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RESEARCH ARTICLE

Response of F_1 arabica coffee genotypes descending from disease resistant Ethiopian accessions to coffee berry disease

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ABSTRACT

Coffee is one of Tanzania's primary agricultural export commodities but seriously constrained by *Colletotrichum kahawae* which causes significant yield losses in Arabica coffee during high infection seasons. Use of resistant varieties rather than chemical fungicides which are costly and causing environmental pollution is a more sustainable approach to control the disease. A number of F1 hybrid genotypes developed from Ethiopian accessions of Arabica coffee of known Coffee berry disease (CBD) resistance were evaluated under CBD pressure to observe acquired resistance. Eight Arabica coffee genotypes were inoculated with three types of CBD pathogen isolates. Results revealed high resistance on two of the F1 genotypes (F90/64/4660 x KP423 and F89/64/4660 x KP423) with no infection to all *C. kahawae* isolates. Two Genotypes (F45/64/2049 x KP423 and F24/64/902 x KP423) revealed moderately resistance by scoring less than 50% infected berries, while F45/64/2061 x KP423 and F24/64/886 x KP423 demonstrated moderate susceptibility. The F1 genotypes with less infection are to be included into breeding program for development of resistant varieties. Pathogenicity of three *C. kahawae* isolates obtained from different coffee growing areas in Tanzania was also observed for their effects to the tested genotypes. Isolate 2006/14 from Kibosho – Kombo was more aggressive in terms of infected berries followed by isolate 2019/16 from Ugano-Mbinga. Isolates found to be more aggressive are potential for screening resistance to develop coffee berry diseases varieties in Tanzania.

Keywords: F_1 hybrid genotypes, Ethiopian accessions, Arabica coffee, Coffee berry disease

INTRODUCTION

Arabica coffee in Tanzania is mostly grown in areas from 1200 m.a.s.l and above, where low temperatures and long hours of water films existence on the coffee berry surfaces provide conducive conditions for the coffee berry disease (CBD) development, a disease responsible for about 70 to 80 % yield loss (Silva et al., 2006). Improved management of CBD is clearly important in stabilizing yield. Current control strategies are based mainly on timely application of fungicides (Gichimu and Omondi, 2010), cultural practices, quarantine procedures (Phiri et al*.,* 2001) and host-resistance (Omondi et al*.,* 2000; 2001). However, application of fungicides occasionally exacerbates the disease by reducing the levels of non-pathogenic competitors in the microbial populations (Derso et al., 2000). In addition, continuous application of copper fungicides has been reported to have environmental problems. A study conducted by Lolland and Singh (2004) indicated that bean plants from coffee fields showed a high concentration of copper suggesting possible copper pollution problems.

Epidemics of CBD depend upon susceptibility of Arabica coffee varieties and the presence of *Colletotrichum kahawae* isolates as a disease causative agent. Mouen Bedimo et al. (2010) reported that conidia of the pathogen causing CBD are dispersed by rainfall, and require liquid water or about 100 % relative humidity for germination. Optimum temperatures for germination of conidia, appressoria formation and mycelial growth are 21-23^{0}C (Janardhan and Krishna, 2021).

High temperature above 30 ^{0}C, does not favor growth of the fungus (Chen *et al.,* 2003). Conidia produced in acervuli are released from perithecia and distributed by rainfall or wind to berries in coffee or nearby plants (Bedimo et al*.,* 2007). In the absence of diseased berries, the primary source of inoculum for CBD comes from fruiting bodies of the fungus present on maturing bark of the coffee twigs, flower buds and mummified berries (Jayalakshmi et al., 2021). These inoculum sources activated by rainfall become actively sporulated, produce conidia and resume parasitic activity by infecting young green fruit. Bedimo et al. (2008) observed that green berries are susceptible to the disease at four to fourteen weeks after flowering. Ripening berries are also susceptible while mature berries (16 to 25 week of fruit development) are resistant due to endocarp formation (Silver et al*.,* 2006). In addition to climatic conditions, other authors reported that turgor pressure of *C. kahawae* plays a role in coffee cuticle penetration (Chen et al*.,* 2004). On infected green berries one form of symptoms appears as small dark sunken lesions (active lesions) that can expand and coalesce to cover the whole berry. Under wet conditions, a pinkish mass of spores develop on the lesion surface (Bedimo et al., 2008). Another form of lesion (scab lesion) can be formed under adverse weather conditions or as a result of defensive reaction (Chen et al*.,* 2006; Don et al*.,* 2003).

Promising sources of disease resistance in the germplasm of coffee have been identified in Tanzania (TaCRI 2005; TaCRI 2004; Teri et al*.,* 2004). Worldwide there are long-term prospects for using resistant cultivars to manage the disease (Wamatu et al*.,* 2003). However, progress in understanding the *C. kahawae* pathogen and selection of resistance sources has been delayed by lack of information about the pathogenicity variation of the pathogen in Tanzania and elsewhere (Omondi et al*.,* 2001). The aim of this study is to determine the response of F1 hybrids genotypes for their resistance to coffee berry disease caused by *C*. *kahawae.*

Outcomes from this study will assist breeders to identify F1 hybrid genotypes of Arabica coffee that are resistant to coffee berry disease. The available hybrid genotypes will be used as a source of resistance in breeding programmes.

MATERIALS AND METHODS

Description of experimental site

This study was conducted at Tanzania Coffee Research Institute (TaCRI) in Lyamungu, Hai district (Fig.1), which is located at Latitude 03º24'49'' S and Longitude 37º24'46''E; Altitude 1268 meters above sea level, temperature range 17-28°C, relative humidity 40-90 and an average annual rainfall of 1250 mm. The evaluation was performed during the coffee production season of short rains when berries are formed usually between November and February. *C*. *kahawae* isolates collected from different areas known to be coffee berry disease hotspot were isolated at TaCRI. F1 hybrid varieties were created by crossing parents that are genetically distinct, in this particular case, KP 423 was crossed by Ethiopian varieties as presented in Table 1 and *C*. *kahawae* isolates tested were presented in Table 2.

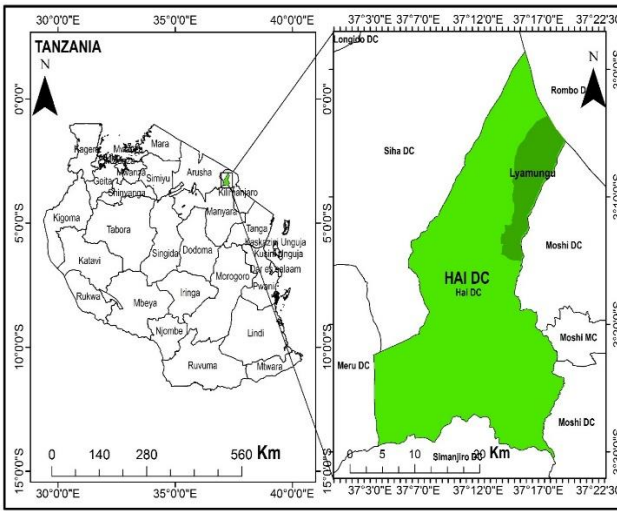

Figure 1. Map of Tanzania showing the location of study area in Lyamungu, Hai district - Kilimanjaro

Table 1. List of Genotypes of Coffea Arabica tested for resistance to *C. kahawae* isolates

Genotypes
F45/64/2061 x KP423
F90/64/4660 x KP 423
F45/64/2049 x KP423
F24/64/902 x KP423
F24/64/886 x KP423
F89/64/4660 x KP 423
VC 298
KP 423

Source: TaCRI coffee germplasm

Table 2. Isolates for *C. kahawae* used to test coffee berry disease resistance

Isolate	Source Plant	Location
2006/14	Arabica Coffee	Kibosho-Moshi, Tanzania
2019/16	Arabica Coffee	Ugano Mbinga, Tanzania
2019/11	Arabica Coffee	Maua Kilema, Tanzania

Source: TaCRI coffee germplasm

Experimental design

A field experiment using a split plot in Randomized Complete Block Design (RCBD) with three replications was established whereby, eight *C. arabica* genotypes were inoculated with three isolates of *C. kahawae*; 2006/14, 2019/16 and 2019/11. The main plots were the genotypes and subplots were the *C. kahawae* isolates, every genotype consisted of three coffee trees. The spacing between coffee trees was 2.5 m and within 2.0 m. One inner plant was inoculated with three *C. kahawae* isolates by covering three different branches using polythene bag, each branch was inoculated with different C*. kahawae* isolate. The three branches used for CBD assessment had 5 to 6 berry clusters per branch (averaging 25 berries per cluster) of green expanding coffee berries at 8-12 weeks old. The other two trees were used as guard rows. One tree in each genotype was inoculated with three different isolates in order to maintain uniformity. Inoculation was done twice at the interval of 48 hours for better infection (Kilambo et al., 1999). During the experiment, rainfall, temperature and humidity were recorded (Appendix 1a, 1b and 1c) which were the main environmental factors for disease infection.

Inoculum preparation

Various genotypes were subjected to CBD infection through inoculation with the *C. kahawae* isolates. Malt Extract Agar (MEA) was used for inoculum increase of the isolates. 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 for multiplication. Plating involved transfer of CBD sporulated lesions from infected berries to the culture media (MEA) and kept at the culture room in the laboratory for a period of about 14 days. Inoculum was then obtained by dislodging and harvesting the conidia by flooding the plate with 5 mL of sterile distilled water.

Data collection

Data were collected based on CBD symptoms such as; type of lesions formed were active lesions (sunken dark), scab lesions (brown color) and no lesion (clean/non infected), size of lesions (mm^2) number of lesions 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. Full expression of disease was determined on KP423 the susceptible variety. Appearance of sunken dark lesions on berries after inoculation was considered as an indication of pathogenicity. Each genotype was assessed based on expression of

disease symptoms on green expanded coffee berries. Monitoring of the reaction was done daily during the first seven days, then after every three days.

Data analysis

Data were subjected to analysis of variance (ANOVA) for determination of statistical difference using GenStat software (16th version, VSN International) and mean separation test using Duncan Multiple Range Test.

Disease incidence was done as per Marasas et al. (1988)

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

Active lesions (deep sunken black color) are indication of susceptible varieties and scab lesions (brownish color) as a sign of tolerance response.

RESULTS

Resistance levels

ANOVA mean squares for all variables tested on genotypes and *C. kahawae* isolates shows significant variation (P<0.001) on percent infected berries, size of lesions, number of lesions and days to lesions appearance. Interaction of genotypes and isolates was significant (P<0.001) on size of lesions and number of lesions, respectively (Table 3).

Table 3. Analysis of variance table of mean squares for the response of genotypes to coffee berry disease infection

Source of variation	Degree of freedom	% Infected berries	Ms Size of lesions	Ms Number of lesions	Ms Days to lesions appearance
Replications	2	107.56	53.01	0.6007	35.58
Genotypes	7	7778.92***	3106.13***	80.1673***	1614.34***
Isolates	2	768.44***	584.86***	8.0617***	491.77***
Genotypes x Isolates	14	554.22	127.56***	4.5061 ***	23.00
Residual	1414	1310.22	14.32	0.697	26.62

*** Significant at 0.001, Df = degrees of freedom, Ms = mean squares, SV = source of variations

Results shows that, percent infected berries, size of lesions, number of lesions and number of days to lesion appearance varied significantly (P<0.001) among the tested genotypes. F1 hybrid genotypes F90/64/4660 x KP423, F89/64/4660 x KP423 and resistant check VC298 were not infected by the *C. kahawae* isolates (2006/14, 2019/16 and 2019/11) (Table 4). F1 Genotypes F45/64/2061 x KP423 and F24/64/886 x KP423 revealed significantly (P<0.001) high percent infected berries (52.89) and (48.00) while the susceptible check KP423 indicated highest percentage infection (86.22). Genotypes F24/64/902 x KP423 and F54/64/2049 x KP423 revealed low percent of berry infection (34.22) and (36.00), respectively. Results revealed significantly (P<0.001) larger size of lesions (4.2mm^2), (1.8mm^2) and (2mm^2) in the check variety KP423, F1 hybrid genotypes (F24/64/886 x KP423) and (F45/64/2061 x KP423). Results revealed significantly (P<0.001) few (9) days to lesions appearance on F1 Genotypes F45/64/2061 x KP423 and F24/64/886 x KP423, while susceptible check KP423 revealed (5) days respectively. The above two genotypes (F24/64/886 x KP423) and (F45/64/2061 x KP423) and the susceptible check KP423 revealed active lesions (A), while genotypes F24/64/902 x KP423 and F54/64/2049 x KP423 revealed scab lesions (S). F1 hybrid genotypes F90/64/4660 x KP423, F89/64/4660 x KP423 and resistant check VC298 were not infected (N) (Table 4).

Table 4. Effects of Arabica Coffee genotypes on coffee berry disease resistance

Genotypes	% infected berries	Size of lesions	Number of lesions	Number of days to lesions appearance	Type of lesions
F90/64/4660 x KP 423	0.00^a	0.00^a	0.00^a	-	N
F89/64/4660 x KP 423	0.00^a	0.00^a	0.00^a	-	N
VC 298	0.00^a	0.00^a	0.00^a	-	N
F24/64/902 x KP423	34.22^b	1.08^c	0.66^b	11	S
F45/64/2049 x KP423	36.00^b	0.67^b	0.88^c	11	S

F45/64/2061 x KP423	52.89[c]	1.95[d]	4.4[d]	9	A
F24/64/886 x KP423	48.00[c]	1.77[d]	0.82[c]	9	A
KP 423	86.22[d]	4.16[e]	1.61[e]	5	A
Mean	32.17	1.20	0.64	9.08	
SE±	2.892	0.118	0.032	0.308	
CV%	9.0	9.8	5.0	3.4	
P-value	0.001	0.001	0.001	0.001	

Means which share at least one similar letter are not statistically different, according to DMRT ($P \leq 0.05$). A=Active lesions, S=Scab lesions N=No lesion. [a] 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.

Aggressiveness of *C. kahawae* Isolates to F1 Arabica Coffee Genotypes

Results showed that isolate 2006/14 was most active significantly ($P<0.001$) inducing high number of infected berries (36.67%) followed by isolate 2019/16 (Table 5). Similarly isolate 2006/14 had significantly ($P<0.001$) higher ability to cause large lesions ($2mm^2$) followed by 2019/16 and 2019/11. Isolates 2019/16 and 2006/14 induced lesions significantly ($P<0.001$) much earlier than isolate 2019/11, which induce lesions slightly later (13 days after inoculation). Isolates 2019/16 and 2006/14 induced active lesions (A) on genotypes while isolate 2019/11 induced scab lesions (S).

Table 5. Effect of *C. kahawae* isolates on F1 genotypes

Isolates	% Infected berries	Size of lesions (mm^2)	Number of lesions	Number of days to lesion appearance	Type of lesions
2019/11	27.83[a]	0.80[a]	0.51[a]	13	S
2019/16	32.00[b]	0.99[b]	0.68[b]	12	A
2006/14	36.67[c]	1.82[c]	0.72[b]	11	A
Mean	32.17	1.20	0.64	12	
SE±	2.892	0.118	0.032	0.308	
CV%	9.0	9.8	5.0	3.4	
Prob.	0.001	0.001	0.001	0.001	

Variables which share at least one similar letter are not statistically different according to DMRT ($P \leq 0.05$). A=Active lesions, S=Scab lesions N=No lesion.

Interaction Effects of *C. kahawae* Isolates and F1 Arabica Genotypes

Table 6 shows interaction of genotypes and isolates on CBD evaluation parameters. Genotypes F90/64/4660 x KP423 and F89/64/4660 x KP423 and resistant check VC298 had no lesion appearance after inoculation, therefore, ranked with (R) while the susceptible check KP423 had high percent infected berries (86.67, 81.33 and 77.33) when inoculated with *C. kahawae* isolates (2006/14, 2019/16 and 2019/11), thus ranked with (S). Two F1 hybrid genotypes (F45/64/2049 x KP423 and F24/64/902 x KP423) revealed low percent infected berries when inoculated with the various isolates (2019/11, 2019/16 and 2006/14) and placed under moderately resistance class (MR). Genotypes F24/64/886 x KP423 and F45/64/2061 x KP423 ranked under moderate susceptibility (MS) after inoculated with *C. kahawae* isolate (2006/14).

Pathogenicity levels of three of *C. kahawae* isolates

Ability of *C. kahawae* isolates to cause infection on coffee genotypes is presented in Table 7. F1 genotypes F90/64/4660 x KP423 and F89/64/4660 x KP423 resist infection from all three isolates (2006/14, 2019/16 and 2019/11). Results of ability of the three isolates in causing lesions are summarized in Table 7. 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 produce scab lesions (S) the indication of tolerating disease infection. For a susceptible check KP423 active lesions were developed due to its susceptibility to *C. kahawae* isolate used. The level of the pathogenicity of *C. kahawae* isolates is measured by the ability to cause active lesions and taking few days in causing infection (Kilambo et al. 2013).

Table 6. Mean values for the interaction effects of F1 Arabica genotypes and *C. kahawae* isolates on various coffee berry disease resistance parameters under field conditions

Genotypes	Isolates	%infected berries	No. of lesions	Size of lesions (mm2)	Days to lesion appearance	Reaction Classification
F90/64/4660 x KP423	2006/14	0.00^{a}	0.00^{a}	0.00^{a}	-	R
F90/64/4660 x KP423	2019/16	0.00^{a}	0.00^{a}	0.00^{a}	-	R
F90/64/4660 x KP423	2019/11	0.00^{a}	0.00^{a}	0.00^{a}	-	R
F89/64/4660 x KP423	2006/14	0.00^{a}	0.00^{a}	0.00^{a}	-	R
F89/644660 x KP423	2019/16	0.00^{a}	0.00^{a}	0.00^{a}	-	R
F89/64/4660 x KP423	2019/11	0.00^{a}	0.00^{a}	0.00^{a}	-	R
VC298	2006/14	0.00^{a}	0.00^{a}	0.00^{a}	-	R
VC298	2019/16	0.00^{a}	0.00^{a}	0.00^{a}	-	R
VC298	2019/11	0.00^{a}	0.00^{a}	0.00^{a}	-	R
F24/64/902 x KP423	2006/14	40.00^{cd}	0.55^{b}	1.32^{de}	11	MR
F24/64/902 x KP423	2019/16	34.67^{bc}	0.53^{b}	1.09^{cd}	11	MR
F24/64/902 x KP423	2019/11	29.33^{b}	0.91^{def}	0.83^{bc}	12	MR
F24/64/886 x KP423	2006/14	54.67^{efg}	1.07^{ef}	2.80^{hi}	8	MS
F24/64/886 x KP423	2019/16	48.00^{ef}	0.76^{bcd}	1.73^{ef}	10	MR
F24/64/886 x KP423	2019/11	37.33^{bc}	0.63^{bcd}	0.77^{bc}	11	MR
F45/64/2061 x KP423	2006/14	57.33^{fg}	1.44^{gh}	2.81^{hi}	7	MS
F45/64/2061 x KP423	2019/16	48.00^{de}	1.17^{fg}	2.03^{fg}	9	MR
F45/64/2061 x KP423	2019/11	41.33^{cd}	0.73^{bcd}	1.00^{bcd}	10	MR
F45/64/2049 x KP423	2006/14	40.00^{cd}	1.19^{fg}	0.79^{bc}	10	MR
F45/64/2049 x KP423	2019/16	33.33^{bc}	0.88^{cde}	0.64^{bc}	11	MR
F45/64/2049 x KP42	2019/11	29.33^{b}	0.59^{bc}	0.57^{b}	11	MR
KP423	2006/14	86.67^{i}	1.55^{h}	6.81^{j}	3	S
KP423	2019/16	81.33^{hi}	2.11^{i}	2.43g^{h}	4	S
KP423	2019/11	77.33^{h}	1.19^{fg}	3.24^{i}	6	S
Mean		32.17	0.64	1.20		
SE±		2.892	0.032	0.118	9	
CV%		9.0	5.0	9.8	0.308	
P-value		0.151	0.001	0.001	3.4	

Means which share at least one similar letter are not statistically different, according to DMRT ($P \leq 0.05$). A=Active lesions, S=Scab lesions N=No lesion. [a]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

DISCUSSION

The study revealed genotypes F90/64/4660 x KP423 and F89/64/4660 x KP423 to be highly resistant to the infection of all *C. kahawae* isolates involved while genotypes F45/64/2049 x KP423 and F24/64/902 x KP423 were moderately resistant. These results are also supported by those of Leroy et al. (2006) and Bertrand et al. (2011) that, F1 hybrids of Arabica coffee have genetic and agronomic advantages such as heterosis disease resistance and good cup quality that are acceptable to coffee consumers. This study confirmed useful information for strengthening of the coffee breeding programme in Tanzania. Isolates 2006/14 and 2019/16 of *C. kahawae* should be used in research for screening resistance of different *C. arabica* varieties. Genotypes with no infection or low percent incidences were placed under resistant/moderately resistant, hence, to be involved in breeding programme.

Table 7. Active and Scab types of lesions record of berries infected with different isolates of *C. kahawae*

Genotypes	Isolate 2006/14			Isolate 2019/16			Isolate 2019/11		
	AcL %	ScL %	TpL	AcL%	ScL%	TpL	AcL %	ScL %	TpL
F45/64/2061 x KP423	42.22	19.12	A	35	17.32	A	6	38	S
F90/64/4660 x KP423	0	0	N	0	0	N	0	0	N
F45/64/2049 x KP423	10.32	31	S	8	28	S	7.68	23	S
F24/64/902 x KP423	6	35.32	S	5	27	S	9	20.32	S
F24/64/886 x KP423	40	17.32	A	25	23	A	8.55	30.13	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	23.82	12.85		19.96	11.92		13.9	13.93	

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

Appendix 2 showed the appearance of berries portraying the susceptible genotypes with active lesions on green berries, while Appendix 3 indicating few scab lesions and active lesions.

This study revealed high relationship between the size of lesion, days from inoculation to lesion appearance and the percent berry infection. Large size of lesions was observed where there was high percent berry infection, also took few days from inoculation to symptoms appearance as observed on the interaction of susceptible variety KP423 with all three isolates.

Days to the first CBD symptoms appearance were noticed to be earlier on susceptible genotypes (KP423 and F1 hybrid F24/64/886 x KP423 that were inoculated with isolate 2006/14 and 2019/16. 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 reported earlier by Varzea et al*.* (1999; 1993); when studying pathogenicity variability of *C. kahawae* strains.

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 lesion, large size of lesion (mm^2) and few days to CBD symptoms appearance. The formation of scabs is an indication of tolerance mechanism to inhibit infection by *C. kahawae* pathogen, this is reported by Kilambo (2008); Chen et al*.* (2006) and Silva et al*.* (2006).

This study has brought added information that is very valuable for strengthening the coffee breeding programme and also coffee industry in Tanzania. The results revealed that *C. kahawae* isolate 2006/14 from Kibosho – Kombo was more aggressive in terms of infected berries followed by isolate 2019/16 from Ugano-Mbinga. Isolates 2006/14, 2019/16 of *C. kahawae* should be used in future studies for screening resistance of *C. Arabica*. According to Vieira et al., (2019a) and Vieira et al., (2019b), *C. kahawae* aggressiveness is a genetically controlled traits, with a significant reliance on environmental factors.

Assessment of the interaction effect between the F1 Arabica coffee genotypes and *C. kahawae* isolates was significant on the size, number of lesions and days to lesion appearance. Since the assessed parameters are reportedly having consequential associations to the isolate's pathogenicity, it is overbearing to infer about pathogen's aggressiveness as a function of coffee genotype.

This information set, ahead of CBD resistance assessments, the need for using multiple *C. kahawae* isolate when coffee genotypes are to be evaluated. This study was however conducted in one agro-ecological zone of Tanzania, namely Northern Zone, and so the inferences being made here are specific to this zone. Further studies on screening of CBD resistance on multi-location bases are recommended so as to sort out the effect of environment and/or interaction between environment and genotypes.

CONCLUSION

This study sorts out four F1 hybrid genotypes, two (F90/64/4660 x KP423 and F89/64/4660 x KP423) with ascertained CBD resistance, and two more (F45/64/2049 x KP423 and F24/64/902 x KP423) for further consideration due to the fact that they are moderately resistant. From the study, the most pathogenic *C. kahawae* isolate was found to be *C. kahawae* isolates 2006/14 followed by 2019/16,

therefore confirmed to be useful in screening for CBD resistance.

CONFLICT OF INTERESTS

The authors declare no conflict of interests

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AUTHORS' CONTRIBUTIONS

GK performed the conception or design of the work, data collection, data analysis, interpretation and was a major contributor in writing the manuscript (drafting the article). SN and DM performed a critical revision of the article, providing critical comments concerning the discussion of results, conclusions, and recommendations. All authors read and approved the final manuscript.

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