

*Full Length Research Paper*

# **Detached berries response to coffee berry disease (*Colletotrichum kahawae*) of F1 hybrid genotypes developed from Ethiopian accessions of Arabica coffee**

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**Arabica Coffee (*Coffea arabica* L.) is a cash crop that supports the livelihoods of many Tanzanians, but its productivity is limited by various factors, including Coffee Berry Disease (CBD) caused by *Colletotrichum kahawae*. CBD can lead to up to 90% coffee yield losses in Tanzania. There are different control measures for CBD, but the use of resistant cultivars has been mentioned to be a more sustainable method than the use of copper-based fungicides. This study was carried out to determine the level of CBD resistance within the F1 population and to identify top-performing hybrids. The experiment was conducted using a split-plot design under a Randomized Complete Block Design with three replications in the Pathology laboratory at the Tanzania Coffee Research Institute (TaCRI). Detached green berries of eight coffee genotypes were inoculated with three different isolates of *C. kahawae* (2006/14, 2019/16, and 2019/11). Disease severity on detached berries was assessed at 7, 11, and 15 days after inoculation. The results revealed significant variation ( $P \leq 0.05$ ) among the coffee genotypes. Two genotypes, F90/64/4660 x KP423 and F89/64/4660 x KP423, showed a very high level of resistance with absolutely no disease development, equivalent to the resistant check KP423. Genotypes F24/64/902 x KP423 and F54/64/2049 showed moderate susceptibility, while the susceptible check KP423 developed typical CBD symptoms. The interaction between genotypes and *C. kahawae* isolates revealed significant differences in the number and size of lesions formed. This study verified the resistance of the tested F1 hybrid genotypes, which have the potential to be exploited for CBD-resistant varieties in Tanzania.**

**Key words:** Arabica coffee, coffee genotypes, coffee berry disease, *Colletotrichum kahawae* Isolates.

## **INTRODUCTION**

In Tanzania, coffee is a major agricultural export product, accounting for 5% of total exports. Coffee is primarily

grown by smallholder farmers, who contribute to over 95% of the crop's production (TCB, 2012). There are

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various factors affecting coffee production, including coffee berry disease (CBD) and coffee leaf rust (CLR), both of which pose significant threats (Baffes, 2005). Coffee berry disease is caused by the fungus *C. kahawae*, leading to yield losses ranging from 30% to 90% in Tanzania. This disease also escalates production costs for coffee farms, ultimately resulting in reduced returns (Mbwambo et al., 2021). CBD was first documented in Tanzania in 1964 and has subsequently spread to all coffee-producing regions. Following its initial discovery in Ethiopia, coffee berry disease rapidly propagated throughout major coffee-growing areas, excluding lower elevation regions (Gimase et al., 2019). The majority of Arabica coffee-producing areas are situated at high altitudes, characterized by high humidity and low temperatures. These environmental conditions are optimal for the development of *C. kahawae*, as temperatures exceeding 30°C do not support fungal growth (Chen et al., 2003; Cabral et al., 2020).

In the 2003/04 period, the Tanzania Coffee Research Institute (TaCRI) initiated a program to create coffee hybrids at the Coffee Research Station (CRS). *C. arabica* accessions were gathered from various countries, including Ethiopia, Sudan, Kenya, Tanzania, Reunion, India, Portugal, Indonesia, and South Africa. TaCRI's crop improvement initiative strives to continuously develop coffee cultivars with enduring resistance to coffee berry diseases, aiming to enhance coffee productivity and quality. To date, fifteen improved tall and four compact Arabica varieties have been introduced, with additional varieties in the development pipeline (Kilambo et al., 2014). These varieties were derived from a limited number of resistant coffee varieties present in TaCRI germplasm, specifically Catimors, Hibrido de Timor (HdT), and Rume Sudan, which are recognized as progenitors of CBD and CLR resistance (Mtenga et al., 2008). Since the 1950s, genetic material from 2000 Arabica accessions has been utilized to create CBD-resistant varieties (TaCRI, 2003). The initial *Colletotrichum kahawae* strains employed for resistance assessment were isolated from coffee fields in Lyamungu and were found to exhibit lower pathogenicity compared to prior reports of *C. kahawae* strain variations (Kilambo et al., 2008). In Tanzania, *C. kahawae* has been identified to possess multiple strains displaying varying levels of aggressiveness (Kilambo et al., 2005, 2013). The aggressiveness variance among the pathogen population can be attributed to geographic origins (Varzea et al., 2002). This variability in pathogen aggressiveness constitutes a crucial aspect in evaluating coffee varieties across diverse conditions (Omondi et al., 2001). Therefore, there is a potential risk in developing CBD-resistant varieties without subjecting accessions to the most aggressive strains/isolates, which might lead to the emergence of weaker varieties. Consequently, in breeding programs, it is imperative to carefully select the most

suitable accessions within varieties to develop superior coffee strains exhibiting CBD resistance (Mtenga and Reuben, 2012). The objective of this study was to assess the level of CBD resistance within F1 genotypes, thereby identifying the top-performing hybrids.

## MATERIALS AND METHODS

This study was conducted at TaCRI's Pathology laboratory in Lyamungu, Hai district. It is located at latitudes 03°24'49" South and longitudes 37°24'46" East and 1268 meters above sea level.

### Experimental plant materials

The experimental materials used were detached berries of commercial coffee variety KP 423, which is susceptible to CBD and VC 298 a resistant variety, as control checks. Six F1 populations, derivatives of Ethiopian coffee accessions, having high yielding and KP 423, which have good cup quality namely, F45/64/2061 x KP 423, F90/64/4660 x KP 423, F45/64/2049 x KP 423, F24/64/902 x KP 423, F24/64/886 x KP 423, and F89/64/4660 x KP 423 were used.

### Inoculum preparation and inoculation

#### *Inoculum preparation*

For increasing the volume of collected and preserved *C. kahawae* isolates, the Malt Extract Agar (MEA) was prepared as described by Kilambo et al. (2008). These isolates 2006/14, 2019/16 and 2019/11 were sourced from TaCRI isolates collection which were collected from Kibosho-Kombo, Mbinga-Ugano and Maua-Kilema areas respectively. The ten months stored isolates were activated by inoculating on freshly green coffee berries in the field for sporulation, and then plated on solidified MEA in petri dishes kept in culture room for multiplication for a period of 14 days. Inoculum was then obtained by dislodging and harvesting the conidia by flooding the plate with 5 ml of sterile distilled water. The cell suspensions were made using sterile distilled water and its concentration of  $2 \times 10^6$  conidia/ml as described by van der Vossen et al. (1976) were adjusted using a haemocytometer (Caligiore-Gei and Valdez, 2015). Detached coffee berries were inoculated with *C. kahawae* isolates using a hand sprayer, spraying them twice at 48 hours' interval with the inoculum of each isolates.

### Experimentation

The laboratory condition was set at the temperatures of 19-21°C, and R.H of approximately 100%. A split plot under Randomized Complete Block Design was used with three replications whereby eight genotypes were the main plot and three *C. kahawae* isolates sub factors. A set of 20 detached green expanded berries were kept in a sandwich box having tissue paper with sterilized distilled water to prevent berries from shriveling. In sandwich boxes, berries were arranged at a spacing of 1.0 x 1.0 cm. One replication therefore contained a total of 24 sandwich boxes. The whole experiment consequently had a total of 72 sandwich boxes.

### Procedure of assessment and data collection

The number of green coffee berries infected with CBD was

**Table 1.** Analysis of variance Table of mean squares.

Source of variation	Df	% infected berries	Size of lesions (mm <sup>2</sup> )	Number of lesions	Days to lesions appearance
Blocks	2	92.36	1.377	0.8528	77.42
Genotypes (G)	7	71299.65***	444.959***	78.3016***	1152.98***
Isolates (I)	2	300.69***	111.325***	13.9382***	142.12***
G x I	14	382.64	64.386***	4.6549***	26.12
Residual	1414	590.97	1.500	0.7520	24.75

\*\*\*, \*\* and \* Significant at 0.001, 0.01 and 0.05 successively.

recorded at 7, 11, and 15 days after artificial inoculations. Data collected were based on CBD symptoms such as: - number of lesions, type of lesions formed, active sunken dark lesions (A), scab lesions (S) and (N) for non-infected berries, size of lesions (mm<sup>2</sup>) and days from inoculations to symptoms appearance. Disease incidence was calculated by counting the number of CBD infected berries against uninfected berries, and then the percentage berry infected calculated. Potential genotypes in terms of CBD resistance were identified based on the disease incidence; the ones with lower incidence were marked.

#### Means of analyzing data

Data were subjected to analysis of variance (ANOVA) for determination of statistical difference using GenStat software (16<sup>th</sup> version, VSN International) and means of disease severity were separated using Duncan Multiple Range Test. Resistance categories were then classified using disease incidence as; resistant (R) 0 – 25, moderately resistant (MR) 26 – 50, moderately susceptible (MS) 51 – 75) and susceptible (S) 76 – 100 as applied by Mtenga (2006). Disease incidence was performed as per Marasas et al. (1988) formula.

$$\text{Disease Incidence} = \frac{\text{Number of infected berries in the cluster}}{\text{Total number of berries in the cluster}} \times 100$$

## RESULTS

Table 1 shows ANOVA mean squares for all variables tested to determine the effects of Arabica coffee genotypes and *C. kahawae* isolates. The mean of squares revealed significant effects ( $P < 0.001$ ) of genotypes and isolates on all variables presented (percent infected berries, size of lesions, number of lesions, and days to lesions appearance). However, interaction of genotypes and isolates was only significant ( $P < 0.001$ ) on size of lesions and number of lesions, respectively.

#### Effect of coffee berry disease on resistance level of the F1 population

Results on effect of CBD on Arabica Coffee genotypes on

CBD resistance for the percent infected berries, size of lesions, number of lesions and number of days to lesion appearance for each Arabica coffee genotype are presented in Table 2. F1 hybrid genotypes F90/64/4660 x KP423, F89/64/4660 x KP423 and the resistant check VC289 demonstrated higher resistance level to all *Colletotrichum kahawae* isolates as they had no (0.00) scores of CBD infection. Genotypes F45/64/2061 x KP423 and F24/64/886 x KP423 revealed significant difference at ( $P < 0.001$ ) high percent infected berries (56.67) and (63.89) while Susceptible check KP423 indicated highest percentage level of infection which is 88.89. Results revealed significant difference at ( $P < 0.001$ ) of larger size of lesions (4.7mm<sup>2</sup>), (1.8mm<sup>2</sup>) and (1.4mm<sup>2</sup>) in the check variety KP423, F24/64/886 x KP423 and F45/64/2061 x KP423, respectively. Some F1 hybrid genotypes F24/64/886 x KP423 and F45/64/2061 x KP423 were observed to have significant effect ( $P < 0.001$ ) on few days 8 and 9 to lesions appearance. Active type of lesion (A) was revealed by F1 hybrid genotypes F24/64/886 x KP423 and F45/64/2061 x KP423 upon infection. F1 hybrid genotypes F24/64/902 x KP423 and F45/64/2049 formed high percent of scab lesions (S) which indicated disease tolerance, therefore categorized as moderately resistance. Days to the first CBD symptoms appearance were noticed earlier on susceptible genotypes (KP423 and F1 hybrid F24/64/886 x KP423 that were inoculated with isolate 2006/14 and 2019/16 than isolate 2019/11.

#### Results on aggressiveness of *C. kahawae* isolates

Results in Table 3 show the mean values for the percentage of infected berries, size of lesions, number of lesions, type of lesions formed and number of days to lesion appearance caused by three different *C. kahawae* isolates. Isolates 2006/14, 2019/16 and 2019/11 had a significant effect ( $P < 0.001$ ) on size of lesions of 1.7, 1.2, and 1.0 mm<sup>2</sup> on the F1 hybrid genotypes. Moreover, isolates 2019/16 and 2006/14 induced lesions significantly earlier at ( $P \leq 0.05$ ) on 9 and 8 days after inoculation

**Table 2.** Effect of coffee berry disease on the Arabica coffee genotype tested.

Genotypes	% Infected berries	Size of lesions (mm <sup>2</sup> )	Number of lesions	Number of days to lesion appearance <sup>a</sup>	Type of lesions
F90/64/4660 x KP 423	0.00 <sup>a</sup>	0.00 <sup>a</sup>	0.00 <sup>a</sup>	-	N
F89/64/4660 x KP 423	0.00 <sup>a</sup>	0.00 <sup>a</sup>	0.00 <sup>a</sup>	-	N
VC 298	0.00 <sup>a</sup>	0.00 <sup>a</sup>	0.00 <sup>a</sup>	-	N
F24/64/902 x KP423	36.11 <sup>b</sup>	0.94 <sup>b</sup>	0.59 <sup>b</sup>	11	S
F45/64/2049 x KP423	41.67 <sup>c</sup>	0.73 <sup>b</sup>	1.05 <sup>c</sup>	11	S
F45/64/2061 x KP423	56.67 <sup>d</sup>	1.38 <sup>c</sup>	1.09 <sup>c</sup>	9	A
F24/64/886 x KP423	63.89 <sup>e</sup>	1.78 <sup>d</sup>	1.36 <sup>d</sup>	8	A
KP 423	88.89 <sup>f</sup>	4.71 <sup>e</sup>	1.63 <sup>e</sup>	5	A
Mean	35.90	1.19	0.71	9	
SE(±)	1.387	0.054	0.0421	0.302	
CV%	3.9	4.5	5.9	3.4	
P-value	0.001	0.001	0.001	0.001	

Means with similar letter in the same column are not statistically different at  $P \leq 0.05$ ; A=Active lesions; S=Scab lesions; N=No lesion; <sup>a</sup>Cells in the table where there is no numerical value (-) is because no lesions were ever formed within the set observation period of maximum 15 days.

**Table 3.** Effect of *C. kahawae* isolates on CBD on detached berries.

Isolates	% infected berries	Size of lesions (mm <sup>2</sup> )	Number of Lesions	Number of days to lesion appearance	Type of lesions
2019/11	33.33 <sup>a</sup>	0.72 <sup>a</sup>	0.52 <sup>a</sup>	10	S
2019/16	36.04 <sup>b</sup>	1.17 <sup>b</sup>	0.79 <sup>b</sup>	9	A
2006/14	38.33 <sup>c</sup>	1.68 <sup>c</sup>	0.83 <sup>b</sup>	8	A
Mean	35.90	1.19	0.71	9	
SE(±)	1.387	0.054	0.421	0.302	
CV%	3.9	4.5	5.9	3.4	
P-value	0.001	0.001	0.001	0.003	

Means with similar letter in the same column are not significant different at  $P \leq 0.05$ ; A=Active lesions; S=Scab lesions; N=No lesion.

respectively than isolate 2019/11 which induced lesion at 10 days after inoculation. Isolates 2006/14 and 2019/16 caused active lesions (A) on tested F1 genotypes compared to isolate 2019/11 which revealed scab lesions (S). However, all tested isolates 2006/14, 2019/16 and 2019/11 caused significant disease effect at ( $P < 0.001$ ) whereby on percentage berries infection was 38.33, 36.04, and 33.33, respectively.

#### Interaction effects of F1 Arabica genotypes and *C. kahawae* isolates on various CBD resistance parameter

Results in Table 4 indicated that, F1 Hybrids F90/64/4660 x KP423, F89/64/4660 x KP423 and the resistant check 298 were not infected by all isolates of the CBD pathogen; this made them to fall under the resistant classification

(R). F1 hybrid F45/64/886 x KP423 when interacted with three isolates 2019/11, 2019/16 and 2006/14 revealed a significant difference at ( $P \leq 0.05$ ) on high percent infected berries of 56.67%, 65.20, and 70.01, respectively, and placed under moderately susceptible (MS); while F1 genotype F45/64/2049 x KP423 when interacted with isolate 2019/16 revealed 41.7% and was ranked under moderately resistant (MR). The susceptible check KP423 had significant ( $P \leq 0.05$ ) high infected berries of 95, 90 and 81.7% when interacted with isolates 2006/14, 2019/16 and 2019/11, respectively, and placed under susceptible class (S). F1 hybrid 45/64/6061 x KP423 when inoculated with isolate 2019/16 had the largest lesion size of 2.1 mm<sup>2</sup>, while other F1 hybrid genotypes had an average lesion size of 1 mm<sup>2</sup>. The highest number of lesions of 2 was revealed when the susceptible check KP423 interacted with isolates 2006/14 and 2019/16. Highest number of lesions of 1.05, 1.75, and

**Table 4.** The interaction effects of F1 Arabica genotypes and *C. kahawae* isolates on various CBD resistance parameters under laboratory conditions.

Genotype	Isolates	% berry infection	Size of lesions mm <sup>2</sup>	Number of lesions	Days to lesions appearance <sup>a</sup>	Reaction classification
F90/64/4660 x KP423	2006/14	0.00a	0.00a	0.00a	-	R
F90/64/4660 x KP423	2019/16	0.00a	0.00a	0.00a	-	R
F90/64/4660 x KP423	2019/11	0.00a	0.00a	0.00a	-	R
F89/64/4660 x KP423	2006/14	0.00a	0.00a	0.00a	-	R
F89/64/4660 x KP423	2019/16	0.00a	0.00a	0.00a	-	R
F89/64/4660 x KP423	2019/11	0.00a	0.00a	0.00a	-	R
VC298	2006/14	0.00a	0.00a	0.00a	-	R
VC298	2019/16	0.00a	0.00a	0.00a	-	R
VC298	2019/11	0.00a	0.00a	0.00a	-	R
F24/64/902 x KP423	2006/14	36.67bc	1.20cd	0.52bc	11	MR
F24/64/902 x KP423	2019/16	35.00b	0.63b	0.43b	11	MR
F24/64/902 x KP423	2019/11	36.67bc	0.98bcd	0.78cde	11	MR
F45/64/2049 x KP423	2006/14	45.00d	0.67b	1.33hi	10	MR
F45/64/2049 x KP423	2019/16	41.67cd	0.70bg	1.20ghi	11	MR
F45/64/2049 x KP423	2019/11	38.33bc	0.82bcd	0.62bcd	11	MR
F24/64/886 x KP423	2006/14	70.01h	2.98f	1.25hi	7	MS
F24/64/886 x KP423	2019/16	65.20gh	1.32d	1.13fghi	8	MS
F24/64/886 x KP423	2019/11	56.67ef	1.03bcd	0.88defg	9	MS
F45/64/2061 x KP423	2006/14	60.00fg	1.07bcd	1.82kl	9	MS
F45/64/2061 x KP423	2019/16	56.67ef	2.12e	1.467ij	9	MS
F45/64/2061 x KP423	2019/11	53.33e	0.95bcd	0.82cdef	10	MS
KP423	2006/14	95.00j	7.55h	1.75jk	4	S
KP423	2019/16	90.00j	4.60g	2.083i	5	S
KP423	2019/11	81.67i	1.98e	1.05efgh	6	S
Mean		35.90	1.192	0.714	8.84	
CV%		3.9	4.5	5.9	3.4	
SE(±)		1.387	0.0536	0.421	0.302	
p-value		0.028	0.001	0.001	0.393	

Means with the similar letter in the same column are not significant different, at  $P \leq 0.05$ . <sup>a</sup>Cells in the table where there is no numerical value (-) is because no lesions were ever formed within the set observation period of maximum 15 days; R= Resistance; MR= Moderate resistance; MS= Moderate susceptible; S= Susceptible.

2.08 were observed on KP423 when interacted with all tested isolates 2019/11, 2006/14 and 2019/16, respectively, followed by F1 hybrid F45/64/886 x KP423 when inoculated with isolates 2006/14 and 2019/16 took 7 and 8 days to lesion appearance, respectively. F1 hybrid F24/64/902 x KP423 revealed low percent infected berries of 35, small size of lesion of 1 and 11 days to lesion appearance when infected by isolate 2019/16 (Table 4).

#### Levels of pathogenicity of the three isolates of *Colletotrichum kahawae*

Table 5 summarizes results of ability of the three isolates

in causing lesions. When the scored active lesions were more than 50% of infected berries, then the genotype was ranked under active type (A) while scab type of lesion (S) was ranked for the genotype which had more than 50% scabs from the total infected berries. Resistant varieties were ranked (N) which means not infected. All three isolates were pathogenic in susceptible genotype KP423 by causing active lesions (A) on green berries. Genotypes F45/64/2061 x KP423 and F24/64/886 x KP423 were weaker to isolates 2006/14 and 2019/16 by forming active lesions (A) upon infection, in contrast with isolate 2019/11 where they produced scab lesions (S), the indication of tolerating disease infection. However, type of lesions differed depending on the genotype. For a susceptible check KP423 active lesions were developed

**Table 5.** Active and scab types of lesions record of berries infected with different isolates of *Colletotrichum kahawae*.

Genotype	Isolate 2006/14			Isolate 2019/16			Isolate 2019/11		
	AcL %	ScL %	TpL	AcL%	ScL%	TpL	AcL %	ScL %	TpL
F45/64/2061 x KP423	35	25	A	40.22	16.43	A	5	48.35	S
F90/64/4660 x KP423	0	0	N	0	0	N	0	0	N
F45/64/2049 x KP423	13	32	S	11.52	30.13	S	3.22	35.13	S
F24/64/902 x KP423	16.44	20.21	S	8	28	S	4.32	25	S
F24/64/886 x KP423	40	17.32	A	30	18	A	2.55	36.13	S
F89/64/4660 x KP423	0	0	N	0	0	N	0	0	N
VC 298	0	0	N	0	0	N	0	0	N
KP423	92	0	S	86.68	0	A	80	0	A
Mean	24.55	11.82		22.05	11.57		11.89	18.08	
S. e									

AcL = active lesions, ScL = Scab lesions, TpL = Type of lesions (A=active, S=scabs. N=nil/no infection).

due to its vulnerability to all isolates of *C. kahawae* used.

## DISCUSSION

### Effect of coffee berry disease on resistance level of the F1 population

This study confirmed useful information for strengthening of the coffee breeding programme in Tanzania. Isolates 2006/14 and 2019/16 of *C. kahawae* could be used in research for screening resistance of different *C. arabica* varieties. Genotypes with no infection or low percent incidences were placed under resistant or moderately resistant, hence, to be involved in breeding programme. This study also revealed a high relationship between the sizes of lesions, days from inoculation to lesion appearance and the percent berry infection. Large size of lesions were observed where there is high percent of berry infection, and also took few days from inoculation to symptoms appearance as observed on the interaction of susceptible variety KP423 with all three isolates.

Previous findings documented close connection between pathogenicity and earliness in CBD symptom appearance (Kilambo et al., 2008; Varzea et al., 2002). A similar effect was also reported earlier by Varzea et al. and Varzea et al. (1999, 1993) when studying pathogenicity variability of *C. kahawae* strains.

### Interaction effects of F1 Arabica genotypes and *C. kahawae* isolates on various CBD resistance parameters

The number, size and type of lesions noticed to be caused by CBD isolates on the coffee genotypes, such as, on KP423 indicated susceptibility on its high percent

infected berries, high number of lesions, large size of lesion (mm<sup>2</sup>) and few days to CBD symptoms appearance. The formation of scabs is an indication of a tolerance mechanism; namely the capacity to inhibit infection by *C. kahawae* pathogen, this also reported by Kilambo et al. (2008), Chen et al. (2006) and Silva et al. (2006).

The presence of appropriate coffee green berry stage, favourable weather conditions and a pathogenic *C. kahawae* isolate can assist in assessing the response of a coffee genotype for its resistance, moderate resistance, moderate susceptible and susceptibility (Kilambo et al., 2013). During the first four weeks, the berry does not increase in size; it remains at the "pinhead" stage which is normally resistant to CBD (Mulinge, 1970). Pinard et al. (2012) also reported that the expanding berry period of 4-16 weeks after flowering, is the most susceptible stage. The availability of essential humidity, free water (rainfall) on berry surface and temperature, all enhance favorable conditions for infection, and development of CBD as was confirmed on KP423 the susceptible variety and VC 289 the resistant variety.

### Levels of pathogenicity of the three isolates of *Colletotrichum kahawae*

The obtained results are in line with Kilambo et al. (2013) who stated that, *C. kahawae* isolate's pathogenicity level is measured by the ability to cause active lesions; and that it takes only a few days in causing infection. The variation in disease development (infection period) is related to the stage of berry development, soft green berries are infected earlier than hard green berries; this was also reported by Mulinge (1970). The observed significant interaction effect between genotype KP423 and isolate 2019/11 on percent infected berries provides

further support to the need for testing coffee varieties against *C. kahawae* isolates from diverse geographic locations.

## Conclusion

The study confirmed the resistance of four F1 hybrid genotypes to coffee berry disease, with two genotypes (F89/64/4660 x KP423 and F90/64/4660 x KP423) exhibiting high resistance (R), while the other two (45/64/2049 x KP423 and F24/64/902 x KP423) showed moderate resistance (MR). These genotypes displayed attributes such as low percent berry infection, small lesion size, minimal lesion count, longer duration from inoculation to symptom appearance, and a higher percentage of scab lesions compared to active lesions on infected berries.

## Recommendation

In selecting *C. kahawae* isolates for identifying *C. arabica* varieties resistant to the pathogen, it is crucial to consider early appearance of CBD symptoms, large lesion size, and a high number of lesions. Notably, the most pathogenic isolates were identified as *C. kahawae* 2006/14 and 2019/16, solidifying their utility in identifying CBD-resistant varieties. The study also proposes F1 hybrid genotypes as potential sources of resistance genes for CBD in breeding programs aimed at developing future commercial coffee cultivars. Furthermore, there is a need for additional studies to assess the pathogenicity of *C. kahawae* isolates from various agro-ecological zones.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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