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Potential of the Colombian Coffee Collection as a Source of Genetic Resistance to *Colletotrichum kahawae* JM Waller and PD Bridge

Julio Quiroga-Cardona [1,2,*], Vitor Manuel Pinto Várzea [3], Esther Cecilia Montoya-Restrepo [4], Álvaro León Gaitán-Bustamante [5] and Claudia Patricia Flórez-Ramos [1]

[1] Plant Breeding, National Coffee Research Center, Cenicafé, Manizales 170009, Colombia; claudia.florez@cafedecolombia.com
[2] Faculty of Agricultural Sciences, University of Caldas, Manizales 170001, Colombia
[3] LEAF—Linking Landscape, Environment, Agriculture and Food, Instituto Superior de Agronomia, University of Lisbon, 1349-017 Lisbon, Portugal; vitorvarzea@sapo.pt
[4] Biostatistics, National Coffee Research Center, Cenicafé, Manizales 170009, Colombia; esthercecilia.montoya@cafedecolombia.com
[5] Plant Pathology, National Coffee Research Center, Cenicafé, Manizales 170009, Colombia; alvaro.gaitan@cafedecolombia.com
* Correspondence: julio.quiroga@cafedecolombia.com

Abstract: *Coffee berry disease* (CBD) is not present in the Americas and presents a potential risk for growing coffee. Therefore, Colombia, which has been in scientific cooperation with the *Centro de Investigação de Ferrugens do Cafeeiro* (CIFC) of Portugal for more than 30 years, has been evaluating the genetic resistance of nine populations of *C. arabica* to 13 isolates of *Colletotrichum kahawae* JM Waller and PD Bridge, which are diverse in terms of aggressiveness and geographical origin. The phenotypes observed in the interaction between *C. arabica* and *C. kahawae* were used to develop a statistically reliable scale (p-value ≥ 0.001) to categorize resistance in *C. arabica* into five classes, and this scale was used to classify the nine populations of *C. arabica* evaluated. The results allowed us to corroborate the potential of Timor Hybrid CIFC 1343 (TH CIFC 1343) as a source of genetic resistance to CBD and to identify new genetic sources not yet explored for the development of varieties in Colombia that may eventually mitigate the effects of CBD in the face of increasing rainfall events and minimum temperatures due to climate change, which can favor disease development. Additionally, the results suggest that the existence of races in the *C. arabica*–*C. kahawae* complex is probable, and a selection of genotypes was identified as a possible differential series of races in *C. kahawae*.

Keywords: *coffee berry disease* (CBD); *Colletotrichum kahawae*; *Coffea arabica*; resistance; isolate; aggressiveness; races; genetic improvement; source of resistance; coffee breeding; phenotyping

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1. Introduction

Coffee berry disease (CBD) is caused by *Colletotrichum kahawae* JM Waller and PD Bridge [1,2]. This fungus is classified among the ten main genera that cause diseases in crops and is currently restricted only to the African continent [3–5]. *C. kahawae* affects *Coffea arabica* crops, causing production losses of more than 80% when weather conditions favor pathogen development and preventive measures are not taken to control it [6–8]. The effects of *C. kahawae* on *C. arabica* cultivation are far superior to those caused by *Hemileia vastatrix* Berk and Broome [9]. Its aggressiveness is highly variable across isolates and geographic origins; it presents a clearly defined population structure and is composed of three geographic groups, namely Angola, Cameroon, and East Africa [10–12].

CBD is dispersed across altitudes, affecting coffee plantations established at altitudes as low as 1000 m above sea level (masl) [13,14]. However, the most severe effects occur in coffee plantations established at altitudes above 1400 masl [4,15] in combination with

climate conditions that favor epidemics, including relative humidity close to the saturation point (>95%) [13,16–18] and temperatures ranging between 15 °C and 25 °C [18].

In Africa, in countries where varieties with genetic resistance to CBD are not cultivated, the disease must be controlled with the application of chemically synthesized products [19]. In Kenya, no fewer than eight applications are recommended during the year, distributed in the rainy season, between five and six applications in the first half and between two and three in the second half (personal communication, Dr. Elijah Gichuru, Coffee Research Institute—Kenya). Additionally, fungicide application should be complemented with cultural crop management practices, including pruning and the regulation of conditions that produce favorable microclimates [16,18,20].

From an economic and environmental conservation perspective, the development of varieties with genetic resistance to diseases is the most suitable strategy for ensuring the production and profitability of crops. Proof of this are the unprecedented impacts achieved in Colombia, where the adoption of highly productive varieties of *C. arabica* that are resistant to *H. vastatrix* has had positive impacts on the economy for coffee growers and on environmental sustainability [21,22]. It has also been demonstrated that CBD can be controlled through the development of varieties resistant to *C. kahawae* [16,19,23,24]. This resistance can be found in different sources of *C. arabica*, some of which are derived from interspecific hybridization, such as Timor Hybrid (TH) [25].

TH has been noted as the main source of genetic resistance to diseases that limit *C. arabica* production, and for this reason, it has been used extensively in genetic improvement programs around the world [26]. In Africa, progeny derived from the TH have been shown to be resistant to CBD, providing effective and durable genetic control of epidemics caused by *C. kahawae* [19,27–29].

In the Americas, specifically in Colombia, the Coffee Research Center, Cenicafé, has developed varieties with resistance to CBD from TH CIFC 1343, and the processes of selecting genotypes with resistance to this disease have been based on the following three strategies:

1. **Indirect selection or assisted selection by molecular markers (MAS)**. In Colombia, SSR markers associated with the *Ck-1* gene of resistance to CBD are currently used [30–32]. This biotechnological tool has made it possible to identify the *Ck-1* gene in rust-resistant varieties developed in Colombia [33].
2. **Direct selection through field tests**. Advanced progeny evaluations of TH CIFC 1343 derivatives were carried out from scientific cooperation agreements with the Coffee Research Institute, formerly the Coffee Research Foundation (CRI), Ruiru, in Kenya, between 1975 and 1977 [34,35]. Later, in Kenya, they developed the varieties Ruiru 11 and Batian from derivatives of TH CIFC 1343 [24,36].
3. **Direct selection via the hypocotyl inoculation method**. The advantage of this method is that it allows the identification of resistance to CBD in advanced nonclonal genotypes [37] and presents the highest correlation (>0.73) with the behavior of the disease under natural crop or epidemic conditions [38]. The first tests using this method were carried out 50 years ago at the CRI in Kenya [39]. The results made it possible to identify the resistance of TH CIFC 1343 to *C. kahawae*, and evaluation is currently being carried out at the CIFC.

Historically, the method of evaluating resistance to CBD via the hypocotyl inoculation method uses severity scales [40,41]. However, when scales of this type are used, their interpretation can become controversial [42]. Using already developed scales of evaluation of CBD resistance [40,41] allows for the identification of highly resistant and completely susceptible genotypes with great ease. However, the classification of individuals with intermediate levels of resistance is not clear [40,41], and its complexity lies mainly in the very nature of the resistance character and how it is inherited, aspects of which there has been no clear consensus on to date [9,30,43–45].

In 1997, the Cenicafé genetic improvement program and the CIFC began evaluating nine populations of *C. arabica* for their resistance to different *C. kahawae* isolates, which are diverse in aggressiveness and geographic origin. The purpose of this research was (1) to

develop a scale for the determination of classes of resistance to CBD in improved genotypes and (2) to identify resistance to this disease in unexplored genotypes of the Colombian coffee collection (CCC), which have potential as progenitors of new varieties of coffee.

2. Materials and Methods

2.1. Location

The evaluated populations were established at the Naranjal Experimental Station (04°58′ N, 75°39′ W), which is located in the municipality of Chinchiná (Caldas, Colombia) at 1381 m above sea level; the average temperature is 21.4 °C, the annual precipitation is 2782 mm, and the average relative humidity is 77.5%. Evaluations of resistance to *C. kahawae* were carried out in the laboratories of the CIFC in Portugal for 25 years (1997–2022).

2.2. Genotypes Evaluated

In total, 512 genotypes derived from nine populations were evaluated (Table 1). The Caturra variety, which is susceptible to *C. kahawae*, was used as a control genotype.

Table 1. Populations evaluated and number of genotypes for each population.

	Origin of the Population	Population ID	Number of Genotypes
1	TH CIFC 2252	CCC.650	1
2	TH CIFC 1343	CV.1, CV.2, CV.3, CV.4, CV.6, CV.7, CV.8, CV.9	8
3	Cat. × TH CIFC 832/1	CCC.907, CCC.916, CCC.933, CCC.936, CCC.937, CCC.939, CCC.945	33
4	Cat. × TH CIFC 1343	H.3001, H.3004, H.3005, H.3029, H.3074, H.3083, H.3096	275
5	TH CIFC 1343 × Typica/Bourbon	CCC.1, CCC.2, CCC.4, CCC.12, CCC.43, CCC.129, H.3008, H.3010, H.3102	36
6	Ethiopian accessions resulting from surveys carried out by the FAO and ORSTOM between 1654 and 1966.	CCC.16, CCC.30, CCC.81, CCC.115, CCC.149, CCC.150, CCC.156, CCC.158, CCC.160, CCC.161, CCC.162, CCC.164, CCC.165, CCC.166, CCC.167, CCC.168, CCC.169, CCC.171, CCC.176, CCC.181, CCC.184, CCC.186, CCC.192, CCC.193, CCC.194, CCC.195, CCC.197, CCC.198, CCC.199, CCC.200, CCC.202, CCC.203, CCC.212, CCC.213, CCC.216, CCC.218, CCC.221, CCC.239, CCC.244, CCC.290, CCC.294, CCC.295, CCC.301, CCC.302, CCC.305, CCC.308, CCC.314, CCC.315, CCC.318, CCC.330, CCC.334, CCC.337, CCC.340, CCC.341, CCC.344, CCC.346, CCC.354, CCC.359, CCC.363, CCC.388, CCC.396, CCC.412, CCC.414, CCC.415, CCC.419, CCC.420, CCC.421, CCC.425, CCC.428, CCC.462, CCC.464, CCC.467, CCC.468, CCC.469, CCC.471, CCC.472, CCC.473, CCC.478, CCC.479, CCC.480, CCC.483, CCC.484, CCC.490, CCC.498, CCC.509, CCC.515, CCC.516, CCC.517, CCC.519, CCC.520, CCC.521, CCC.522, CCC.523, CCC.524, CCC.525, CCC.527 CCC.529, CCC.531, CCC.534, CCC.535, CCC.557, CCC.1112, CCC.1113, CCC.1114, CCC.1115, CCC.1145, CCC.1146, CCC.1147, CCC.1148, CCC.1149, CCC.1150, CCC.1151, CCC.1152, CCC.1153, CCC.1154, CCC.1155	116
7	Interspecific hybrids between *C. arabica* × diploid species	CCC.955, H.4026, H.4027, H.4158, H.44203, H.4213, H.4303, H.4309	33
8	Typica/Bourbon and their mutations	AM.252, CCC.1, CCC.12, CCC.129, CCC.2, CCC.4	9
9	Genotypes F3–F6, derived from the cross between the Ethiopian accession CCC.1146 × Cat.	Cat. × CCC.1146	1

Cat.: Caturra; TH: Timor hybrid; CCC: Colombian coffee collection.

2.3. Isolates of *C. kahawae*

The evaluated populations were inoculated with 13 isolates of *C. kahawae*, which are diverse in aggressiveness and belong to the biological collection of the CIFC (Table 2).

Table 2. Geographical origins of *C. kahawae isolates* and their classification by aggressiveness.

Geographic Origin	Isolate ID	Level of Aggressiveness	References
Angola	*Ang29*	High	[12,46]
	A68	*na*	*na*
Cameroon	*Cam1*	High–Medium	[10,12,46]
	CC	*na*	*na*
Malawi	*M*	*na*	*na*
	M2	Low	[10,12]
Kenya	*Que2*	Medium–Low	[10,12,46]
Uganda	*Uga1*	Medium	[12]
Rwanda	*R*	*na*	*na*
Zimbabwe	*Z12*	High–Medium	[12,46]
	Z9	*na*	*na*
	Zim1	Medium–Low	[10,12,46]
	Zim12	*na*	*na*

na: information not available.

2.4. Evaluation of Resistance via Hypocotyl Testing

In each of the evaluations (genotype isolate), between 100 and 120 hypocotyls were tested using the methodology developed by [38,40] and modifications made by the CIFC. Hypocotyls 3 to 6 cm long (5–6 weeks of development) were arranged in a humid chamber on nylon sponges moistened with water and subsequently inoculated with a solution of *C. kahawae* conidia in suspension at an approximate concentration of 2×10^6 conidia per milliliter of water.

Conidia were obtained from in vitro cultures of *C. kahawae* in malt extract agar medium (GEM) [Oxoid, 3.4%] and were grown for eight days at 22 °C. The culture was subsequently resuspended in 5 mL of sterile distilled water. The suspension was filtered through layers of sterile muslin cloth to remove the mycelia. The solution was sprayed on each hypocotyl with an atomizer coupled to an air pressure pump (Pumpentyp Siehe unterseite Pumpe VDE 0530). The inoculated hypocotyls were kept in a humid chamber for 48 h, and a second inoculation was subsequently carried out under the same conditions.

During the first four days of incubation, the humid chambers remained at an average temperature of 22 °C, then subsequently at 19 °C; the relative humidity was close to the saturation point and the photoperiod was 12 h. The infection process was monitored for 4 weeks; during this time, the hypocotyls were classified according to the severity scale [41] (Table 3).

Table 3. CBD phenotypic severity scale in *C. arabica* hypocotyls and description of the development of symptoms caused by *C. kahawae*.

Grade	Symptoms
0	Absence of symptoms (immunity).
1	Development of small greenish lesions up to 1–2 narrow brown lesions, lesions up to 0.5 mm wide.
2	Development of brown lesions that exceed 0.5 mm. Lesions coalesce. The formation of black lesions is not frequent; however, they can occur.
3	Large brown lesions with numerous black spots and/or black lesions. Black lesions can completely surround the stem without death of the upper part.
4	Black lesions completely surround the stem. The upper part of the hypocotyl dies.

2.5. Data Analysis

Each hypocotyl evaluated (observation unit) was classified as resistant if it developed a degree of infection of 1, 2, or 3 and as susceptible if it was Grade 4 according to the severity scale (Table 3) [41]. From each of the populations and isolates (treatments), groups of 10 observation units (sampling units) were randomly formed, with the percentage of resistant hypocotyls as a variable of interest, i.e., of the 10 observation units per sampling unit, the percentage of those that were not classified as Grade 4. For each combination (genotype isolate), the average percentage of resistant hypocotyls was estimated, with their respective intervals. For a confidence coefficient of 95% and with the percentile distribution of the averages, a classification by resistance classes was established.

For each resistance class, the average percentage of resistant hypocotyls was estimated, and the Duncan test at 5% was used to compare the averages. Once the significant differences between classification groups were obtained (categorization of resistance classes), the classification criterion was applied to the populations derived from the Cat. × TH CIFC 832/1, TH CIFC 2252, TH CIFC 1343, Cat. × TH CIFC 1343, and CCC populations.

3. Results

3.1. Resistance Class Scale

With the described methodology, a scale was obtained for the classification of *C. arabica* genotypes on the basis of their CBD resistance. The scale is made up of four resistance classes, namely high resistance (HR), moderate resistance (MR), low resistance (LR), and very low resistance (VLR), as well as a susceptible class (S). Each resistance class was established according to the percentage of hypocotyls with phenotypic reactions of severity between 1 and 3, that is, classified as resistant (Tables 3 and 4).

Table 4. Scale for the classification of *C. kahawae* resistance in *C. arabica* and the percentage of hypocotyls with phenotypic reactions of severity 1, 2, and 3.

Percentage of Hypocotyls Resistant	Classes of Resistance
≥79%	High (HR)
>50 ≤ 79%	Moderate (MR)
>20 ≤ 50%	Low (LR)
>0 ≤ 20%	Very low (VLR)
=0%	Susceptible (S)

The classification did not include a class that considers the absence of the disease because in the populations evaluated, no immunity to *C. kahawae* was found and to date, there are no reports of immunity in *C. arabica*.

Duncan's comparison test at 5% revealed differences between the averages of the five classes, statistically separating them (Table 5) with a reliability greater than 95%. Therefore, the statistically obtained scale is a reliable tool for phenotypic categorization by classes of *C. kahawae* resistance in *C. arabica*.

Table 5. Average percentage of hypocotyls resistant to *C. kahawae* by class and their respective confidence intervals.

Resistance Class		Repetitions	Mean *	Li **	Ls **
1	High (HR)	300	85.3 a	84.0	86.6
2	Moderate (MR)	1565	55.9 b	54.4	57.5
3	Low (LR)	16,828	37.8 c	37.3	38.4
4	Very low (VLR)	12,740	6.1 d	5.8	6.4
5	Susceptible (S)	709	0.0 e	0.0	0.0

* Averages with different letters differ statistically according to Duncan's test at a significance level of 5% (p-value < 0.001). ** Lower limit (Li) and upper limit (Ls). The confidence intervals Li and Ls correspond to a 95% confidence level for estimating the average.

The classification of a genotype into one of the classes with the highest level of resistance to CBD, HR (Figure 1A) or MR (Figure 1B), is mainly due to the percentage of hypocotyls that were less affected by the disease, that is, Grades 1 and 2 (Table 3). In these cases, the advancement and development of *C. kahawae* in the hypocotyls was restricted to the formation of small lesions, which may eventually coalesce without the disease progressing to the most advanced stages (Grades 3 and 4).

For the HR class, the hypocotyls least affected by CBD (Grade 1) contributed the most to categorization into this group, since they represented greater than 70% of the tested hypocotyls, with 28% developing Grade 2 symptoms and 2% developing Grade 3 symptoms. In Grade 3, lesions that affect the circumference of the hypocotyl were present, and although the disease progressed, the hypocotyl survived until the end of the evaluation.

The MR class presented a lower percentage of Grade 1 hypocotyls (55%) than the HR class and greater percentages of hypocotyls with Grade 2 and Grade 3 symptoms. At least 45% of the hypocotyls presented Grades 2 and 3 symptoms in the MR class, corresponding to a 15% decrease in the number of hypocotyls with Grade 1 symptoms compared with the HR class.

The genotypes in the LR (Figure 1C) and VLR (Figure 1D) classes mainly presented Grade 1 resistance (Table 4), corresponding to 54% and 72% of the hypocotyls, respectively. The remaining hypocotyls were Grades 2 and 3. As in the other resistance classes, the hypocotyls that reached Grade 3 represented the lowest proportion.

The S class (Tables 3 and 4) consisted completely of Grade 4 phenotypes (Figure 1D). This level of CBD development is characterized by the presence of lesions that completely necrotize the stem, followed by the complete death of the hypocotyl tissues.

In the genotypes least affected by *C. kahawae* (Grades 1 and 2), more than 90% of the hypocotyls presented resistance, thus defining the resistance class in which a genotype can be classified. However, the percentage of resistant hypocotyls taken as a reference for categorizing a genotype into one of the four resistance classes included those in the first three degrees of phenotypic qualification (Grades 1, 2, and 3). That is, the resistance class of a genotype is not exclusively due to the concentration of symptoms to a defined degree.

In the hypocotyls cataloged within the HR class, 70% were Grade 1, among which the selection limit of the class (≥79%) was present in 58.8% of the cases, and 10.9% were Grade 2. In other words, it is highly probable (≥95%) to find HR genotypes, and their resistance classification (class) is exclusive to Grade 1.

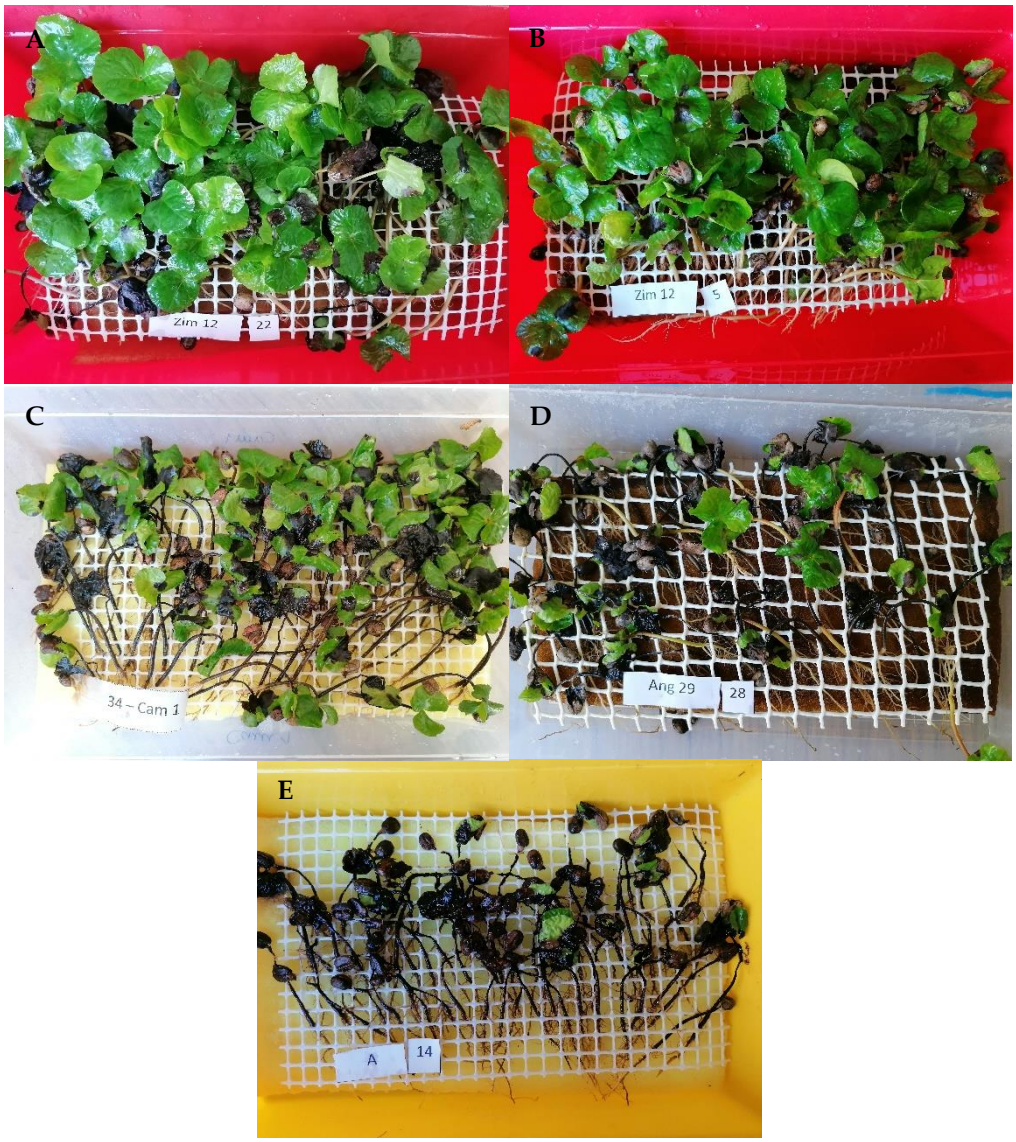

Figure 1. Phenotypic response of *C. arabica* to *C. kahawae* and its classification on the scale of resistance. (**A**) Phenotypic response of the genotypes of the Cat. × TH CIFC 1343 population classified in the high resistance (HR) class to the Zim12 (Zimbabwe) isolate. (**B**) Phenotypic response of the genotypes of the Cat. × TH CIFC 1343 population classified in the moderate resistance (MR) class to the Zim12 (Zimbabwe) isolate. (**C**) Phenotypic response of the genotypes of the Cat. × TH CIFC 1343 population classified into the low resistance (LR) class to the Cam1 (Cameroon) isolate. (**D**) Phenotypic response of the genotypes of the Cat. × TH CIFC 1343 population classified in the very low resistance (VLR) class to the Ang29 (Angola) isolate. (**E**) Phenotypic response of the genotypes of the Cat. × TH CIFC 1343 population classified in the susceptible class to the A (Angola) isolate.

For the MR class, the selection range corresponded to >50% and ≤79% of the hypocotyls as a result of the sum of Grade 1 + Grade 2 + Grade 3 hypocotyls (Tables 3 and 4). However, unlike those in the HR class, the hypocotyls within the selection limit were not concentrated within a specific severity degree. Therefore, the sum of the number of hypocotyls in the two lowest severity grades (Grades 1 and 2) ultimately defines the resistance class (MR).

Among the genotypes of the LR class (>20% and ≤50%), the resistant hypocotyls were equally distributed among the three degrees of severity, similar to MR. However, for a genotype to be considered LR, a greater number of hypocotyls with Grade 2 phenotypic reactions are needed. The VLR class is the product of a higher frequency of Grade 2

phenotypic responses, with fewer Grade 1 and Grade 3 responses. Finally, the S genotypes present exclusively Grade 4 phenotypic reactions.

Of the 512 genotypes evaluated, 98.2% were evaluated with isolates from two of the three documented population origins, Cameroon and East Africa. The East Africa isolates were obtained from five countries (Malawi, Kenya, Rwanda, Uganda, and Zimbabwe) (Table 6). The remaining percentage was evaluated with isolates from the third geographical origin (Angola).

A total of 38.73% of the evaluated progeny developed HR and MR phenotypes to the *Cam1*, *M*, *M2*, *Que2*, *R*, *Z12*, *Z9*, *Zim1*, and *Zim12* isolates, and more severe reactions to *C. kahawae* (LR and VLR) occurred in 40.5% of the progeny when evaluated with the same isolates. Reactions of complete susceptibility (class S) developed in 20.77% of the progeny (Table 6).

In the evaluated populations, no high or medium resistance was observed for isolates originating from Angola and Uganda. The highest proportion of the evaluated genotypes presented high/medium resistance to isolates from Kenya (*Que2*), Zimbabwe (*Zim1*, *Z9*), and Malawi (*M*) (Table 6).

Table 6. Percentage of genotypes evaluated by isolate and the *C. kahawae* resistance classes in which they were grouped.

Isolate	HR	MR	LR	VLR	S	Total
A68	0.00	0.00	0.00	0.00	0.07	0.07
Ang29	0.00	0.00	0.14	0.96	0.82	1.91
Cam1	0.20	1.02	2.39	6.76	12.09	22.47
CC	0.00	0.00	0.00	0.96	0.07	1.02
M	3.83	2.73	1.23	0.55	0.55	8.88
M2	1.43	0.20	0.00	0.07	0.00	1.71
Que2	5.60	6.01	4.51	3.96	1.71	21.79
R	0.07	0.14	0.07	0.48	0.00	0.75
Uga1	0.00	0.00	0.00	0.00	0.07	0.07
Z12	0.27	0.20	0.48	0.34	0.20	1.50
Z9	3.42	3.01	3.69	3.21	2.19	15.51
Zim1	3.89	5.26	5.60	4.44	2.87	22.06
Zim12	0.41	1.02	0.48	0.20	0.14	2.25
Total	19.13	19.60	18.58	21.93	20.77	100.00

HR: high resistance; MR: moderate resistance; LR: low resistance; VLR: very low resistance; S: susceptible.

3.2. Resistance to CBD in TH from the Class Scale

In the populations derived from TH CIFC 832/1, only the LR class was identified for the isolate *Que2* in one of the progeny (HG.106), which also presented VLR to the isolate *Zim1*. The other progeny were classified as VLR and S for the four isolates (*Cam1*, *Que2*, *Z9*, *Zim1*). The S class predominated in the population derived from TH CIFC 832/1, similar to the results obtained for the Caturra variety (Figure 2).

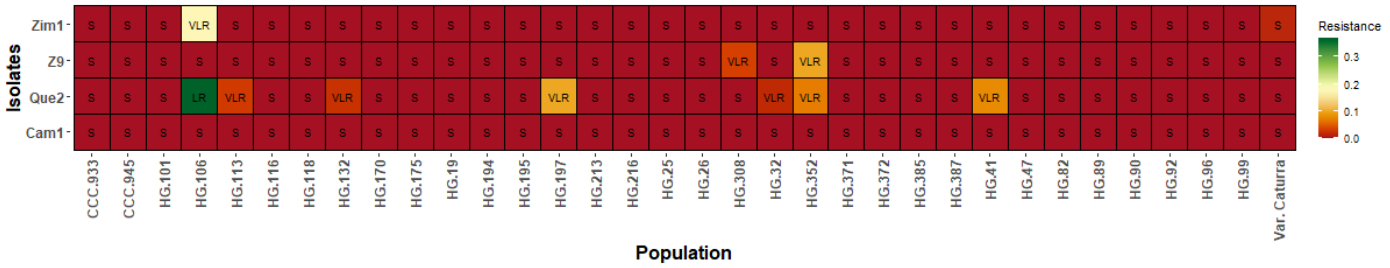

Figure 2. Classification of resistance to the *Cam1*, *Que2*, *Z9*, and *Zim1* isolates of *C. kahawae* in the genotypes of the Cat. × TH CIFC 832/1 population. HR: high resistance; MR: moderate resistance; LR: low resistance; VLR: very low resistance; S: susceptible.

In the results obtained from the classification of resistance to *C. kahawae* in TH CIFC 1343, the CV.9 genotype presented an HR phenotype for the *Cam1* isolates, which have been reported as being highly aggressive, and for the *Que2* and *Zim1* isolates, which are of medium–low aggressiveness. TH CIFC 2252 presented an MR phenotype for the *Que2* isolate and susceptibility to the other isolates (Figure 3).

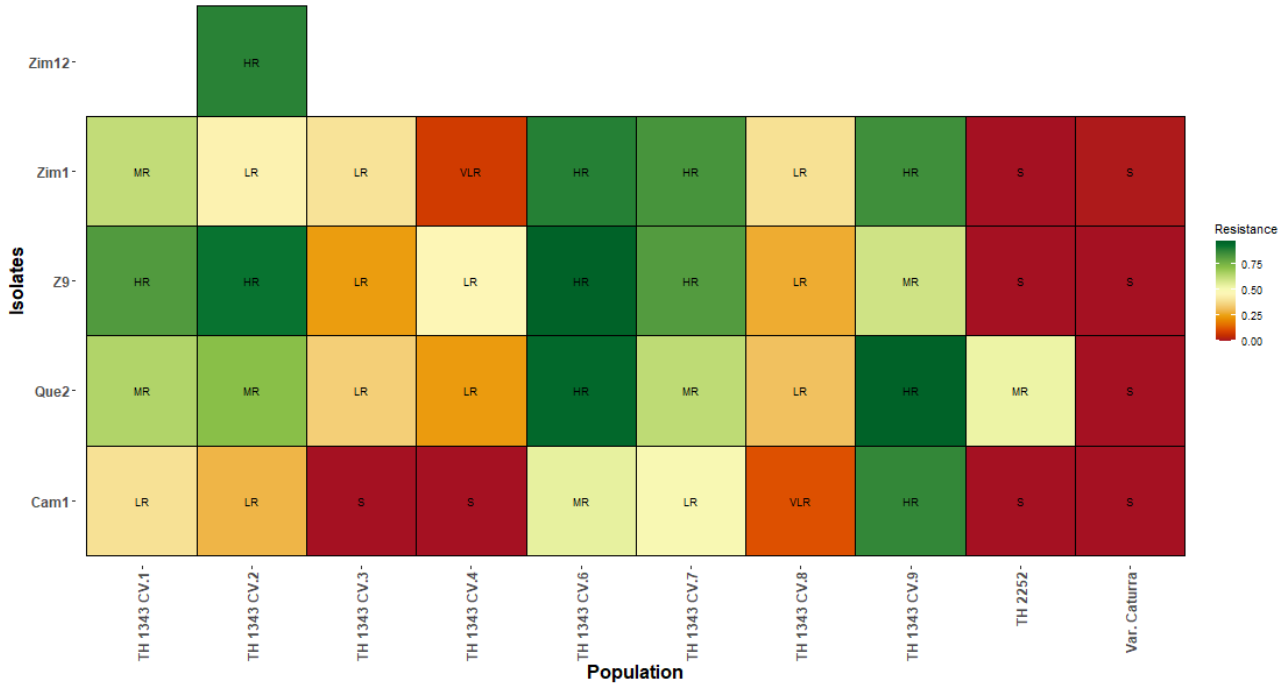

Figure 3. Classification of resistance to the *Cam1*, *Que2*, *Z9*, *Zim1*, and *Zim12* isolates of *C. kahawae* in TH CIFC 1343 and TH CIFC 2252. HR: high resistance; MR: moderate resistance; LR: low resistance; VLR: very low resistance; S: susceptible.

3.3. CBD Resistance Classes in Cat. × TH CIFC 1343

The populations derived from Cat. × TH CIFC 1343 consisted of 275 genotypes of advanced generations (F_3–F_6), which were analyzed and classified according to the phenotypic classes of resistance determined in this study (Table 4). This population was evaluated with 11 isolates of different levels of aggressiveness and diverse geographic origins. All the phenotypic classes of resistance to *C. kahawae* were identified in the genotypes evaluated, ranging from genotypes of the HR class, through to the intermediate classes of resistance, to the VLR and susceptible classes (Table 7).

Table 7. Total percentage of genotypes evaluated by isolate and the *C. kahawae* resistance classes in which the Cat. × TH CIFC 1343 population was grouped.

Isolate	HR	MR	LR	VLR	S	Total
Ang29	0.00	0.00	7.41	51.85	40.74	100.00
Cam1	0.37	4.40	9.16	34.07	52.01	100.00
CC	0.00	0.00	0.00	93.33	6.67	100.00
M	43.75	31.25	14.06	5.47	5.47	100.00
M2	87.50	12.50	0.00	0.00	0.00	100.00
Que2	27.76	26.24	19.77	18.25	7.98	100.00
R	9.09	18.18	9.09	63.64	0.00	100.00
Z12	15.79	15.79	36.84	21.05	10.53	100.00
Z9	24.28	21.97	17.34	21.39	15.03	100.00
Zim1	19.10	24.34	24.72	19.85	11.99	100.00
Zim12	12.90	48.39	22.58	9.68	6.45	100.00

HR: high resistance; MR: moderate resistance; LR: low resistance; VLR: very low resistance; S: susceptible.

3.4. Resistance to C. kahawae Isolates in Cat. × TH CIFC 1343

3.4.1. Resistance to the *Ang29* Isolate

Two classes of resistance to the *Ang29* isolate of Angolan origin were found, namely LR and VLR. A total of 7.41% of the progeny were grouped in the LR class; that is, between 20% and 50% of their hypocotyls presented Grade 1, 2, or 3 reactions. The progeny identified in this class are *CU.1842* and *BH.1247* (Figure 4). The remaining hypocotyls developed phenotypic reactions of the VLR class (51.85%) or were completely susceptible (40.74%).

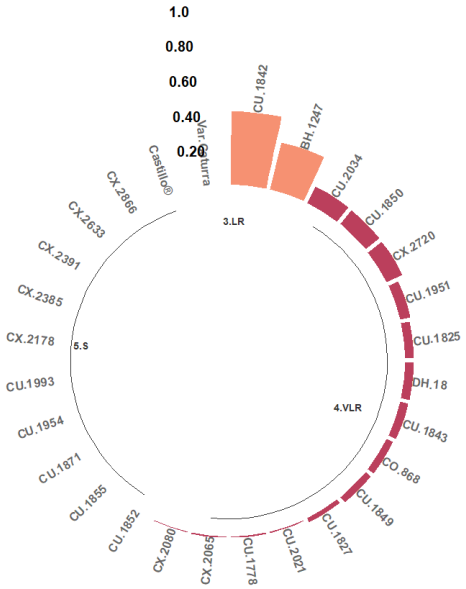

Figure 4. Distribution of resistance classes to the *C. kahawae* Ang29 isolate in progeny derived from the Cat. × TH CIFC 1343 population. LR: low resistance; VLR: very low resistance; S: susceptible. The amount of resistance to CBD as determined by the resistance scale is represented by the numbers on the *Y*-axis.

3.4.2. Resistance to the *Cam1* and CC Isolates

The resistance classes for these isolates were more diverse than those for *Ang29*. For the *Cam1* isolate, 0.37% of the genotypes were categorized as HR (*CU.1997*) and 4.4% were categorized as MR (*A.323*, *BK.261*, *B.998*, *DH.155*, *DH.145*, *BI. 486*, *A.437*, *DG.1365*,

DT.210, *DG.1376*, *CX.2171*, and *BH.914*) or LR (*DH.124*, *DH.77*, *DG.1025*, *CX.2714*, *DG.1386*, *DG.1399*, *CX.2073*, *A.41*, *B.996*, *CU.1994*, *A.222*, *BG.89*, *CU.1796*, *A.328*, *CU.1826*, *CIN* (*B.998*), *BH.1247*, *BK.40*, *CU.1903*, *CX.2369*, *DH.82*, *CX.2354*, *BI.720*, *NR.270*, and *CX.2224*). The remaining percentage was classified as VLR or susceptible (Figure 5A). For the *CC* isolate, all genotypes were classified into the VLR or S classes (Figure 5B).

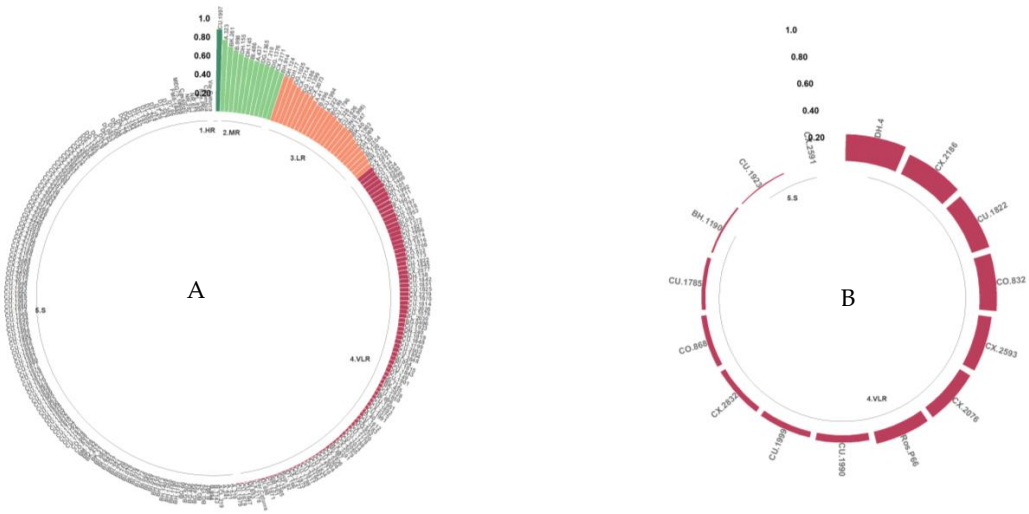

Figure 5. Distribution of resistance to *C. kahawae* isolates *Cam1* (**A**) and *CC* (**B**) in the progeny derived from the Cat. × TH CIFC 1343 population. HR: high resistance; MR: moderate resistance; LR: low resistance; VLR: very low resistance; S: susceptible. The amount of resistance to CBD as determined by the resistance scale is represented by the numbers on the *Y*-axis.

3.4.3. Resistance to the *M* and *M2* Isolates

For the isolates from Malawi (*M* and *M2*), more than 75% of the population evaluated was classified as HR or MR. Very few genotypes were grouped into the least resistant classes (Figure 6A,B). In the resistance tests with *M2*, no S class genotypes were found.

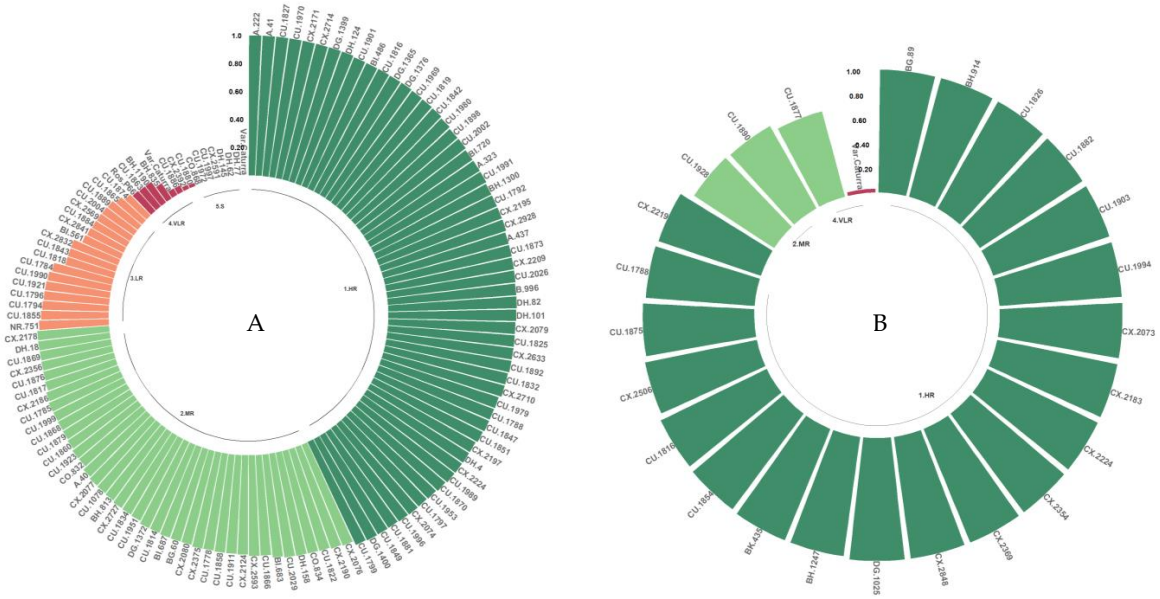

Figure 6. Distribution of resistance to *C. kahawae* isolates *M* (**A**) and *M2* (**B**) in the progeny derived from the Cat. × TH CIFC 1343 population. High resistance; MR: moderate resistance; LR: low resistance; VLR: very low resistance; S: susceptible. The amount of resistance to CBD as determined by the resistance scale is represented by the numbers on the *Y*-axis.

3.4.4. Resistance to the *Que2* Isolate

For the Kenyan isolate, the HR, MR, and LR classes were observed. The genotypes with the highest resistance corresponded to 73.76% of the population evaluated with *Que2*, most notably *BH.914*, *BK.40*, *CU.1994*, *CX.2183*, *CX.2354*, *DG.1025*, *DT.210*, and *IF.1601*, along with *A.241*, *CU.2583*, *DG.1365*, *DH.82*, *PL.892*, *CU.1824*, *B.998*, *BK.261*, *CX.2848*, *DH.101*, *CU.2002*, *CX.2171*, *CU. 1903*, *CX.2710*, *A.328*, *BG.89*, *DH.155*, *CX.2714*, *CX.2928*, *DT.166*, *BI.321*, *CU.1854*, *DH.124*, *CU.1969*, *BK.435*, *DT.149*, *CU.1832*, *BI.073*, *CU.1983*, *BH.1247*, *DH.173*, *CX.2583*, *CU.1817*, *DN.240*, *BH.1300*, *CX.2537*, *CX.2383*, *CX. 2209*, *CU.1849*, *CU.1992*, *BI.078*, *DG.1400*, *A.222*, *CU.1892*, *CU.1819*, *CU.2012*, *B.1143*, *DG.1399*, *CU.1827*, *CX.2219*, *BI.076*, *DG.1376*, *DN.79*, *CU.1816*, *CX.2190*, *NR.270*, *DT.274*, *CU.1814*, *CU.1953*, *CU.1996*, *DH.77*, *CU.1882*, *DT.267*, *CX.2077*, and *BI.120*. Only 18.25% of the genotypes were categorized in the VLR class, and only 7.98% were grouped in the S class (Figure 7).

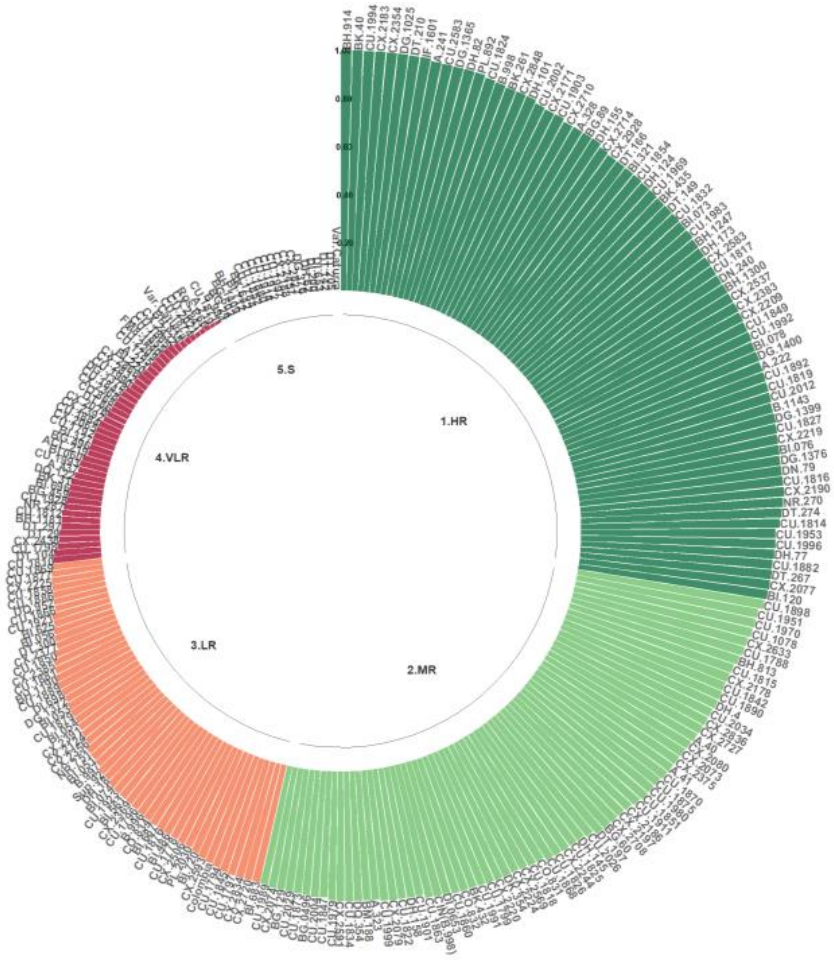

Figure 7. Distribution of resistance to *C. kahawae* isolate *Que2* in the progeny derived from the Cat. × TH CIFC 1343 population. HR: high resistance; MR: moderate resistance; LR: low resistance; VLR: very low resistance; S: susceptible. The amount of resistance to CBD as determined by the resistance scale is represented by the numbers on the *Y*-axis.

3.4.5. Resistance to the R Isolate

Four different classes of resistance to *C. kahawae* of Rwandan origin were observed. In this sense, 36.36% of the progenies (*CU.1816*, *CU.1825*, *CU.1827*, and *CU.1842*) were grouped into the HR, MR, and LR classes, and 63.64% were in the VLR class (Figure 8). No genotypes were identified that were classified in class S for the *R* isolate.

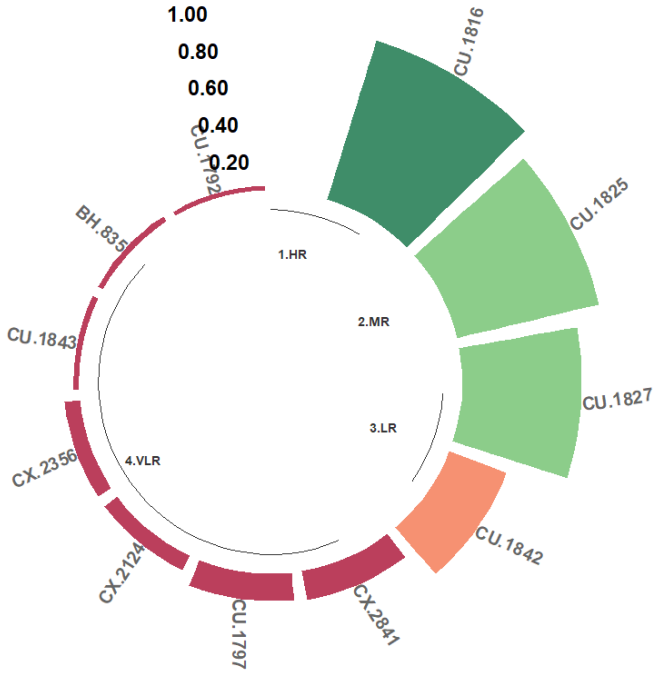

Figure 8. Distribution of resistance to *C. kahawae* isolate *R* in the progeny derived from the Cat. × TH CIFC 1343 population. HR: high resistance; MR: moderate resistance; LR: low resistance; VLR: very low resistance; S: susceptible. The amount of resistance to CBD as determined by the resistance scale is represented by the numbers on the *Y*-axis.

3.4.6. Resistance to the *Z12*, *Z9*, *Zim1*, and *Zim12* Isolates

The resistance classes observed for isolates *Z12*, *Z9*, *Zim1*, and *Zim12*, which originated in Zimbabwe, were mainly HR, MR, and LR (Figure 9A–D). Of the evaluated genotypes, 87.4% were classified as HR, MR, or LR, of which 20.4% were HR. The S class constituted 12.6% of the genotypes evaluated with these isolates.

3.5. Resistance in Other Populations

When accessions of Ethiopian origin from the CCC were evaluated, all four resistance classes and the susceptibility class were observed, with high and medium resistance (HR and MR) to isolates from Cameroon and Angola, which are reported to be highly aggressive (*Cam1*, *Ang29*), which is particularly noteworthy (Table 2). Similarly, for isolates of high/medium and medium/low aggressiveness from Kenya and Zimbabwe, between 4.0% and 10.8% of genotypes were classified in the HR class and between 3.7% and 17.8% in the MR class. The remaining genotypes were grouped into the least resistant classes (Table 8). In the Cat. × Ethiopian population, the evaluated genotypes were grouped into the least resistant classes, LR and VLR, with no genotypes classified into the HR and MR classes.

Genotypes derived from TH CIFC 1343 and the Typica/Bourbon group were classified as HR and MR for the isolates of Kenyan and Zimbabwean origin and MR for the *Cam1* isolate. Other genotypes were classified into the VLR class. In the population derived from hybridization between *C. arabica* and a diploid *Coffea* species, *C. canephora*, a higher percentage of genotypes were grouped into the HR class than in the other populations. Similarly, a higher percentage of genotypes were classified as MR for the *Cam1* isolate, which is considered highly aggressive (Table 8).

In the Typica/Bourbon group, resistance was distributed across all the resistance classes and the susceptibility class. Notably, some genotypes were entirely classified as HR to isolates of Zimbabwean origin (Table 8).

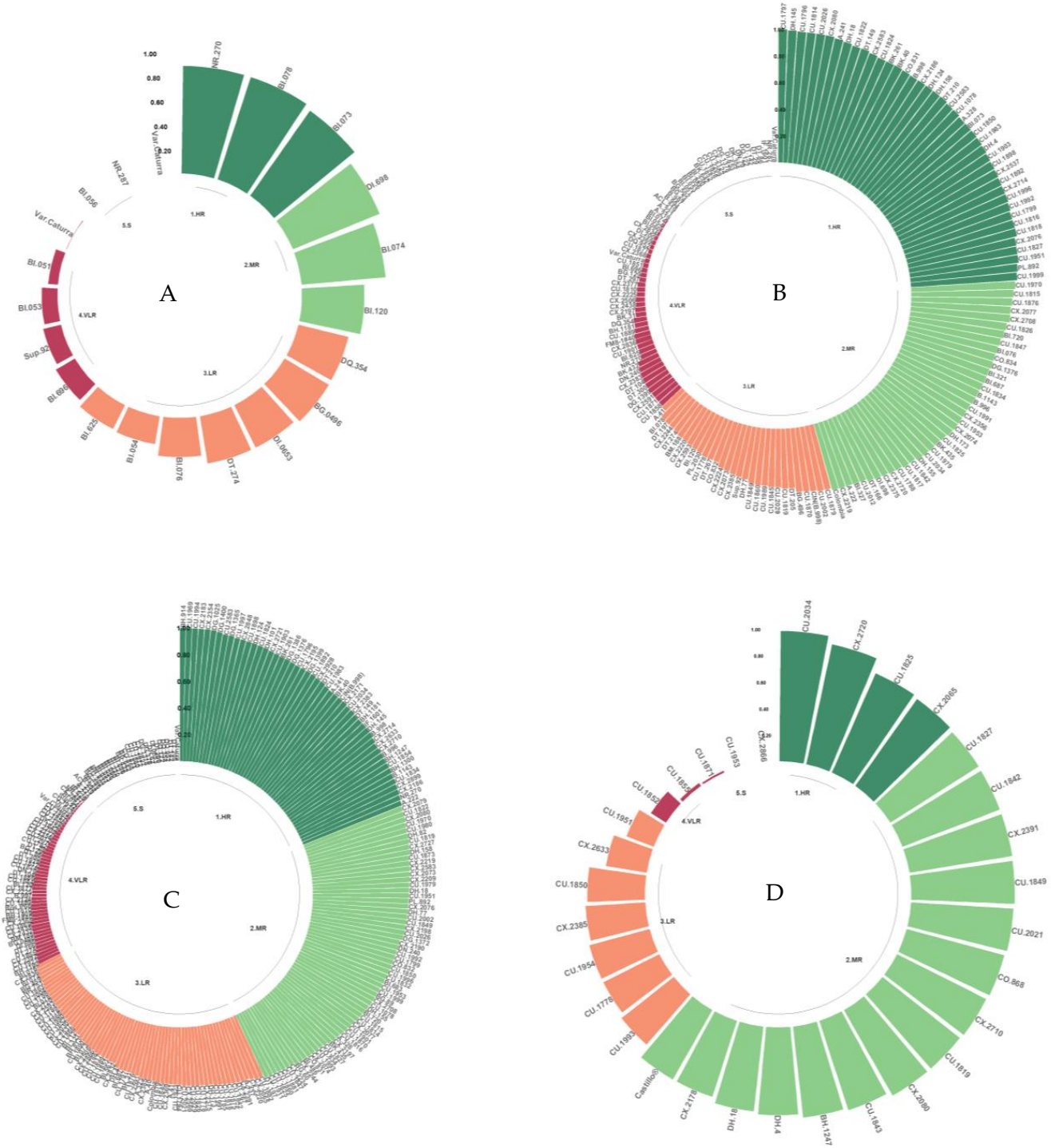

Figure 9. Distribution of resistance to *C. kahawae* isolates *Z12* (**A**), *Z9* (**B**), *Zim1* (**C**), and *Zim12* (**D**) in the progeny derived from the Cat. × TH CIFC 1343 population. HR: high resistance; MR: moderate resistance; LR: low resistance; VLR: very low resistance; S: susceptible. The amount of resistance to CBD as determined by the resistance scale is represented by the numbers on the *Y*-axis.

Table 8. Total percentage of genotypes evaluated by isolate and the *C. kahawae* resistance classes in which the populations of the Colombian coffee collection were grouped.

Population	Isolate	HR	MR	LR	VLR	S	Total
Cat. × Ethiopian	*Cam1*	-	-	-	100.00	-	100.00
	Que2	-	-	100.00	-	-	100.00
	Z9	-	-	100.00	-	-	100.00
	Zim1	-	-	100.00	-	-	100.00
Ethiopian	*Ang29*	-	6.35	12.70	26.98	53.97	100.00
	Cam1	1.98	2.97	10.89	26.73	57.43	100.00
	Que2	10.89	17.82	17.82	21.78	31.68	100.00
	Z12	5.00	3.75	10.00	32.50	48.75	100.00
	Z9	-	13.64	22.73	13.64	50.00	100.00
	Zim1	5.88	9.80	15.69	15.69	52.94	100.00
	Zim12	4.00	-	12.00	28.00	56.00	100.00
TH CIFC 1343 × Typica/Bourbon	*Cam1*	-	2.78	5.56	11.11	80.56	100.00
	Que2	11.11	38.89	25.00	19.44	5.56	100.00
	Z9	11.11	11.11	47.22	25.00	5.56	100.00
	Zim1	5.56	25.00	27.78	25.00	16.67	100.00
Interspecific hybrids	*Ang29*	-	-	-	100.00	-	100.00
	Cam1	-	12.90	9.68	22.58	54.84	100.00
	Que2	32.26	12.90	3.23	19.35	32.26	100.00
	Z12	75.00	25.00	-	-	-	100.00
	Z9	29.63	11.11	7.41	11.11	40.74	100.00
	Zim1	29.03	9.68	6.45	9.68	45.16	100.00
	Zim12	-	50.00	-	-	50.00	100.00
Typica/Bourbon	*Cam1*	11.11	11.11	55.56	-	22.22	100.00
	Que2	33.33	11.11	22.22	22.22	11.11	100.00
	Z12	100.00	-	-	-	-	100.00
	Z9	-	14.29	57.14	-	28.57	100.00
	Zim1	11.11	22.22	33.33	11.11	22.22	100.00
	Zim12	100.00	-	-	-	-	100.00

Cat.: Caturra; TH: Timor hybrid; HR: high resistance; MR: moderate resistance; LR: low resistance; VLR: very low resistance; S: susceptible.

3.6. Evidence for the Existence of Races in C. kahawae

Although there is no conclusive evidence for the existence of races in *C. kahawae*, our results suggest that they likely occur in the *C. arabica–C. kahawae complex*. This behavior in pathogen specificity on the host (*C. arabica*) was observed in genotypes derived from Cat. × TH CIFC 1343, wild genotypes, and interspecific hybrids (*C. arabica* × *C. canephora*) evaluated with isolates of high and medium/low aggressiveness (Table 9). While some genotypes were classified in class S, some isolates were also grouped in the HR class or others.

Table 9. Percentage of hypocotyls affected by CBD with high resistance (Grade 1) and complete susceptibility (Grade 4) in the *C. arabica*–*C. kahawae* complex in genotypes from four populations.

Population	Genotype	*Ang29*	*Cam1*	*M*	*M2*	*Que2*	*Z9*	*Zim1*
Cat. × TH 1343	DH.18	95 (S)	99 (S)	-	-	-	97 (HR)	-
Cat. × TH 1343	CX.2080	99 (S)	100 (S)	-	-	-	99 (HR)	-
Cat. × TH 1343	CU.1997	-	-	100 (S)	-	100 (S)	-	97 (HR)
Cat. × TH 1343	CU.1898	-	100 (S)	90 (HR)	-	-	-	97 (HR)
Cat. × TH 1343	CU.1873	-	100 (S)	93 (HR)	-	-	-	-
Cat. × TH 1343	CX.2195	-	99 (S)	94 (HR)	-	-	-	90 (HR)
Cat. × TH 1343	CU.1980	-	100 (S)	97 (HR)	-	-	-	-
Cat. × TH 1343	CU.1969	-	100 (S)	99 (HR)	-	-	-	99 (HR)
Cat. × TH 1343	A.241	-	100 (S)	-	-	99 (HR)	98 (HR)	-
Cat. × TH 1343	CU.1882	-	100 (S)	-	100 (HR)	-	-	-
Cat. × TH 1343	CU.2026	-	91 (S)	-	-	-	98 (HR)	-
Cat. × TH 1343	CX.2183	-	97 (S)	-	92 (HR)	-	-	97 (HR)
Cat. × TH 1343	CX.2369	-	-	-	99 (HR)	-	97 (S)	-
Cat. × TH 1343	CX.2506	-	99 (S)	-	91 (HR)	-	93 (S)	-
Cat. × TH 1343	CX.2848	-	94 (S)	-	94 (HR)	-	-	-
Cat. × TH 1343	DT.149	-	95 (S)	-	-	91 (HR)	-	-
Cat. × TH 1343	PL.892	-	100 (S)	-	-	99 (HR)	-	-
Ethiopian	CCC.1148	-	92 (S)	-	-	-	-	92 (HR)
Ethiopian	CCC.203	-	100 (S)	-	-	94 (HR)	-	-
TH 1343 /Typica /Bourbon	CJ.211	-	100 (S)	-	-	-	95 (HR)	-
HI	HE.811	-	99 (S)	-	-	-	92 (HR)	-

Cat.: Caturra; HI: interspecific hybrid; CCC: Colombian coffee collection; HR: high resistance; S: susceptible.

These genotypes can eventually be considered a differentiating series of possible races in *C. kahawae*.

4. Discussion

4.1. CBD Resistance Class Scale

In countries where CBD is absent, direct [40,47,48] and indirect [30,31,49] laboratory techniques are the tools that support quick decision-making, identifying genotypes with genetic resistance to the disease. However, direct techniques such as those used to identify resistance to CBD [40,47,48] and the information obtained can represent a challenge for interpretation when the method of qualification and registration of the information is not standard [42].

In this sense, the direct evaluation technique using hypocotyls [38,40,41] qualifies the phenotypic reaction against *C. kahawae* by registering the degree of disease development [40,41]. However, the *C. arabica* genetic improvement program in Colombia has shown the difficulty of interpreting these scales; while they unequivocally identify completely resistant and susceptible genotypes, classifying the intermediate categories is more complex. This difficulty has represented a challenge for plant breeders in Colombia; however, it has not been an impediment to advancing the development of varieties resistant to CBD. On the other hand, from the very moment of the conception of the hypocotyl methodology to evaluate CBD resistance in the 1970s, it was evident that identifying intermediate categories of resistance

to CBD represented greater complexity, as suggested by Cook [40], who developed the method of evaluating resistance to CBD via the hypocotyl method.

These categories of intermediate resistance to CBD, which are highly complex with respect to classification, suggest that resistance to the disease is controlled by genes with an additive dominant effect and in some cases, with a recessive effect [43,49], but there is no current consensus in the scientific community on the type of resistance mechanisms present [9]. Therefore, having a standard protocol for objective data interpretation or the evaluation of resistance via hypocotyl tests is highly beneficial for genetic improvement programs, since this method presents a greater correlation (>0.73) with CBD incidence values under field conditions [38].

The classification scale of genetic resistance to CBD for *C. arabica* presented in this study was obtained from the rigorous evaluation of 331,386 hypocotyls using the standard evaluation method, corresponding to four times as many hypocotyls than that used when the method was first developed [48,49]. The scale groups resistance to CBD into five classes, HR, MR, LR, VLR, and S (Table 4), on the basis of the assessment of the degree of disease progression, with greater than 95% reliability [41]. Before this report, there was no scale that would allow this classification to be carried out with statistical support.

This advance in the categorization for the objective classification of resistance to CBD from a rapid, direct technique and with high correlation with the expression of the disease under epidemic conditions contributes to making decisions in the selection of genotypes resistant to *C. kahawae.* This contribution to the classification of the phenotypic expression of resistance to CBD complements the advances achieved in Kenya and Ethiopia in the 1970s and 1980s [38,40,48], which to date have supported the processes of genetic improvement for resistance to CBD in *C. arabica*.

The scale proposed here classifies resistance in *C. arabica*, regardless of the isolate, because it was obtained from different sources of genetic resistance; isolates with different levels of aggressiveness, high, medium, and low [10,12,46], and three geographical origins, Angola, Cameroon, and Kenya [4,11] were selected. Additionally, the adoption of the scale by genetic improvement programs for resistance to CBD will complement the classifications obtained from the average degree of infection [41,50] and may become a single and standard language, which can easily be interpreted by plant breeders and phytopathologists from countries seeking to advance the development of varieties of *C. arabica* with resistance to *C. kahawae* using the hypocotyl test.

On the other hand, this is the first time that a country without the disease has developed a statistically reliable tool to perform the phenotypic preselection of genotypes with resistance to CBD, which will doubtlessly benefit other countries in their selection processes.

4.2. Aggressiveness of C. kahawae Isolates in TH

No immunity to CBD was identified in the populations evaluated. The resistance expressed by the evaluated genotypes was distributed among the different resistance classes (Table 4). This behavior can be attributed to the very nature of the inheritance of the genes responsible for resistance [43,51], complemented by the evident genetic diversity in resistance to different isolates of *C. kahawae.* In this manner, what has been suggested by other researchers is confirmed [52].

Indirectly, the phenotypic response of the genotypes to specific isolates can be an indicator of the aggressiveness of each of the isolates. This result is in agreement with that reported for isolates originating from Angola and Cameroon, which have been classified as highly aggressive [10,12,46]. On the basis of the percentages of hypocotyls in the least resistant classes (VLR and S in the populations evaluated), *C. kahawae* from Angola (*Ang29*) and Cameroon (*Cam1*, *CC*) can be considered the most aggressive isolates among the isolates referenced in this research. Among the hypocotyls evaluated with these isolates, more than 86% developed lesions forming a ring around the entire hypocotyl or completely died. These symptoms are equivalent to the maximum expression of the disease [41].

Isolates of Rwandan origin have also been identified as highly aggressive [10,12]. The Cat. × TH CIFC 1343 population studied here was evaluated with the *R* isolate, which is different from the isolate used in studies where its level of aggressiveness was reported [10,12]. However, our results, which are based on the distribution of resistance classes, suggest that its pathogenicity does correspond to a highly aggressive isolate.

Aggressiveness was observed in isolates *Zim12*, *Zim1*, and *Z9* from Zimbabwe and *Que2* from Kenya, consistent with previous reports [12,46]. The advanced populations derived from Cat. × TH CIFC 1343 presented a greater level of resistance to these isolates compared to the isolates from Angola, Cameroon, and Rwanda.

The lowest aggressiveness was associated with isolates from Malawi and Zimbabwe (*M2*, *M*, and *Z12*). The phenotypic response of the hypocotyls was characterized by more than 84% of the hypocotyls having small lesions without them coalescing. In the population derived from TH CIFC 1343, these isolates had the least effect, as has been previously reported in evaluations of varieties considered susceptible [12].

In general terms, diverse degrees of aggressiveness in isolates from the same geographic origin was observed, as has been previously documented [12,43]. This same behavior was observed in progeny derived from Cat. × TH CIFC 1343, in which different levels of resistance were found in different isolates from the same geographical origin, such as isolates of Zimbabwean origin.

4.3. Diversity of Resistance to CBD in the CCC Genotypes

TH is the main source of resistance to limiting and potential diseases for growing coffee, and its genetic value is so important that it has been used as a source of resistance by different genetic improvement programs around the world [25]. TH is diverse [26], and in Colombia, varieties resistant to rust and CBD have been developed from TH CIFC 1343 [35,53,54].

Although the inheritance of resistance to CBD is complex [43,50], the results of evaluations in progenies derived from TH CIFC 1343 clearly show that it is possible to obtain lasting resistance to *C. kahawae*, as described previously with the hybrid variety Ruiru 11 in Africa [19,24,28,29]. This antecedent highlights the importance of this genetic resource (*TH CIFC 1343*) as a source of resistance to cherry disease [30,50,53,55]. On the other hand, it is important to note that this same TH is the source of resistance to rust in the coffee varieties developed in Colombia.

However, the universally documented resistance of TH, the variety from which it developed, is not synonymous with immunity to *C. kahawae* [39,52,55,56]. Therefore, knowledge of resistance in derived varieties is beneficial for strengthening crop protection strategies in the event of the eventual arrival of the pathogen in countries where the disease has not yet been reported. One of those strategies is the knowledge and use of various sources of resistance. In the genotypes of Ethiopian origin used in this study, as with those that constitute the other populations evaluated here, resistance was found to be distributed across the different resistance classes (Table 4), including the HR class (*ET.37C9*, *ET.56*, *E.537*, *E.538*, *E.139*, *Rume Sudan*, *E.53*, *E.264*, *E.288*, and *E.417*), and the resistance of some of these genotypes, such as *ET.56* and *Rume Sudan*, has been previously reported [57–59].

Within the Typica/Bourbon group, Local Bronce.8, Local Bronce.12, Jackson.2 and Villalobos were noteworthy and were grouped in the highest resistance classes. Genotypes of the Local Bronce population have already been reported to present some degree of resistance to CBD [57–59]. We found no previous reports documenting resistance to Jackson and genotypes of Ethiopian origin. From the group of interspecific hybrids, the genotypes with the highest degree of resistance were obtained from *C. arabica* × *C. canephora*, and our findings agree with previous studies [52].

Although the possible existence of physiological races has been suggested in *C. kahawae* [52], to date there is no conclusive evidence to support this hypothesis. However, the results obtained from the Cat. × TH CIFC 1343 population suggest that the existence of races in the *C. arabica*–*C. kahawae* complex is probable (Table 9). This is how genotypes

completely susceptible (Grade 4) to isolates such as *Ang29* and *Cam1*, and at the same time, highly resistant to other isolates from, for example, Kenya, Malawi, and Zimbabwe, were identified (Table 3), indicating contrasting resistance reactions to different isolates. Similar results have been reported with isolates from Angola, Malawi, and Kenya [52].

From the perspective of our findings, the evaluation of *C. arabica* varieties for resistance to CBD is highly valuable, and none of the research centers other than those on the African continent have evaluated their gene banks for resistance to a potential disease such as CBD (or if so, their research data have not been published), except for the data previously reported by Cook and van der Vossen [47,48]. Therefore, contrary to expectations, countries that produce *C. arabica*, such as Colombia, have been working for more than 40 years to prepare their coffee growing industry in the event of the eventual arrival of *C. kahawae*. This is why we emphasize that management and control strategies have not been the sole responsibility of African countries, where the disease is currently present [9].

Cenicafé has advanced in the search for and incorporation of various sources of resistance to CBD (unpublished data). In the case of TH, the incorporation of disease resistance mechanisms in the varieties developed by Cenicafé has been validated since the 1970s [39]. More recently, varieties resistant to rust and currently planted by coffee growers in Colombia have been found to express *Ck-1*, which confers resistance to CBD [33] and displays a wide spectrum of resistance to different isolates with different levels of aggressiveness and geographical origins [32].

In this research, improved progeny with the potential to be incorporated into a genetic diversity strategy and the central axis of rust-resistant varieties in Colombia were also evaluated. The results confirm that there is a diversity of resistance to *C. kahawae*; however, Colombia and the producing countries should continue working on strengthening their strategies for the incorporation of resistance mechanisms of different origins for the development of future varieties.

4.4. Potential of Genetic Resistance to CBD in a Climate Change Scenario

C. kahawae is a pathogen that is dispersed mainly by rain [60], and the disease is potentiated by specific weather conditions [18]. Therefore, the development of varieties resistant to CBD is an important challenge and requires a detailed understanding of the pathogen and local patterns of the real and probable climate conditions that can affect crop development [61].

The historical records of climate in Colombia since 1980 have made it possible to classify the hourly distribution of rainfall as light (0.1 to 10.0 mm h^{-1}), light to moderate (10.1 to 20.0 mm h^{-1}), moderately strong (20.1 to 40.0 mm h^{-1}), strong (40.1 to 60.0 mm h^{-1}), and torrential (>60.0 mm h^{-1}) [62], and under a climate change scenario, a significant increase in the number of moderate and strong rainfall events in Colombia is projected [62,63]. Additionally, an increase is expected in the minimum and maximum historical temperatures in the Colombian coffee region [63].

These predicted changes in rain and temperature events could favor pathogen dispersion and disease development, with its most severe effects on susceptible varieties. Possible increases in rainfall and minimum temperatures warn of the need to work on strategic fronts that allow us to face potential diseases such as CBD. One of these strategies involves the search for sources of resistance for the development of varieties with genetic resistance to *C. kahawae*.

5. Conclusions

CBD represents a potential risk for coffee growing in the Americas, and the best way to prepare for the eventual arrival of the pathogen is the use of genetic diversity and resistance. The historical evaluations carried out by Cenicafé and the data published here confirm that genetic resistance to CBD is not synonymous with immunity. For this reason, all countries where coffee cultivation constitutes an important economic industry should continue to

join efforts to evaluate their varieties and characterize their germplasm banks to identify possible sources of resistance to potential threats such as CBD.

The proposed CBD resistance classification tool unifies the language between research centers and their breeding programs for genetic resistance to CBD in such a way that it facilitates the evaluation, understanding, and interpretation of data to strengthen the knowledge network regarding CBD. On the other hand, the scale could contribute to the development of molecular markers linked to resistant genes to CBD, thereby allowing their validation under laboratory conditions.

Additionally, the results confirm the importance of TH CIFC 1343 as a source of resistance to CBD, the potential of CCC as a source of genes for resistance to this disease, and new evidence of the probable existence of races or pathotypes in *C. kahawae.*

Similarly, this is the first time that direct tests have shown the possible panorama of susceptibility to *C. kahawae* in coffee growing on the basis of TH CIFC 832/1. For this reason, the countries that support coffee growing using this source of resistance should move toward the use of new genetic sources and strategies to strengthen their coffee farms. Finally, the search for new sources of genes aimed at strengthening the genetic resistance already found in the varieties developed by Cenicafé in Colombia is recommended.

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Data Availability Statement: Restrictions apply to the dataset. The datasets presented in this article are not easily available because they were obtained with resources from the National Federation of Coffee Growers of Colombia and are part of the development program for *C. arabica* varieties with genetic resistance to CBD. Requests to access the datasets should be directed to the National Coffee Research Center (Cenicafé).

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