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# Characterization in populations of *Coffea arabica* L. for resistance to CBD using molecular markers

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## ABSTRACT

Coffee berry disease (CBD) is caused by the fungus *Colletotrichum kahawae* Waller and Bridge. This disease is restricted to the African continent, where it can cause production losses of more than 80% when susceptible varieties are used or when the indicated chemical control is not carried out. For this reason, since 1970, Cenicafé has developed lines resistant to this disease in the absence of the pathogen, a process that has been favored by the discovery and validation of microsatellite markers associated with the *Ck-1* gene for resistance to CBD. In this research, 12 populations of *Coffea arabica* were characterized for their resistance to CBD using the molecular markers Sat235, Sat207 and FR34-6CTG. The molecular markers allowed us to identify that the same allelic form of resistance to CBD is present in lines derived from Timor Hybrid CIFC 1343 (HdT CIFC 1343). Furthermore, the allelic form of resistance associated with the three molecular markers was identified in one line derived from *Coffea canephora*. In lines derived from Caturra x HdT CIFC 1343 it was evident that, when the plants present the allelic forms of resistance identified by the molecular markers, high percentages of hypocotyls resistant to different isolates of *C. kahawae* are observed in the progeny.

**Key words:** *CBD*; *Coffea* spp; resistance genes; marker-assisted selection.

## 1 INTRODUCTION

Coffee berry disease (CBD) is caused by the fungus *Colletotrichum kahawae Waller and Bridge* (Waller, et al., 1993; Cabral et al., 2020). This disease was first detected in eastern Kenya in 1922 (McDonald, 1926), and quickly spread to other coffee producing countries on the african continent becoming one of the main diseases that limit coffee production in Africa (Adugna, 2023). The infection begins during the formation of the flowers, causing them to fall. When this does not occur, the fungus remains latent in the flower until the fruits begin to develop, at which point the fungus causes the mummification and loss of the berries, leading to losses in production and the profitability of the crop (Van der Graaff, 1981; Giddisa, 2016).

It has been estimated that the losses caused by this disease can exceed 80% when the environmental conditions favor the development of the pathogen and when control measures are not applied (Kebati et al., 2016; Motisi; Papaix; Poggi, 2022). In addition, chemical control of the disease can represent up to 40% of the total production costs (Gichuru et al., 2008; Motisi; Ribeyre; Poggi, 2022).

CBD is a potential threat for coffee producing countries where the disease is absent. Therefore, research has focused on determining the diversity of the pathogen (Vieira, et al., 2018), understanding the defense mechanisms in the host and identifying sources of genetic resistance to the disease (Diniz et al., 2017; Da Silva et al., 2019).

The genetic resistance to CBD present in *C. arabica* is controlled by three genes. The *T gene* (*Ck-1*) of the HdT, the *R gene* (*Ck-2, Ck-3*) of Rume Sudan, and the recessive *gene k,* present in the varieties K7, Pretoria and Rume Sudan (Vossen; Walyaro, 1980; Gichuru et al., 2008; Gimase et al., 2020). By introgressing these genes into a commercial variety, costs related to chemical control of the disease are not incurred, thus positively impacting economic profitability and environmental sustainability (Gichuru et al., 2008).

However, the accumulation of genes that confer resistance to diseases within a genotype and their identification through conventional methods are limited by the dominant effects of the factors that govern resistance, and in many cases, the phenotypic response can be affected by interactions with the environment (Motisi; Ribeyre;Poggi, 2019). Similarly, there are limitations due to the time needed to improve coffee plants, the high costs of field evaluations, and the lack of precision in the strategies used to restore the genetic background of the cultivars, which not only ensures resistance to coffee leaf rust (CLR), but also improves productivity, physical qualities of the grains and the sensory quality of the drink created from the improved varieties (Moncada et al., 2016). The use of molecular markers linked to resistance genes can help to overcome these barriers, making it possible to identify resistant genotypes at an early stage of development, increasing the speed and efficiency of the selection process and decreasing associated costs (Lashermes et al., 2016; Moncada et al., 2016).

To date, ten markers linked to the *Ck-1* gene for CBD resistance have been identified. Of these, eight are AFLP, and two are microsatellites, namely, Sat207 and Sat235, the latter being the closest to the genomic region that contains the gene (Gichuru et al., 2008). Recently, two SNP markers correlated with the resistance genes present in the Rume Sudan variety, *Ck-2* and *Ck-3,* were identified (Gimase et al., 2020). The joint use of these markers allowed the identification of 11 components lines of the varieties in the Ruiru 11 and Batian varieties that carry the *Ck-1*, *Ck-2* and *Ck-3* genes (Gimase et al., 2021).

Since their identification, the Sat207 and Sat235 microsatellites have been used to characterize the germplasm collections of the Federal University of Viçosa (UFV), the Empresa de Pesquisa Agropecuaria de Minas Gerais (EPAMIG), and the *Coffea Research Foundation* (CRF) in Kenya and the germplasm bank and the Ethiopian collection of *C. arabica* in Tanzania. These studies made it possible to identify genotypes resistant to CBD, which could be used as progenitors for the development of coffee varieties in genetic improvement programs (Alkimim, 2017; Silva et al., 2018).

Various authors have also reported the use of the Sat207 and Sat235 markers to select promising genotypes resistant to CBD in populations with different degrees of generational advancement (Alkimim et al., 2017; De Almeida et al., 2021; Benti et al., 2021). Recently, through bioinformatic analysis, Yu et al. (2021), determined that in the same region of the chromosome where the markers Sat207 and Sat235 are located, there are at least 10 genes related to resistance to pests and diseases, among which could be the gene associated with resistance to CBD, *Ck-1*.

In Colombia, in the absence of the pathogen *C. kahawae*, a strategy for the selection of genotypes resistant to CBD was implemented, this strategy began in the early 1970s, when F3 progeny were sent to the CRF in Kenya to evaluate their resistance under field conditions. In 1989, through the agreement established between the Centro de Investigaciones de Café (Cenicafé) and the Centro de Investigação das Ferrugens do Cafeeiro (CIFC) in Portugal, evaluations of genotypes for resistance to *C. kahawae* were implemented.

With the identification of the molecular markers Sat207 and Sat235, which are associated with the resistance gene present in HdT (Gichuru et al., 2008). Their validation in Cenicafé and the identification of another marker (FR34-6CTG), which is associated with the region that contains the *Ck-1* gene (Guzmán; Moncada, 2012), the use of these microsatellites was routinely implemented in the selection of resistant genotypes in the absence of the disease. Today, it is known that the component lines of the commercial varieties Castillo® General and its seven regional components, Tabí, Cenicafé 1, Castillo® Zona Norte, Castillo® Centro and Castillo® Sur, have the markers FR34-6CTG and Sat235, which are indirect indicators of their resistance to CBD (Maldonado; Ángel, 2020).

Between 2013 and 2020, a regional adaptation test was carried out in eight departments in 13 coffee growing municipalities of Colombia, where 93 advanced lines were selected from Cenicafé genetic improvement program for their resistance to CLR, high productivity, good quality, grain characteristics and excellent beverage sensory attributes. These selected lines constitute the set of genotypes with the potential to reconstruct the current commercial cultivars or to form a new one. Therefore, the objective of this research was to characterize the CBD resistance of the 93 lines selected in regional tests by the presence of markers linked to the *Ck-1* resistance gene.

## 2 MATERIAL AND METHODS

A total of 1512 plants were evaluated for their characters of agronomic interest and the presence of the molecular marker Sat235 linked to the CBD resistance gene *Ck-1* in its dominant allelic form. Subsequently, the elite plants identified and with the presence of the molecular marker Sat235 were genotyped with the molecular markers Sat207 and FR34-6CTG. The parents of the 12 populations were genotyped with the three molecular markers. These plants belonged to 93 advanced lines of 12 populations originating from crosses that included the HdT CIFC 1343, *C. canephora* and wild introductions of Ethiopian origin that make up the Colombian Coffee Collection (CCC) (Table 1).

The populations were established at the Naranjal Experimental Station in the municipality of Chinchiná (Caldas, Colombia) (LN 10° 25´18"; LW 73° 39´29"). The site is located at 1407 masl, with an average annual rainfall of 2586 mm, a temperature of 20.9 °C and a solar brightness of 1738 h/year.

From each selected plant, the first and second pairs of leaves were collected from branches in the middle and upper thirds of the tree. The leaf tissue was frozen at -80 °C and subsequently lyophilized. The dehydrated leaves were macerated in mortars with porcelain pestles, liquid nitrogen was added to facilitate the process, and oxidation was limited by rehydration of the tissue. The DNA was extracted following the methodology proposed by Maldonado and Ángel (2020). Subsequently, the samples were amplified with the markers Sat207, FR34-6CTG and Sat235 via PCR, according to the protocol reported by Guzmán and Moncada (2012) and modified by Maldonado and Ángel (2020). The sequences of the primers used are presented in Table 2. The fragments amplified with the marker Sat235 were run on 2% agarose gels, stained with ethidium bromide (0.1 µg/ml) in a horizontal electrophoresis chamber at 70 volts per 60 minutes and visualized through a Gel Doc XR (Bio-Rad) ultraviolet light documentation system. The amplified fragments of the markers Sat207 and FR34-6CTG were run on 6% polyacrylamide gels with 7 M urea and 0.7X TBE and stained with silver nitrate following the methodology established by Maldonado and Ángel (2020). Each sample was classified as resistant (presence of allelic dominant form the molecular marker), susceptible (presence of allelic recessive form the molecular marker) or heterozygous. The classification genotypic of resistance or susceptibility was carried out by taking into account the results obtained by Gichuru et al. (2008); Guzmán and Moncada, (2012), and Maldonado and Ángel (2020).

The parents that gave rise to the population derived from the cross between Caturra x HdT CIFC 1343 were sent to the CIFC in Portugal for the direct analysis of resistance to CBD through tests of the hypocotyls Van der Graaff (1981) (Table 3).

Each genotype was inoculated with at least three isolates from different origins, such as Cameroon (Cam1), Malawi (M, M2), Kenya (Que2), Rwanda (R) and Zimbabwe (Zim1, Z9).

## 3 RESULTS

All parents that gave rise to the 12 characterized populations were evaluated with the molecular markers (Sat235, Sat207, and FR34-6CTG). The molecular markers associated with the CBD resistance gene (*Ck-1*) were identified in HdT 1343 and some plants of *C. canephora* (CCC.1013, CCC.1003, and CCC.996) (Table 4).

In populations 9, 10, 11, and 12, the presence of the molecular marker Sat235 was identified in the three allelic forms (homozygous, heterozygous, and recessive) of the *Ck-1* resistance gene. The Population 12 was the one with the highest number of homozygous plants (87.8%), followed by populations 9 and 10. These populations presented 20 and 22% of homozygous plants, respectively.

Population 11 only presented plants with heterozygous and recessive allelic forms, with the recessive allelic form predominating (92.9%). The population with the highest percentage of heterozygous plants was population 9 (Table 5). Populations 1, 2, 3, 4, 5, 6, 7 and 8 only presented the recessive allelic form.

In population 9, plants from the two lines that make it up were selected. Plants with the presence of the molecular marker Sat235 in its homozygous allelic form also presented the molecular markers Sat207 and FR34-6CTG. In population 10, the presence of Sat235 molecular marker was identified in 10 of the 47 lines evaluated, derived from interspecific hybridization between *C. arabica* var. Caturra and *C. canephora*. In line MEG6.65 #0612, the three markers linked with resistance were observed (Table 6). The progenitor of this line is the *C. canephora* accession CCC.1013, which also presented the resistance markers Sat235, Sat207 and FR34 (Table 4).

The results of the hypocotyl tests carried out in the F3/F4 generations that gave rise to the genotypes evaluated in this study, revealed that among those samples with alleles associated with resistance, there was resistance to isolates from Zimbabwe, Kenya, Rwanda and Malawi (Table 7). However, only the 998 plant was resistant to isolates from Cameroon (Cam1) (Table 7).

**Table 1:** Populations and number of lines per filial generation evaluated for the molecular markers Sat235, Sat207 and FR34-6CTG.

| Id. Population | Populations | No. Plants | No. Lines | Filial Generation | | |
|---|---|---|---|---|---|---|
| | | | | F4 | F5 | F6 |
| 1 | [[(Cat. x HdT 1343) x (Cat. x HdT 1343)] x [Catuaí x (Cat. x CCC.27)]] | 13 | 1 | 1 | - | - |
| 2 | [[Cat. x (Cat. x *C. canephora*)] x [Catuaí x (Cat. x CCC.27)]] | 80 | 5 | 5 | - | - |
| 3 | [[(Cat. x HdT 1343) x (Cat. x HdT 1343)] x (CCC.81 x Catuaí)] | 17 | 1 | 1 | - | - |
| 4 | [[(Cat. x HdT 1343) x (Cat. x HdT 1343)] x CCC.1159] | 16 | 1 | 1 | - | - |
| 5 | [[(Cat. x HdT 1343) x (Cat. x HdT 1343)] x CCC.1162] | 15 | 1 | 1 | - | - |
| 6 | [[Cat. x (Cat. x *C. canephora*)] x CCC.1155] | 13 | 1 | 1 | - | - |
| 7 | [[Cat. x (Cat. x *C. canephora*)] x CCC.173] | 34 | 2 | 2 | - | - |
| 8 | [Cat. x [(Cat. x *C. canephora*) x Cat.] | 20 | 2 | 2 | - | - |
| 9 | [CCC.1166 x (Cat. x HdT 1343)] | 144 | 2 | 2 | - | - |
| 10 | [(Cat. x *C. canephora*) x Cat.] | 705 | 47 | 10 | 37 | - |
| 11 | [(Cat. x HdT 1343) x CCC.50] | 14 | 1 | 1 | - | - |
| 12 | Cat. x HdT 1343 | 441 | 29 | - | 10 | 19 |
| | Total | 1512 | 93 | 27 | 47 | 19 |

Cat: Caturra. HdT 1343: Timor hybrid CIFC 1343. CCC: Colombian Coffee Collection.

**Table 2:** Sequences of the primers used for the molecular markers associated with the CBD resistance gene *Ck-1*.

| Marker | Primers | Amplicon (pb) | Reference |
|---|---|---|---|
| Sat207 | F: GAAGCCGTTTCAAGCC<br>R: CAATCTCTTTCCGATGCTCT | 90 | Gichuru et al. (2008) |
| FR34-6CTG | F: AAGCCGTTTCAAGCCATA<br>R: GCTTCTGTCTCAACACAACA | 176 | Guzmán and Moncada (2012) |
| Sat235 | F: TCGTTCTGTCATTAAATCGTCAA<br>R: GCAAATCATGAAAATAGTTGGTG | 225 | Gichuru et al. (2008) |

**Table 3:** Severity scale and classes for the preselection of genotypes by resistance to CBD as determined by the hypocotyl test by Van der Graaff (1981).

| Scale | Class |
|---|---|
| 0 | No symptoms |
| 1 | From small greenish lesions to 1 or 2 narrow brown lesions, lesions up to 0.5 mm wide. |
| 2 | More than 2 brown lesions or coalescing brown lesions. The width of the lesions exceeds 0.5 mm. |
| 3 | Large brown lesions with numerous black spots and/or black lesions. Black lesions can completely surround the stem, but the upper part remains alive. |
| 4 | Black lesion surrounding the stem. The top dies. |

**Table 4:** Evaluation of the molecular markers Sat235, Sat207 and FR34-6CTG associated with the *Ck-1* gene resistance gene in the parents of the 12 populations characterized.

| Parent | Sat235 | | | Sat207 | | | FR34-6CTG | | |
|---|---|---|---|---|---|---|---|---|---|
| | 1 | 0 | 1/0 | 1 | 0 | 1/0 | 1 | 0 | 1/0 |
| HdT CIFC 1343 | 1 | | | 1 | | | 1 | | |
| Caturra | | 0 | | | 0 | | | 0 | |
| CCC.1013 | 1 | | | 1 | | | 1 | | |
| CCC.1003 | 1 | | | 1 | | | 1 | | |
| CCC.980 | | 0 | | | 0 | | | 0 | |
| CCC.996 | 1 | | | 1 | | | 1 | | |
| CCC.976 | | | 1/0 | | | 1/0 | | | 1/0 |
| CCC.982 | | 0 | | | 0 | | | 0 | |
| CCC.27 | | 0 | | | 0 | | | 0 | |
| CCC.81 | | 0 | | | 0 | | | 0 | |
| CCC.1159 | | 0 | | | 0 | | | 0 | |
| CCC.1162 | | 0 | | | 0 | | | 0 | |
| CCC.173 | | 0 | | | 0 | | | 0 | |
| CCC.1166 | | 0 | | | 0 | | | 0 | |
| CCC.50 | | 0 | | | 0 | | | 0 | |

*0: Presence of the molecular marker associated with the susceptibility allelic form.

*1: Presence of the molecular marker associated with the homozygous resistant allelic form.

*1/0: Presence of the molecular marker associated with the heterozygotic allelic form.

## 4 DISCUSSION

The evaluation of the molecular markers Sat235, Sat207 and FR.34-6CTG, showed that 58.3% of the lines derived from HdT CIFC 1343 and 21% that originated from the interspecific cross [(Cat. x *C. canephora*) x Cat.] presented the allelic form resistance of gene *Ck-1*. These results demonstrated the importance of using markers linked to the *Ck-1* resistance gene for the characterization of genotypes with the potential to be parents of resistant varieties, as well as for the selection of improved lines. Additionally, it has been corroborated by various investigations. For example, Alkimim et al. (2017), identified three HdT genotypes from the germplasm collection of the Federal University of Viçosa (UFV) that carry the *Ck-1* gene. Silva et al. (2018), identified 99 HdT accessions from the UFV collection with multiple CLR and CBD resistance genes.

**Table 5:** Evaluation of the molecular marker Sat235 associated with the *Ck-1* resistance gene in the 12 populations and the percentage of plants identified with the different allelic form.

| Id. Population | Cruzamiento | No. Plants | Molecular Marker Sat235 | | |
|---|---|---|---|---|---|
| | | | 1 | 0 | 1/0 |
| 1 | [[(Cat. x HdT 1343) x (Cat. x HdT 1343)] x [Catuaí x (Cat. x CCC.27)]] | 13 | - | 100.0 | - |
| 2 | [[Cat. x (Cat. x *C. canephora*)] x [Catuaí x (Cat. x CCC.27)]] | 80 | - | 100.0 | - |
| 3 | [[(Cat. x HdT 1343) x (Cat. x HdT 1343)] x (CCC.81 x Catuaí)] | 17 | - | 100.0 | - |
| 4 | [[(Cat. x HdT 1343) x (Cat. x HdT 1343)] x CCC.1159] | 16 | - | 100.0 | - |
| 5 | [[(Cat. x HdT 1343) x (Cat. x HdT 1343)] x CCC.1162] | 15 | - | 100.0 | - |
| 6 | [[Cat. x (Cat. x *C. canephora*)] x CCC.1155] | 13 | - | 100.0 | - |
| 7 | [[Cat. x (Cat. x *C. canephora*)] x CCC.173] | 34 | - | 100.0 | - |
| 8 | [Cat. x [(Cat. x *C. canephora*) x Cat.] | 20 | - | 100.0 | - |
| 9 | [CCC.1166 x (Cat. x HdT 1343)] | 144 | 22.2 | 41.0 | 36.8 |
| 10 | [(Cat. x *C. canephora*) x Cat.] | 705 | 20.3 | 78.3 | 1.4 |
| 11 | [(Cat. x HdT 1343) x CCC.50] | 14 | - | 92.9 | 7.1 |
| 12 | Cat. x HdT 1343 | 441 | 87.8 | 9.1 | 3.1 |
| Total | | 1512 | | | |

Cat: Caturra. HdT 1343: Timor hybrid CIFC 1343. CCC: Colombian Coffee Collection.

*0: Presence of the molecular marker associated with the susceptibility allelic form.

*1: Presence of the molecular marker associated with the homozygous resistant allelic form.

*1/0: Presence of the molecular marker associated with the heterozygotic allelic form.

**Table 6:** Evaluation of the molecular markers associated with the *Ck-1* resistance gene in populations 9 and 10, and their allelic forms.

| Id. Population | Population | Id. Plant | Id. Line | Molecular Markers | | |
|---|---|---|---|---|---|---|
| | | | | Sat235 | Sat207 | FR34-6CTG |
| 9 | [CCC.1166 x (Cat. x HdT1343)] | 295 | MEG3.11 #0209 | 1 | 1 | 1 |
| 9 | [CCC.1166 x (Cat. x HdT1343)] | 380 | MEG3.12 #1746 | 1 | 1 | 1 |
| 10 | [(Cat. x *C. canephora*) x Cat.] | 377 | MEG6.67 #0669 | 1 | 0 | 0 |
| 10 | [(Cat. x *C. canephora*) x Cat.] | 078 | MEG6.67 #0665 | 1 | 0 | 0 |
| 10 | [(Cat. x *C. canephora*) x Cat.] | 237 | MEG6.67 #0663 | 1 | 0 | 0 |
| 10 | [(Cat. x *C. canephora*) x Cat.] | 278 | MEG6.67 #0206 | 1 | 0 | 0 |
| 10 | [(Cat. x *C. canephora*) x Cat.] | 203 | MEG6.67 #0133 | 1 | 1/0 | 1/0 |
| 10 | [(Cat. x *C. canephora*) x Cat.] | 127 | MEG6.67 #0129 | 1 | 0 | 0 |
| 10 | [(Cat. x *C. canephora*) x Cat.] | 061 | MEG6.67 #0126 | 1 | 0 | 0 |
| 10 | [(Cat. x *C. canephora*) x Cat.] | 492 | MEG6.67 #0119 | 1 | 0 | 0 |
| 10 | [(Cat. x *C. canephora*) x Cat.] | 181 | MEG6.67 #0114 | 1 | 1/0 | 1/0 |
| 10 | [(Cat. x *C. canephora*) x Cat.] | 377 | MEG6.65 #0612 | 1 | 1 | 1 |

In the case of populations with different degrees of generational advancement, the application of molecular markers allowed to corroborate the presence of the allelic form associated with the *Ck-1* gene in the progeny of the variety Ruiru 11 and its parents Gichimu et al. (2014). Maldonado and Ángel (2020), used molecular markers to evaluate the resistance to CBD governed by the *Ck-1* gene in all the component lines of the commercial varieties used in Colombia. The authors found that the derivatives of HdT CIFC 1343 possess the resistance alleles of the markers Sat235 and FR34-6CTG. De Almeida et al. (2021), analyzed the molecular markers in an F2 population derived from the crossing of HdT UFC443-03 x Catuaí, and reported that six progeny contained genes linked to CLR and CBD resistance.

Benti et al. (2021), recognized the *Ck-1* gene with molecular markers in different component lines of 10 coffee varieties in Ethiopia. Thus, the use of these markers favors the selection of improved lines through the recognition of the *Ck-1* gene in different degrees of generational advancement, increasing the efficiency of genetic improvement programs.

**Table 7:** Hypocotyl test and evaluation of molecular markers associated with the *Ck-1* resistance gene with its three allelic forms in population 12.

| Id. Population | Id. Plant | Resistance in the hypocotyl test in percent | | | | | | | | ID Line | Molecular Markers | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Cameroon | Malawi | | Kenya | Rwanda | Zimbabwe | | | | | | |
| | | Cam1 | M | M2 | Que2 | R | Z1 | Z9 | Zim1 | | Sat235 | Sat207 | FR34-6CTG |
| 12 | 996 | 0.00 | - | - | 0.46 | - | 0.86 | 0.64 | - | MEG3.13 #0194 | 1 | 1 | 1 |
| | | | | | | | | | | MEG3.13 #0196 | 1 | 1 | 1 |
| 12 | 998 | 0.66 | - | - | 0.99 | - | - | 1.00 | 0.89 | MEG3.15 #1352 | 1 | 1 | 1 |
| | | | | | | | | | | MEG3.15 #1368 | 1 | 1 | 1 |
| | | - | 0.65 | - | 0.95 | - | - | 0.87 | 0.91 | MEG3.14 #0818 | 1 | 1 | 1 |
| 12 | 1239 | 0.13 | 0.70 | - | 0.91 | - | - | 0.89 | 0.91 | MEG3.15 #1770 | 1 | 1/0 | 1/0 |
| | | | | | | | | | | MEG3.15 #1514 | 1 | 1 | 1 |
| | | | | | | | | | | MEG3.15 #1506 | 1 | 1 | 1 |
| 12 | 1160 | 0.07 | 0.91 | - | 0.96 | - | - | 0.98 | 0.96 | MEG3.14 #1030 | 1 | 1 | 1 |
| 12 | 1315 | 0.00 | 0.75 | - | 0.76 | - | - | 0.81 | 0.66 | MEG3.15 #1449 | 1 | 1 | 1 |
| | | | | | | | | | | MEG3.15 #1448 | 1 | 1 | 1 |
| 12 | 1289 | 0.01 | 0.88 | - | 0.98 | - | - | 0.63 | 0.97 | MEG3.14 #0695 | 1 | 1 | 1 |
| | | | | | | | | | | MEG3.14 #0996 | 1 | 1 | 1 |
| 12 | 1361 | 0.00 | - | - | - | 0.74 | - | 0.85 | 0.99 | MEG3.15 #1703 | 1 | 1 | 1 |
| | | | | | | | | | | MEG3.15 #1705 | 1 | 1 | 1 |
| | | | | | | | | | | MEG3.15 #1460 | 1 | 1 | 1 |
| | | | | | | | | | | MEG3.15 #1458 | 1 | 1 | 1 |
| | | | | | | | | | | MEG3.15 #1455 | 1 | 1 | 1 |
| 12 | 997 | 0.27 | 0.99 | 0.96 | 0.95 | 0.87 | | 0.85 | 0.88 | MEG3.14 #0882 | 1 | 1 | 1 |
| 12 | 1233 | 0.34 | 0.84 | - | 0.88 | - | - | 0.84 | 0.8 | MEG3.14 #0792 | 1 | na | 1/0 |
| | | 0.10 | 0.66 | - | 1.00 | - | - | 0.94 | 0.98 | MEG3.14 #0893 | 1 | 1/0 | 1/0 |
| | | | | | | | | | | MEG3.14 #1267 | 1 | 1 | 1 |

*0: Presence of the molecular marker associated with the susceptibility allelic form.
*1: Presence of the molecular marker associated with the homozygous resistant allelic form.
*1/0: Presence of the molecular marker associated with the heterozygotic allelic form.
*na: non-amplified allelic form.

The results for the molecular markers Sat235, Sat207 and FR34-6CTG for the lines derived from *C. canephora,* indicated the presence of the allelic form associated with resistance to CBD. These findings have also been reported in previous investigations, where the presence of the sequences encoding the molecular markers Sat207 and Sat235, was observed among other markers reported in *C. arabica* in the genome of *C. canephora* (Yu et al., 2021). This result supports the conclusion that several characteristics of agronomic importance present in varieties of *C. arabica*, such as Ruiru 11 in Kenya, IAPAR 59 in Brazil, and the Colombia, Castillo® and Cenicafé 1 varieties in Colombia, leading to resistance to CLR, CBD and nematodes, have been introgressed to *C. arabica* from *C. canephora,* through the HdT.

Previous studies on *C. kahawae* revealed that this species has little genetic variability and no sexual reproduction, with a clearly defined population structure in three groups: Angola, Cameroon and East Africa (Silva et al., 2012). To date, no physiological races of *C. kahawae* have been found in *C. arabica;* however, differences have been reported in the interactions between this fungus and some genotypes of *C. arabica*. However, isolates of *C. kahawae* have been found to differ in their aggressiveness regardless of their geographic origin (Gichuru, 2008; Vieira et al., 2019).

Thus, Vieira et al. (2019), evaluated 26 isolates de *C. kahawae*, classifying them into three classes according to their aggressiveness: high, medium and low. By comparing these results with those obtained in the present investigation, it was observed that the HdT CIFC 1343 population was resistant to isolates of medium-high aggressiveness, such as Que2; moderate-low aggressiveness, such as Zim1; and low aggressiveness, such as M2. Only one genotype presented resistance to the Cam1 isolate, which is reported as having medium to high aggressiveness (Table 7).

## 5 CONCLUSIONS

The results corroborate the potential of the microsatellites Sat235, Sat207 and FR34-6CTG for the identification of the resistance allele associated with the *Ck-1* gene in the lines derived from the HdT CIFC 1343, which is an invaluable genetic resource and of high interest for coffee growers. Additionally, based on indirect selection through molecular markers linked to resistance gen *Ck-1*, it is possible to identify plants with a high level of resistance to CBD isolates when direct tests are carried out through hypocotyl evaluations. Plants here evaluated, with the presence of allelic forms of resistance, showed high resistance to the isolates from Malawi, Kenya, Rwanda, and Zimbabwe.

## 6 AUTHORS' CONTRIBUTION

Conceptualization idea: Flórez, C.; Lopez, L.F.; Methodology design: Lopez, L.F.; Quiroga, J.C; Flórez, C.; Data collection: Lopez, L.F.; Ramirez, C.A.; Arango, N.O.; Data analysis and interpretation: Lopez, L.F.; Quiroga, J.C; Flórez, C.; And writing and editing: Lopez, L.F.; Quiroga, J.C; Flórez, C.].

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