, through the sequence information of a HdTCIFC 832/2 BAC library (Cação et al. 2013; Barka et al. 2020; Almeida et al. 2021) cloned two coffee resistance genes in HdT. They share conserved sequences with others S_H genes and displays a characteristic polymorphic allele conferring different resistance phenotypes. Two resistance gene analogs (RGAs) containing the motif of leucine-rich repeat-like kinase (LRR-RLK) were identified and were highly expressed during both the compatible and incompatible coffee-*H. vastatrix* interactions.

To gain a more comprehensive understanding of the *C. arabica*-CLR interaction, Porto et al. (2019) sequenced the genome of *H. vastatrix* race XXXIII using the Pacific Biosciences PacBio RS II and Illumina HiSeq 2500 platforms and assembled all reads to obtain a high-quality reference genome. The obtained genome comprised 547 Mb, with 13,364 predicted genes that encode 13,034 putative proteins with transcriptomic support. Putative secretome was analyzed with 615 proteins and 111 effector candidates specific to *H. vastatrix* were identified. Of these, 17 were analyzed by RT-qPCR over time during the infection process. During the pre-haustorial phase (24 hai) of an incompatible interaction (HdT CIFC 832/1 inoculated with *H. vastatrix* race XXXIII), five effector genes were significantly induced. This illustrated the probable role of these effectors in the recognition of *C. arabica* resistance genes.

Another nine genes were significantly induced after haustorium formation (48 and 72 hai) in a compatible reaction (Caturra CIFC 19/1 inoculated with race XXXIII). The authors inferred that these effectors were likely to be translocated to host cells via haustories.

Through genetic engineering, Cacas et al. (2011) isolated and validated the coffee *non-race-specific disease resistance NDR1*, previously identified as a participant in the defense mechanism against *H. vastatrix*. The coffee *NDR1* gene was expressed in the Arabidopsis knock-out null mutant ndr1-1. The *ArabidopsisNDR1* ortholog is a well-known master regulator of the hypersensitive response that is dependent on R proteins. Upon challenge with *Pseudomonas syringae*, coffee *NDR1* was able to restore the resistance phenotype in the mutant genetic background. They showed that the *NDR1* is a key regulator initiating hypersensitive signaling pathways and that there is an *NDR1*-dependent defense mechanism conservation between Arabidopsis and coffee plants. Thus, the authors proposed that the coffee *NDR1* gene might be a main target for genetic manipulation of the coffee innate immune system and achieve broad-spectrum resistance to *H. vastatrix* races. Additionally, they also provided a methodology for isolating and validating other genes for resistance to *H. vastatrix*.

Genomic and transcriptomic studies related to CLR have provided tools to accelerate coffee breeding programs. With the reference genomes of C*offea*, as well the pathogen genome, our knowledge of this plant-pathogen interaction will increase, and we will have diverse opportunities to control this disease, such as the use of RNAi for key rust genes or genome editing of susceptible genes in coffee plants (Cui et al. 2020).

4.5.2 Coffee Berry Disease (CBD)

CBD is caused by the hemibiotrophic fungus *Colletotrichum kahawae* Waller and Bridge and can quickly destroy 50–80% of developing berries (6–16 weeks after anthesis) in susceptible arabica cultivars during prolonged wet and cool weather conditions (van der Vossen and Walyaro 2009). The pathogen can infect all organs of the host, but the outbreak of the disease with visible symptoms occurs during the expanding stage of berry development, producing sunken, black, anthracnose-like lesions on the green pulp (Hindorf and Omondi 2011). Although the application of fungicides can provide adequate control, the use of coffee resistant varieties is the most appropriate and sustainable management strategy against this disease. Despite being restricted to Africa, it is a concern for other coffee-producing regions, such as Latin America and Asia, where preventive breeding studies for CBD are in progress (Diniz et al. 2017).

Studies based on inheritance have reported three CBD resistance genes, the dominant *T* and *R* genes and the recessive *k* gene. The *T* gene comes from HdT, the *R* from *C. arabica* (var Rume Sudan), and the *k* from Rume Sudan and *C. arabica* K7 (van der Vossen and Walyaro 1980; Gimase et al. 2020).

Gichuru et al. (2008) characterized the *T* gene (Ck-1) through a genetic mapping approach from segregating populations for resistance to CBD. In that study, two SSRs and eight AFLP markers were strongly linked to the *T* gene. In another study, through a GWAS approach, using an F_2 population from the parents Rume Sudan (resistant) and SL28 (susceptible), two SNPs were associated with resistance conferred by the *R* gene (Gimase et al. 2020). These two associated SNPs, Ck-2 and Ck-3, explained 12.5 and 11% of total phenotypic variation, respectively. Additionally, these SNPs are linked to genes involved in the resistance mechanism of plants against pathogens, such as the response to abscisic acid (ABA) and cell wall metabolism. These three markers, Ck-1, Ck-2, and Ck-3, were validated and used in progenies with introgression of resistance to CBD and displayed their essential role in reducing the time required to develop resistant cultivars (Alkimin et al. 2017; Gimase et al. 2021).

Understanding the molecular basis governing coffee resistance to *C. kahawae* is fundamental to gain insights into the distinctive processes underlying the plant resistance response and aid breeding efforts. Therefore, the expression of two defense-related genes (*RLK* and *PR-10*) was analyzed by RT-qPCR in *C. arabica*-*C. kahawae* compatible and incompatible interactions (Figueiredo et al. 2013). For this, two coffee genotypes, Catimor 88 (resistant) and Caturra (susceptible) were infected by *C. kahawae*, and the samples were collected at 12, 48, and 72 h after inoculation (hai). The expression of the *RLK* gene, which is predicted to encode a receptor-like kinase, increased in the resistant genotype with the highest transcriptional level at 48 h post-inoculation. *PR10*, which appears to be related to the jasmonic acid-dependent resistance pathway and to accumulate in host cells in incompatible interactions, increased during the inoculation time-course in the resistant genotype. Additionally, 10 genes (*S24*, *14-3-3*, *RPL7*, *GAPDH*, *UBQ9*, *VATP16*, *SAND*, *UQCC*, *IDE*, and *β-Tub9*) were selected as reference genes for interaction studies in the pathosystem that allowed the precise quantification of gene expression of resistance to CBD.

In another study, to elucidate the involvement of phytohormone pathways in coffee resistance and susceptibility to *C. kahawae*, Diniz et al. (2017) evaluated the expression by RT-qPCR of salicylic acid, jasmonic acid, and ethylene biosynthesis, reception, and responsive-related genes. Again, the genotypes Catimor 88 and Caturra after *C. kahawae* challenge were used and samples were collected at time points during the infection process based on a cytological analysis of fungal growth and associated host responses (6, 12, 24, 48, and 72 hai). The expression patterns of the salicylic acid pathway-related genes were quite similar for both coffee varieties, suggesting that this hormone is less relevant in this pathosystem. Conversely, some genes related to jasmonic acid pathway, such as *12-oxoplytodienotae reductase 1-like* (*OPR3*) and *pathogenesis-related 10* (*PR10*), showed earlier and greater magnitude in the resistant genotypes. According to the authors, this evidence supported the role of the jasmonic acid pathway activation in defense responses and inhibition of fungal growth. In relation to the genes involved in the ethylene pathway, the stronger activation of the *ethylene-responsive factor 1* (*ERF1*) gene at the beginning of the necrotrophic phase in the susceptible variety suggested the involvement of this pathway in tissue senescence.

4.5.3 Bacterial Halo Blight (BHB)

Bacterial halo blight (BHB), caused by the bacteria *Pseudomonas syringae* pv. *garcae* (Amaral et al. 1956), affects coffee production mainly at high altitudes and in mild temperature regions where cold winds impact the plants, favoring the incidence of the disease (Patrício and de Oliveira 2013). This disease has already been reported in Brazil, Kenya, Ethiopia, Uganda, and China (Ramos and Shavdia 1976; Chen 2002; Zoccoli et al. 2011; Xuehui et al. 2013; Adugna et al. 2012). BHB is also a major problem in nurseries, where density and excessive moisture on the surfaces of the leaves provide an ideal environment for pathogen development. The lack of control, in this case, can result in 100% damage (Zoccoli et al. 2011). Control is achieved using copper and antibiotics, which increases production costs and is frequently inefficient in the field, in addition to its possible environmental impacts (Zambolim et al. 2005; Patrício and de Oliveira 2013). In this limited scenario, cultivars resistant to BHB are a sustainable alternative and can reduce costs.

In a *C. arabica* wild type collection (FAO 1968), substantial genetic variability for BHB resistance was identified. The accessions of this collection have levels of resistance to BHB that vary between highly resistant, resistant, moderately resistant, moderately susceptible, susceptible, and highly susceptible (Mohan et al. 1978). Using this information, GWAS for resistance to BHB identified five SNPs associated with resistance (Ariyoshi et al. 2019). The genes linked to the SNPs identified in this study, *serine/threonine-kinase* and *NB-LRR*, have pathogen-specific recognition functions and trigger defense responses, such as cell death (Zong et al. 2008; Coll et al. 2011). These genomic regions of resistance have a high potential for application in MAS and as a target for gene editing.

A genetic diversity study and pathogenicity test among 25 strains of *P. syringae* pv. *garcae* were performed using enterobacterial repetitive intergenic consensus-PCR (ERIC-PCR) and Repetitive element sequence-based-PCR (REP-PCR) techniques. For the pathogenicity tests, the strains were inoculated in the leaves of the susceptible coffee cultivar Mundo Novo (Maciel et al. 2018). In the combined analysis of ERIC- and REP-PCR, the Brazilian strains obtained from 1958 to 1978 grouped separately from the remaining strains and the Kenyan strains grouped separately from the Brazilian strains. Regarding the pathogenicity tests, the strains could be divided into four aggressiveness classes (highly aggressive, aggressive, moderately aggressive, and less aggressive). These results could contribute to the development of rapid and reliable methods for early detection of the pathogen to minimize their negative impacts on coffee production.

4.5.4 Nematodes

Root-knot nematodes (RKN), which belong to the genus *Meloidogyne*, are one of the most limiting factors in coffee cultivation. Because of its wide distribution, combined

with its high reproductive capacity and aggressiveness, this group of nematodes could be responsible for 15% of the total reduction in relation to the 20% of losses caused by all phytonematodes (Amorim et al. 2016). Among the 19 nematode species reported in association with the crop (Aribi et al. 2018; Villain et al. 2018), the most common, harmful, and well-known species are *Meloidogyne exigua, Meloidogyne coffeicola, Meloidogyne incognita*, and *Melodoigyne paranaensis*. Together, *M. incognita* and *M. paranaensis* can destroy up to 80% of the roots of *C. arabica* (Bertrand and Anthony 2008). They are distributed worldwide and are considered the most important parasitic nematodes in coffee (Aribi et al. 2018), causing yield losses of approximately 36% worldwide (Fatobene et al. 2017).

The use of resistant cultivars and clones is considered a key strategy in the management of nematodes in *Coffea*, because once the area is infested, the management of nematode populations can be costly and have low sustainability. Resistance to *M. incognita* has been found in *C. arabica, C. canephora*, and *C. congensis* (Gonçalves et al. 1988, 1996; Sera et al. 2006; Fatobene et al. 2017). However, the sources of resistance to *M. paranaensis* are scarce but have been observed in *C. arabica* and *C. canephora* (Anthony et al. 2003; Sera et al. 2006; Fatobene et al. 2017). Nevertheless, cultivars such as IPR 100 originated by crossing Catuaí and the hybrid Catuaí x BA-10 coffee and IPR 106 (Icatu), demonstrated resistance to *M. incognita* and *M. paranaensis* (Ito et al. 2008; Sera et al. 2017; Shigueoka et al. 2017). Breeding programs are also exploring Ethiopia's wild accessions as a source of resistance to nematodes because of their greater genetic diversity than that of breeding materials (Silvestrini et al. 2007). Resistance to *M. incognita, M. exigua* and *M. paranaensis* (Fatobene et al. 2017; Holderbaum et al. 2020) has been detected in selected accessions.

Sources with high resistance to *M. exigua* have been found in several *Coffea* species, including *C. arabica, C. canephora, C. congensis, C. dewevrei, C. liberica, C. racemosa, C. kapakata, C. eugenioides, C. salvatrix, C. stenophylla*, and *C. sessiliflora* (Curi et al. 1970; Fazuoli and Lordello 1977, 1978; Anthony et al. 2003; Fatobene et al. 2017). Cultivars with an HdT background, such as IAPAR 59 and IAC 125-RN Acauã, MGS, Catiguá, and Paraiso MG H491-1 are highly resistant to *M. exigua* (Muniz et al. 2009; Pereira and Baião 2015). Furthermore, *C. canephora* Apoatã IAC-2258 is recommended as a rootstock and is resistant to the three most important coffee nematodes (Salgado et al. 2005; Sera et al. 2006; Andreazi et al. 2015).

Nematodes use molecular tools that are successful in infection and plant parasitism. They synthesize proteins that make up a complex parasitism methodology, inducing cell wall modifications and even control of host gene expression (Maluf et al. 2008). In this sense, one of the key points in the development of a new resistant cultivar is the selection of R genes capable of overcoming sophisticated nematode parasitism. The search for R genes in coffee pathosystems is one of the priorities of breeding programs. *Mex-1* was the first and only nematode resistance gene identified in *C. arabica*, providing HR-type responses to *Meloidogyne* infection (Noir et al. 2003; Anthony et al. 2005). Additionally, 14 AFLP markers were identified and associated with resistance to *M. exigua*, from which a genetic map of the *Mex-1*

locus was prepared, with markers for the selection of resistant genotypes (Noir et al. 2003). The *Mex-1* locus is located on chromosome 3, which houses the S_H3 gene that confers resistance to coffee rust (Saucet et al. 2016).

Despite reports of sources of resistance to *Meloidogyne*, the transcriptome of these pathosystems with coffee plant interactions has barely been studied. Albuquerque et al. (2017) evaluated the initial expression (4, 5 and 6 days after-inoculation of 88 differentially expressed genes between resistant and susceptible coffee to *M. incognita*. This study reports the overexpression of genes encoding miraculin (defense), *RGLG1* (protein degradation), *SENA* (cell death associated with senescence), *NLR* (immunity signaling), *CaWRK11* (transcription factor), and *OBP* (wall modification). by analyzing *C. arabica* resistance to *M. incognita* and *M. paranaensis*, recent studies from our group suggest that the response is different and may be caused by differences in nematode aggressiveness, which can result in different molecular patterns and effectors secreted by these nematodes. The response of resistant plants is mostly earlier, occurring at 5 dai with *M. incognita*, and the response to *M. paranaensis* was mostly later, at 14 dai, but it was higher than that triggered by *M. incognita.* These studies increase our knowledge of coffee resistance responses to *M. incognita* and *M. paranaensis* and provide new genes to improve plant resistance.

Although some genes have already been shown to participate in the process of coffee resistance to RKN, many other studies must be conducted, because the plant-nematode interaction is complex and encompasses many unknown genes. Genetic transformation of coffee with resistance genes can not only produce resistant cultivars, but also help to understand how the resistance process is developed by plants.

4.5.5 Coffee Leaf Miner (CLM)

Coffee leaf miner (CLM), caused by *Leucoptera coffeella* (Guérin-Mèneville 1842), is considered one of the most important pests of coffee plantations, causing serious economic damage in the main coffee-producing regions. During their life span, the moth lays its eggs in the adaxial part of the leaves. The larvae that hatch form mines in the mesophile during their feeding, which can lead to leaf fall and consequently a reduction of up to 75% in photosynthetic rates, with productivity losses from 30 to 80%, depending on the infestation level (Dantas et al. 2020). The use of insecticides has been widely used to control CLM, however, it is not completely efficient. Chemical control also increases production costs and can affect natural predators of *L. coffeella.*

Traditional breeding for CLM control has been a challenge because there are no sources of resistance in *C. arabica* or *C. canephora.* However, introgression of *C. arabica* with resistant *C. racemosa* originated from the Aramosa hybrids, which have been incorporated in breeding programs to select for CLM resistance. From these hybrids, the cultivar *C. arabica* Siriema has been released to coffee farmers, presenting resistance to CLM (Matiello et al. 2015).

Alternatively, search for R genes in other species could contribute to the genetic improvement of the coffee tree. One of these genes, which is widely known to play an important role against pests in several species, is the *Cry* gene identified in *Bacillus thuringiensis* (Bt). This gene has the potential to protect coffee trees against the miner bug and has already been used in the genetic transformation of *C. arabica* and *C. canephora*. The *cry1Ac* gene encodes proteins CryIA (c), CryIB, and CryIE, whereas *cry10Aa*, present in the *B. thuringiensis* serovar *israelensis* (Bti), translates the protein Cry10Aa, which is highly toxic to insects (Guerreiro Filho et al. 1998; Méndez-López et al. 2003).

In addition to *Cry* genes, Mondego et al. (2005) isolated and characterized genes associated with resistance during the infestation of the miner bug in progeny resulting from a cross between *C. canephora* and *C. racemosa*. Five short repetitions in tandem (STRs) or microsatellites were characterized as a starting point to unravel the mechanisms of resistance to CLM. Additionally, Mondego et al. (2005) reported the participation of the *CoMir* gene, which codes for proteinase-inhibiting proteins and may be involved in the process of resistance to minor bugs.

Through a database of EST sequences, Cardoso et al. (2014) validated 18 genes differentially expressed in resistant and susceptible plants to miner bugs. These authors reported that positively regulated genes include those responsible for defense mechanisms, hypersensitivity responses, and genes involved in cell function and maintenance, suggesting that resistance to miner bugs is not triggered by a larger resistance gene. Additionally, they found that the differential expression between resistant and susceptible genotypes was observed in the absence of miner bugs, indicating that the defense already exists in resistant plants.

In a pioneering study on the genetic transformation of *C. canephora*, Leroy et al. (2000) produced plants genetically transformed with *cry1Ac* genes. More than 50 transformation events were obtained, showing different levels of Cry protein production, as well as tolerance resistance to the miner bug in greenhouse tests (Leroy et al. 2000). In field tests, several events showed stable resistance during 4 years of experimentation, demonstrating the great potential of using *Cry* genes in the control of CLM (Perthuis et al. 2005).

4.5.6 Coffee Berry Borer (CBB)

Among the different pests that can affect coffee production, coffee berry borer (CBB), *Hypothenemus hampei* (Ferrari 1867) (Coleoptera: Curculionidae), is the most important pest for coffee farmers. Both green and ripe berries are susceptible to attack by *H. hampei*. Damaged fruits suffer seed rot, which affects maturation and early fall on the ground as well. Additionally, there is a loss of quality with depreciation of the product because the number of coffee grains damaged by CBB is considered a defect for coffee quality. The insect feeds and reproduces within the coffee seeds inside the coffee berry, which makes control by the application of insecticides inefficient. For several years, farmers have used organochlorides to control

CBB, but because of safety, health, and environmental reasons, this practice is now forbidden in the main coffee-producing regions. Strategies of biocontrol, such as the use of the parasitoids C*ephalonomia stephanoderis*, *Phymastichus coffea*, and *Prorops nasuta*, did not produce acceptable control of CBB. Biological control of CBB using the fungus *Beauveria bassiana* (Balsamo) has been effective using natural and industrial conidial suspensions, which are sprayed on the fruits. However, the efficiency of *B. bassiana* in controlling CBB depends on environmental conditions, such as high humidity during application. Furthermore, *B. bassiana* CBB control is still hampered by the slow infection process that allows the adult insects to live for enough time to damage the coffee berry, and by the challenges and costs for conidial industrial-scale production (Infante 2018).

Genetic resistance to CBB is not easily available in the coffee gene pool or is difficult to incorporate using conventional breeding. Neither *C. arabica* nor *C. canephora* exhibited natural resistance to *H. hampei*. Despite some reports on CBB resistance in *C. eugenioides* and *C. kapakata* (Sera et al. 2010), the differences in ploidy levels between the allotetraploid *C. arabica* and other diploid *Coffea* species, as well as their incompatibility, are also major limitations associated with conventional coffee breeding for CBB. Therefore, genetic engineering is one of the main strategies employed to transfer CBB resistance to *Coffea.*

De Guglielmo-Cróquer et al. (2010) transformed *C. arabica* (Catimor) by introducing the *cry1Ac* gene again through microjet bombardment methodology (biobalistics). The presence of the gene has been reported in regenerated explants, without the need for selection genes and reporters. Of the 60 regenerated explants, seven had the *cry1Ac* gene in their genome, as confirmed by PCR. However, subsequent studies to verify the performance of the transgenes have not been conducted at the field level.

In the search for alternatives to CBB control using *cry* genes, the high toxicity of *B. thuringiensis* serovar *israelensis* against first instar larvae of CBB has been demonstrated. Bioassays against first-instar *H. hampei* from *cry10Aa* and *cyt1Aa* provided estimated larval mortality rates of 20% and 50%, respectively. Additionally, a combination of *cry10Aa* and *cyt1Aa* resulted in 100% larval mortality. Therefore, the development of coffee varieties resistant to CBB using transgenic technology with these genes may represent an alternative to manage this important pest (Villalta-Villalobos et al. 2016). More recent work by Valencia-Lozano et al. (2019) developed a protocol for obtaining transgenic *C. arabica* plants using biobalistics. The authors introduced the *Cry10Aa* gene into the coffee genome, which presents CBB toxicity, and regenerated explants expressed the protein in a stable manner.

The first characteristic of agronomic importance in the transformation of *C. canephora* coffee trees (clone 126) was the introduction of the *cry1Ac* gene, which is responsible for the production of CryIA (c), CryIB, and CryIE, which are toxic to the CLM (Leroy et al. 2000). Despite the plants being produced for CLM resistance, these plants could also be tested for their potential toxicity against CBB and to stack resistance genes in *Coffea.*

Transgenic genotypes of *C. arabica* (Catuaí Vermelho) were also produced to develop a CBB-resistant cultivar. The *αAI-1* and *αAI-Pc* genes, originating from beans

and responsible for inhibiting α-amylases, enzymes important in starch degradation and digestion in *H. hampei*, were introduced into the coffee tree using a biobalistic methodology. The assays generated 15 transformed plants, of which six genotypes presented a single copy of the *αAI-1* gene and a seventh genotype presented two copies (Barbosa et al. 2010). Despite the potential for plant protection, subsequent experiments to ascertain the potential of genotypes in the interaction with *H. hampeii* in the field have not been conducted.

In contrast, the CBB transcriptome was performed at three different stages of the development of *H. hampei* infesting coffee (Noriega et al. 2019). They identified genes that encode cuticular proteins (CPs) and enzymes involved in cuticle metabolism, which play important roles in physiological processes, such as protection from insecticide penetration, physical injuries, pathogens, and dehydration (Willis 2010). Additionally, genes encoding OBPs, which are proteins with chemosensory functions, play key roles in the copulation and reproduction of the species. The genes encoding CPs and OBPs are potential candidates for the development of silencing methodologies for gene expression based on RNAi (Antony et al. 2018).

The genome of *H. hampei* has been sequenced (Vega et al. 2015). The *Rdl* gene was identified as conferring resistance to insecticides of the two cyclodiene classes, as well as the genes of the carboxyl esterase family (CE), previously identified as being responsible for promoting resistance to other species of insects (*Drosophila melanogaster*, *Aedes aegypti*, *Anopheles gambiae*, and *Tetranychus urticae*) to insecticides of the class of two organophosphates, as well as genes of the *CYP* and *GST* families (Ranson et al. 2002; Claudianos et al. 2006; Strode et al. 2008; Grbić et al. 2011; Oakeshott 2003). Other genes related to antimicrobial activity were also identified. This information can contribute to the development of management strategies associated with chemical and biological control.

4.6 Final Remarks

In any crop plantation, biotic stresses will always need attention because of the natural co-evolution between the host and pathogens. The surge of new pathogen races, as well as the development of insect resistance to pesticides will always require the dedication of coffee farmers and researchers. Preventive breeding using modern genetic tools can help produce cultivars with multiple pathogen resistance. Therefore, the need to conserve and explore the coffee genetic pool is important to increase the necessary arsenal to combat diseases and pests. Molecular tools can rapidly characterize new R genes and identify markers for quick incorporation into elite cultivars by breeders. Extensive use of MAS or genome editing to shutdown susceptible genes in coffee plants can lead to faster development of new cultivars to better handle biotic stresses. These improved cultivars are fundamental to avoiding outbreaks, decreasing the use of pesticides, and helping farmers in sustainable coffee production.

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