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Responses of Compact Coffee Clones Against Coffee Berry and Coffee Leaf Rust Diseases in Tanzania

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Abstract

The utilization of resistant Arabica coffee (*Coffea arabica*) varieties is considered as the most economical control for coffee berry disease (CBD) and coffee leaf rust (CLR) in Tanzania. The resistance levels of varieties at field and laboratory conditions were assessed through their phenotypic disease reaction response to CBD and CLR. In this study sixteen (16) compact hybrids of *C. arabica* plus four (4) standard cultivars were evaluated under a range of environmental conditions in on-station and on-farm trials in Tanzania. Also four (4) *Colletotrichum kahawae* strains of the pathogen responsible for CBD infection; 2010/1, 2010/2, 2006/7 and 2006/14, and *Hemileia vastatrix* uredospores were used to test the sixteen (16) hybrids through artificial inoculation under controlled conditions (temperatures between 19 to 22 ºC, R.H 100%). Results showed that a significant level of variability ($P < 0.05$) occurred between the sixteen (16) compacts, three (3) standard checks and N39 a commercial susceptible variety across trials. Compact genotype CVT14 (PNI086 x (N39 x Rume Sudan Selfed F_2) showed resistance to the four strains of *C. kahawae* and *Hemileia vastatrix*. Differential reactions on compact genotypes were found to *C. kahawae* and *H. vastatrix* strains existing in different coffee growing regions in Tanzania; genotypes CVT4 (PNI088 x (SL34 x HdT) x Kent x Rume Sudan) and CVT13 (PRO127 x (Blue Mountain Jamaica x Cioccie) x Rume Sudan) showed partial resistance to *C. kahawae* strains 2010/1, the genotypes were susceptible to strains 2010/2 but resistant to strains 2006/7 and 2006/14. This shows that host response reaction can be used as criteria for varietal assessment when evaluated at different locations.

Keywords: compact coffee clones, CBD, CLRD, Tanzania

1. Introduction

Coffee production in Tanzania is still constrained to a large extent by the ability of the growers to control coffee berry disease (CBD) and coffee leaf rust (CLR). These are fungal diseases; CBD is caused by *Colletotrichum Kahawae* Waller and Bridge Sp. Nov., and CLR by *Hemileia vastatrix* Berk et Br. Coffee berry disease attacks all stages of the developing crop from pinhead to ripe fruits (Mulinge, 1970), which may destroy 30 to 90% of the crop if the weather conditions are favourable to its epidemics (Ngulu et al., 1998). Coffee leaf rust disease infection can cause severe leaf defoliation leading to die-back of primary branches, followed by death of the coffee tree.

The traditional Arabica coffee varieties commercially grown in Tanzania are all susceptible to CBD and CLR. This is despite their fine quality coffees, in high demand by buyers and roasters (Wrigley, 1989). The varieties including N39, KP423, KP162 and H66 among others occupy over 80% of the total Tanzanian coffee acreage (Fernie, 1970; Robinson, 1964). They are protected against CBD and CLR by an intensive fungicide spray programme that accounts for up to 50% of the production costs (Gustave et al., 2007; Teri et al., 2004; Omondi et al., 2000). The fungicides are not only uneconomical but also environmentally unsafe.

It is for this reason that selection for resistant coffee types was initiated in Tanzania in stabilizing yields and quality of coffee. Coffee Research at Tanzania Coffee Research Institute (TaCRI) initiated a programme to develop compact hybrids in 2003/04 involving Catimors, Hibrido de Timor (HdT), Rume Sudan and varieties contributing attributes of good quality coffee; N39, KP423 and SL28. Most of Catimor lines carry the dominant resistance S_H6 and S_H9 introgressed from HdT (Betterncourt et al., 1992). These two genes confer resistance to *H.*

vastatrix (Rodrigues Jr. et al., 2000). Studies by van der Vossen and Walyaro (1980) revealed that HdT possesses a gene for CBD resistance on the T-locus, and that dominant R and recessive k genes found in Rume Sudan contribute to CBD resistance in the cross of *Coffea arabica*. The compact hybrid breeding lines developed were established in four on-station and six on-farm trials to study genetic responses under varying environmental conditions in Tanzania. Plant pathogen distribution and variability forms important part in assessing the response of crop varieties under diverse conditions (Brenda, 2011; Gichuru & Omondi, 2010). The aim of this work was to identify compact coffee clones that are resistant to CBD and CLR in specific or across ecosystems.

2. Materials and Methods

2.1 Description of the Study Area

The trial sites were located in low altitude coffee growing areas prone to CLRD (1000 to 1200 m. a.s.l), at medium altitude where CLR and CBD prevail (1200 to 1400 m. a.s.l) and high altitude areas where there is high incidence of CBD (> 1400 m. a.s.l). These sites have considerable variation in pathotypes for CBD and CLR. The study areas are shown in Figure 1.

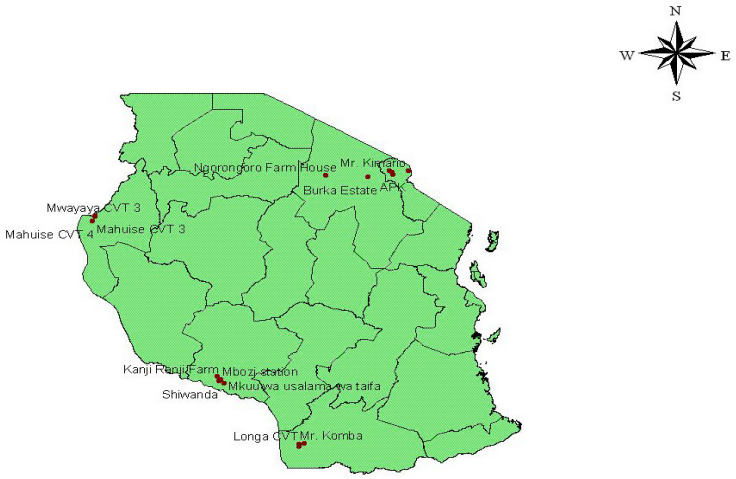

Figure 1. Map of Tanzania showing study areas highlighted with red dots

Specific description of the study locations in terms of altitude, weather conditions (annual precipitation, humidity, temperatures) and soil types are summarized in Table 1.

Table 1. Description of the study locations

Location	Altitude	Weather conditions			Soil type
	(m a.s.l)	Temperature (ºC)	R.H (%)	Rainfall (mm)	
Lyamungu Hai	1268	17-28	40-90	1250	Clay loam
Mbimba Mbozi	1605	14-26	40-90	1332	Clay loam
Ugano Mbinga	1562	16-26	50-92	1220	Clay loam
Ng'uni Hai	1600	*	*	*	Silt loam
APK Hai	1100	*	*	*	Clay
Mokala Rombo	1500	*	*	*	Clay
Khanji Mbozi	1650	*	*	*	Silt loam
Utiri Mbinga	1300	*	*	*	Clay loam
Longa Mbinga	1650	*	*	*	Clay loam

*No records. Clay loam = Moist, loose, mixture of sand, silt and clay; 40% silt: 40% sand: 20% clay. Silt loam = Loam contains; sand, silt and clay. But in this case the proportion of silt is higher. Clay = heavy soils contains more pore spaces, hence can absorbs and retains more water and nutrients. Anonymous (2012).

2.2 Coffee Genotypes

Sixteen compact coffee genotypes, and four check varieties (coffee hybrid SC9, SC13, Catimor PNI088 and N39), were tested for host x pathogen interaction across agro-ecological areas (Table 2). Hybrid seeds of the same breeding lines were produced, raised to the hypocotyl stage (5-6 weeks old, 5-6 cm tall) and tested for genotype x pathogen interaction to CBD and CLR under controlled conditions at temperature of between 19-21 ºC, and R.H. of approximately 100%.

Table 2. Coffee genotypes of compact breeding lines

Code no	Genotype
CVT1	Ctr088 x (N39 x Rume Sudan Selfed F_2)
CVT2	Ctr088 x (SL34 x HdT) x Kent x Rume Sudan
CVT3	Ctr088 x (Padang x (HdT x N39) x Rume Sudan
CVT4	CtrI088 x (SL34 x HdT) x Rume Sudan
CVT5	Ctr088 x (Rume Sudan x Catuai)
CVT6	Ctr088 x (Blue Mountain Jamaica x Cioccie x HdT x Rume Sudan)
CVT7	Ctr088 x (HdT x N39) x SL28) x (N39 x Rume Sudan)
CVT8	Ctr088 x (N39 x HdT) x (N39 x HdT) x Rume Sudan
CVT9	CtrO127 x (Rume Sudan x Catuai)
CVT10	Ctr127 x (N39 x Rume Sudan Selfed F_2)
CVT11	Ctr127 x (N39 x HdT) x HdT
CVT12	Ctr127 x (Padang x (HdT x N39) x Rume Sudan
CVT13	Ctr127 x (Blue Mountain Jamaica x Cioccie) x Rume Sudan
CVT14	Ctr086 x (N39 x Rume Sudan Selfed F_2)
CVT15	Ctr086 x (Rume Sudan x Catuai)
CVT16	Ctr086 x (N39 x Rume Sudan Selfed F_2)
SC9	(N39 x OP729) x HdT) x N39 (SC9) (Control tall cultivar)
SC13	Kent Hb x HdT (SC13) (Control tall cultivar)
PNI088	Catimor PNI088 (Control, compact)
N39	N39 (Control, tall cultivar)

Note: Ctr = Catimor line.

2.3 Collection and Preparation of Colletotrichum kahawae Inoculum

Green coffee berries infected with CBD at the black lesion stage were collected close to trial sites and preserved in a paper bag. Inside the paper bag, green infected berry samples were sandwich between layers of newspaper and kept in a cool box at 18 to 20 ºC for the pathogen to be viable for successful subsequent isolation (Johnston & Booth, 1983). *Colletotrichum kahawae* was isolated from the survey samples using the method described by Beynon et al. (1995). Infected green coffee berries with CBD lesions were surface sterilized by immersing for 15 min in a 5% solution of sodium hypochlorite (available chlorine 0.5% W/V) and rinsed in three changes of sterile distilled water. Infected coffee berry tissues were then removed aseptically and incubated on distilled water agar at 22 ºC. Hyphae from advancing edges of the tentative *C. kahawae* colonies were then transferred to Malt Extract Agar (MEA) (Beynon et al., 1995). The cultures were incubated at 22 ºC for 10 days to allow colony growth. Preliminary confirmatory tests of colony texture of *C. kahawae* isolates on MEA were based on mycological colour chart developed by Rayner (1970). Usually *C. kahawae* colonies have grayish colony texture.

The pathogenicity of *C. kahawae* was tested on 20 fully expanded, soft green berries (≤ 14 weeks old from date of flowering) of the susceptible variety N39. Coffee berries were surface sterilized, placed on damp sterilized

sand in plastic boxes and inoculated with a 0.02 ml drop of the standard *C. kahawae* spore suspension at 2.0 x 10^6 conidia per ml using a pipette. The inoculated green coffee berries were incubated at 25 ºC and observed for CBD symptom development for 14 days (Figure 2). Days to first symptoms appearance and percentages of infected berries were recorded.

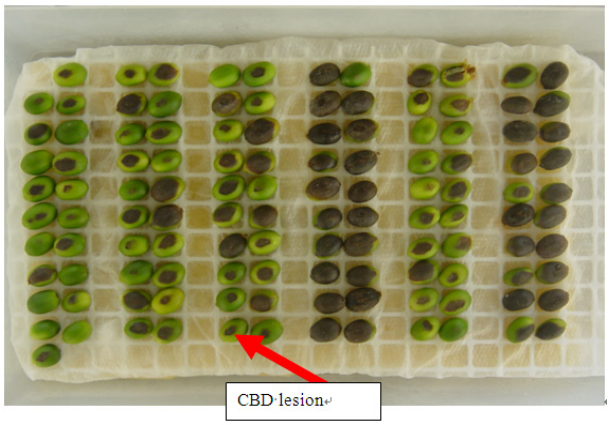

Figure 2. Pathogenicity tests of *Colletotrichum kahawae* isolates on green berries of N39

2.4 Collection and Preparation of Hemileia vastatrix Inoculum

Leaves of N39 with fresh rust lesions of *Hemileia vastatrix* were collected uredospores scraped into conical flask contained sterilized dH_20. Rust uredospores were collected from live plants because *Hemileia vastatrix* is an obligate parasite and it is impossible to grow and multiply through culture media (Kushalappa & Eskes, 1989). The solution was then shaken to allow dispersion of uredospores, and to allow uniform dispersion, a drop of tween 80 is added into a solution and then shaken. Then uredospores concentration calibrated at 1 x 10^6/ml.

2.5 Field Experiments

Multi-location trials located at Lyamungu (1268 m a.s.l), Ugano (1562 m a.s.l), Mbimba (1605 m a.s.l) and Mwayaya (1530 m a.s.l) were used to identify compact coffee clones that are resistant to CBD and CLR pathogens in specific or across ecosystems.

The data were collected in the already existing compact trees that were used to design experimental plots. A completely randomized block design (CRBD) with three replications experimental design was used at Lyamungu, Mbimba and Ugano in the study. Each plot consisted of 24 trees but only the inner 8 trees were considered for precise data collection. The spacing between coffee trees was 2.0 m and within 1.5 m. A set of two branches having five to six berry clusters per branch (about 40 berries per cluster) of green expanding coffee berries at 8 to 12 weeks old, were used for CBD assessment. Disease incidence was done by counting the number of CBD coffee infected berries against uninfected berries, and then the percentage berry infection was computed.

$$\text{Disease incidence} = \frac{\text{number of CBD coffee infected berries in berry clusters}}{\text{total number of berries in berry clusters}} \times 100$$

For CLR the same trees were used as a reading unit to evaluate disease reaction of the genotypes using 0-9 scale by Eskes and Toma-Braghini (1981); whereby 0 describes absence of lesions and 9 describes intense lesions associated with leaf shedding.

The difference in the performance of the varieties of compact genotypes to CBD and CLR were also determined in six on-farm locations. On-farm trials were located at Ng'uni Hai district (1600 m a.s.l), APK Hai district (1100 m a.s.l), Mokala Rombo district (1500 m a.s.l), Khanji Mbozi district (1650 m a.s.l), Utiri Mbinga district (1300 m a.s.l) and Longa Mbinga district (1650 m a.s.l).

2.6 Experiments Under Controlled Conditions

Colletotrichum kahawae strains originated from four (4) ecosystems; Moshi, Kigoma, Mbeya and Mbinga were used to test the level of resistance of sixteen (16) *Coffea arabica* compacts. A set of two branches having five to six berry clusters per branch of green expanding berries were kept in a conical flask having sterilized dH_20 to

sustain their vigour. These were kept in sterile controlled conditions at temperature of 19-21 ºC, and R.H. of approximately 100%. The berries were artificially inoculated with strains of *C. kahawae* suspension at 2 x 10^6 conidia/ml. The quantity of the conidia concentration was determined and standardized using haemocytometer. The *C. kahawae* strains were 2010/1 (Kigoma), 2010/2 (Lunji-Mbeya), 2006/7 (Mbinga) and 2006/14 (Kibosho-Kombo Moshi). One block representing check was sprayed with dH_20 only. The mean percentages of green coffee berries infected with CBD were recorded at 10, 14 and 18 days after artificial inoculation. Similarly F_1 hybrid seed produced through artificial pollination between male parents (Table 2) and the selected compact lines PNI086, PNI088 and PRO127 were used to determine genotype x CBD pathogens interaction using the four *C. kahawae* strains. F_1 of each of the 16 hybrids represented by 40 hypocotyls were raised in a plastic box at a spacing of 5 cm x 5 cm containing sterile sand (Figure 3). The experiment was arranged in the laboratory in a completely randomized design (CRD) with three replications. Hypocotyls at 5-6 weeks or 5-6 cm were sprayed and inoculated with suspension of *C. kahawae* strains at 2.0 x 10^6 conidia/ml twice at 48 hr intervals using the method by van der Vossen et al. (1976). Three weeks after the date of first inoculation, coffee seedlings were individually scored for CBD symptoms which developed on the hypocotyls using a scale with a range of 0-4 which was developed by van der Graff (1982), where 0 is nil and 4 intense CBD infections.

Figure 3. Hypocotyls of the raised F1 hybrids of compact hybrids to determine genotype x *Colletotrichum kahawae* interaction

For each genotype Disease Index Reaction for sixteen (16) F_1 hybrids was determined as follows:

$DIR = 25\frac{\sum i \times ni}{in}$ Where, i is the disease class, *ni* is the number of seedlings in class i and n is the total number of seedlings scored.

Leaves of the terminal node still tender and succulent arranged incompletely randomized (CRD) were used to evaluate the levels of resistance of the compact genotypes using coffee rust races collected from N39 from the four coffee ecosystems. Ten (10) leaves of each of the genotype kept in a sterile box were inoculated by spreading dry coffee rust uredospores on the lower surface of the leaves using sterilized camel's hair brush as described by d'Oliveira (1965). After inoculation the leaves were sprayed with dH_20 and then placed in moist chamber for 48 hr to allow germination of spores and infection. The assessment of the reaction on the leaves was done using a quantitative scale developed by d'Oliveira (1965); where i is without infection and **X** highly variable size of sporulating pustules. The assessment on coffee rust disease reaction was concluded 45 days after the artificial inoculation, the final assessment was considered to confirm the resistance of the genotypes.

The data were analyzed using Genstat software, and mean separation was done using Tukey's multiple tests. Data for CBD and CLR were not transformed as the raw data were symmetrically distributed; between the 16 compact coffee genotypes and N39.

3. Results

3.1 Pathogenicity Tests of Colletotrichum kahawae Isolates on Hypocotyls and Green Berries of N39

Table 3 summarizes the results on pathogenicity test of the *C. kahawae* isolates collected from sites near on-station and on-farm trials. Isolates 2011/2 (2006/14), 2011/6 (2010/2), 2011/7 (2006/7) and 2011/8 (2010/1) had high levels of CBD percent infected green berries than the rest of the strains. Isolate 2006/14 had exceptionally high levels of pathogenicity by inducing CBD on both hypocotyls and green berries in 3-4 days.

Despite high level of pathogenicity of isolate 2011/1, *C. kahawae* isolates 2006/14, 2010/2, 2006/7 and 2010/1 were selected representing four ecosystem to test the reaction of the compact genotypes.

Table 3. Pathogenicity test of *C. kahawae* strains on hypocotyls and green berries

Isolate	Location collected	Days to first symptoms appearance		Reaction on hypocotyls*	Percent infected berries*
		Hypocotyls	Green berries		
2011/1	Lyamungu	6	5	3	90
2011/2 (2006/14)	Kibosho-Kombo	4	3	4	100
2011/3	Rombo-Mokala Juu	7	3	3	75
2011/4	Karatu Ngorongoro	9	3	3	75
2011/5	Mbozi Khanji Estate	10	2	3	80
2011/6 (2010/2)	Mbeya Lunji	9	3	3	85
2011/7 (2006/7)	Mbinga Longa	9	3	3	85
2011/8 (2010/1)	Kigoma Kalinzi	10	3	3	85
Water				0	0
Mean					84
Sx					2.83
LSD					6.92
C.V					9.8

Key: 0 = absence of CBD symptoms; 1 = few (1-2) and small chlorotic lesions; 2 = more than 2 brownish lesions or coalescence brownish lesion; 3 = large brownish lesions with abundant black dots and or black lesions; 4 = dead hypocotyls. *Assessment 14 days after artificial inoculation of *C. kahawae* isolates.

3.2 Reaction of Compact Coffee Genotypes to CBD and CLRD Under Various Ecosystems Under Natural Conditions

Figures 4-5 summarizes the results on reaction of compact genotypes to CBD and CLR for on-station trials under various ecosystems under natural disease infection. Figure 4 show results on percentage CBD infection of the compact genotypes and check varieties. High levels of CBD infection were noted in N39 across the sites, but there was nil infection in the sixteen compact genotypes, SC9, SC13 and Ctr088. Error bars with 0.05 values show significance differences between the sixteen compact genotypes, SC9, SC13, Ctr088 and N39.

Results in Figure 5 show that, N39 had high CLR reaction compared to the rest of the genotypes. There is also significance difference shown by error bars with 0.05 values.

Table 4 shows reaction of compact genotypes to CBD and CLR for on-farm trials across coffee ecosystems under natural disease infection. Results show that across six locations in farmer's plots, the only cultivar which was susceptible to CBD and CLR was N39. The sixteen compact genotypes showed resistance to CBD and CLR under natural conditions. Analysis show high interaction of CBD effect on season, location and genotypes, but for CLR only for location and genotypes (Table 5).

Table 4. Reaction of compact coffee genotypes to *Colletotrichum kahawae* and *Hemileia vastatrix* evaluated under natural conditions across six on-farms in different agro ecological zones for 2010/11

Genotype	Ng'uni				APK				Mokala-Rombo				Khanji				Utiri				Longa			
	CBD		CLR		CBD		CLR		CBD		CLR		CBD		CLR		CBD		CLR		CBD		CLR	
	%	PR	DS	PR	%	PR	DS	PR	%	PR	DS	PR	%	PR	DS	PR	%	PR	DS	PR	%	PR	DS	PR
CVT1	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R
CVT2	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R
CVT3	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R
CVT4	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R
CVT5	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R
CVT6	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R
CVT7	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R
CVT8	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R
CVT9	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R
CVT10	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R
CVT11	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R
CVT12	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R
CVT13	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R
CVT14	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R
CVT15	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R
CVT16	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R
SC9	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R
SC13	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R
Ctr088	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R
N39	45		4		5		8		23		9		65		9		25		9		50		8	

Key: DS = Disease Score; % = CBD Percent infection; PR = Plant Resistance. % CBD infection: 0-5, Resistance; 6-20, Partial Resistance, 21-39, Moderately Susceptible, 40-100 Highly Susceptible. CLR description of scale: 0 = absence of rust lesions (Resistant); 4,5 = number of rust lesions per branch about 7-8 (Moderately Susceptible; 8,9 = Susceptible.

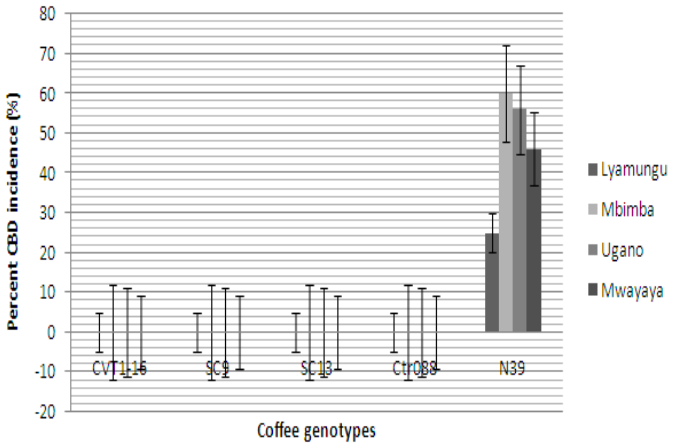

Figure 4. Reaction of the coffee genotypes to coffee berry disease across ecosystems

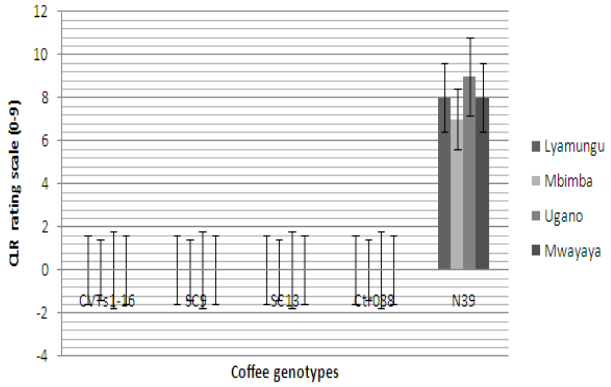

Figure 5. Reaction of the coffee genotypes to coffee leaf rust across ecosystems

Table 5. ANOVA summary to determine effect of season, location and coffee genotypes with CBD and CLR

Source of variation	Degree of freedom	CBD Scores	CLR Scores
Season.location.genotype	341	21.20525***	
Site.genotype	172		23.508187***
Total		4.29516	2.380890

*** Highly significance differences.

Table 6. Reaction of green berries of compact genotypes 18 days after artificial inoculation to *Colletotrichum kahawae* strains

Coffeegenotype	*Colletotrichum kahawae* strains							
	2010/1		2010/2		2006/7		2006/14	
	Infection (%)	Plant Resistance	Infection (%)	Plant Resistance	Infection (%)	Plant Resistance	Infection (%)	Plant Resistance
CVT1	1.7^{d}	R	29.6^{abcd}	MS	9.0^{bc}	PR	28.3^{b}	MS
CVT2	6.8^{cd}	PR	42.3^{ab}	HS	4.7^{bc}	R	0.0^{b}	R
CVT3	25.0^{b}	MS	3.9^{fgh}	R	2.6^{bc}	R	21.7^{b}	MS
CVT4	1.8^{d}	R	2.6^{gh}	R	18.3^{c}	PR	9.5^{b}	PR
CVT5	19.4^{bc}	PR	13.1^{defgh}	PR	6.2^{bc}	PR	5.6^{b}	PR
CVT6	0.8^{d}	R	15.5^{cdefgh}	PR	4.2^{bc}	R	0.0^{b}	R
CVT7	2.8^{cd}	R	19.8^{cdefgh}	PR	5.9^{bc}	PR	4.8^{b}	R
CVT8	1.8^{d}	R	19.8^{cdefgh}	PR	8.9^{bc}	PR	3.8^{b}	R
CVT9	0.0^{d}	R	26.5^{bcd}	MS	8.4^{bc}	PR	0.0^{b}	R
CVT10	2.8^{cd}	R	6.4^{efgh}	PR	4.9^{bc}	R	0.0^{b}	R
CVT11	5.1^{cd}	PR	21.8^{cdef}	PR	8.5^{bc}	PR	14.2^{b}	PR
CVT12	8.2^{bcd}	PR	32.0^{abc}	PR	2.8^{bc}	R	14.5^{b}	PR
CVT13	6.3^{cd}	PR	42.4^{ab}	HS	10.3^{bc}	PR	3.7^{b}	R
CVT14	4.1^{cd}	R	0.0^{h}	R	5.4^{bc}	R	3.6^{b}	R
CVT15	0.0^{d}	R	12.9^{defgh}	PR	0.0^{c}	R	9.2^{b}	PR
CVT16	1.9^{d}	R	17.1^{cdefgh}	PR	2.9^{bc}	R	1.6^{b}	R
SC9	3.9^{cd}	R	22.5^{cde}	PR	6.5^{bc}	PR	8.9^{b}	PR
SC13	2.6^{cd}	R	15.7^{cdefgh}	PR	0.0^{c}	R	4.6^{b}	R
Ctr088	14.0^{bcd}	PR	47.7^{a}	HS	18.5^{b}	PR	16.2^{b}	PR
N39	53.9^{a}	HS	43.9^{ab}	HS	58.8^{a}	HS	86.6^{a}	HS
Mean	8.1		21.8		9.8		21	
Tukey's significant difference (0.05)	3		3.2		2.8		4.3	

Key: Percent infection: 0-5 = Resistance (R); 6-20 = Partial Resistance (PR); 21-39 = Moderately Susceptible (MS); 40-100 = Highly Susceptible (HS) (Fernie, 1963). Means followed by a common letter within a column do not differ significantly ($P < 0.05$).

Table 7. ANOVA summary to determine genotype interaction with *Colletotrichum kahawae* strains using green berries of compact genotypes

Source of variation	Degree of freedom	CBD scores (Mean squares)
Genotypes	19	1.752***
Colletotrichum kahawae strains	4	2.851***
Colletotrichum kahawae strains.genotypes	36	1.963***
Total		2.763

Table 8 presents the results on the reaction of *C. kahawae* strains on hypocotyls of the compact genotypes. The results show a significant ($P < 0.05$) level of variability between the sixteen (16) compact genotypes, three checks and N39. Six genotypes of compact hybrids had DIR scores between 0-25 viz.; CVT2, CVT4, CVT5, CVT7, CVT8 and CVT13. Among the checks, Catimor PNI088 showed outstanding performance in terms of CBD resistance.

Table 8. Disease Index Reaction of the compact genotypes at the hypocotyls stage to four *Colletotrichum kahawae* strains

Coffee Genotype	*C. kahawae* strains							
	2006/14		2010/2		2010/1		2006/7	
CVTI	6.75^{g}	R	20.0^{bc}	R	6.25^{b}	R	32.9^{bc}	MR
CVT2	22.5^{efg}	R	13.75^{bc}	R	7.5^{b}	R	17.5^{bcde}	R
CVT3	37.5^{cdefg}	MR	4.5^{bc}	R	6.25^{b}	R	2.5^{de}	R
CVT4	17.5^{efg}	R	7.3^{bc}	R	0.0^{b}	R	4.4^{cde}	R
CVT5	15.0^{efg}	R	6.25^{bc}	R	0.0^{b}	R	11.65^{bcde}	R
CVT6	28.85^{defg}	MR	20.0^{bc}	R	28.75^{b}	MR	23.75^{bcde}	R
CVT7	9.5^{g}	R	0.7^{c}	R	17.5^{b}	R	16.3^{bcde}	R
CVT8	11.3^{fg}	R	10.3^{bc}	R	21.3^{b}	R	8.8^{bcde}	R
CVT9	48.8^{bcdef}	MR	10.0^{bc}	R	3.8^{b}	R	28.5^{bcde}	MR
CVT10	71.3^{abc}	MS	23.7^{bc}	R	8.8^{b}	R	34.8^{b}	MR
CVT11	66.0^{abcd}	MS	32.9^{b}	MR	12.5^{b}	R	31.7^{bcd}	MR
CVT12	87.8^{ab}	S	24.7^{bc}	R	24.9^{b}	R	33.6^{bc}	MR
CVT13	20.0^{efg}	R	9.7^{bc}	R	6.3^{b}	R	12.9^{bcde}	R
CVT14	52.5^{bcde}	MS	18.8^{bc}	R	27.5^{b}	MR	14.2^{bcde}	R
CVT15	65.0^{abcd}	MS	13.8^{bc}	R	13.8^{b}	R	22.6^{bcde}	R
CVT16	48.8^{bcdef}	MR	26.1^{bc}	MR	27.5^{b}	MR	10.5^{bcde}	R
Catimor PNI 088	0^{g}	R	0^{c}	R	0^{b}	R	0^{e}	R
N39	100^{a}	S	100^{a}	S	100^{a}	S	100^{a}	S
Mean	39.4		19		17.4		22.6	
Tukey's significant difference (0.05)	6.8		5		5.2		5.1	

Key: DIR 0 – 25 = Resistant, 26 – 50 = Moderately Resistant; 51 – 75 = Moderately Susceptible and 76 – 100 = Susceptible. Means followed by a common letter within a column do not differ significantly according to Tukey's ($P \leq 0.05$).

3.3 Reaction of Compact Coffee Clones to CLR Under Controlled Conditions

Table 9 presents the results on the reactions of compact coffee leaves upon artificial inoculation of *H. vastatrix*

uredospores collected from N39. Out of sixteen (16), ten (10) compact genotypes showed immune reaction from the rust inoculum collected from N39 and KP423 variety. N39 was the most susceptible genotype to CLR.

Table 9. Reaction of coffee rust disease on *Coffea arabica* compacts 42 days after artificial inoculation of uredospores collected from N39 and KP423

Genotype	Summary of reaction of genotypes from uredospores collected from N39	Summary of reaction of genotypes from uredospores collected from KP423	Remarks
CVT1	2	0	Genotype infected with all coffee rust race (s)
CVT2	I	2	Great possibility of infection by coffee rust race I
CVT3	i	i	Genotype immune to all coffee rust races
CVT4	i	i	Genotype immune to all coffee rust races
CVT5	2	i	Genotype infected with all possible coffee rust race (s)
CVT6	I	2	Great possibility of infection by coffee rust race I
CVT7	i	i	Genotype immune to all coffee rust races
CVT8	i	i	Genotype immune to all coffee rust races
CVT9	i	i	Genotype immune to all coffee rust races
CVT10	i	i	Genotype immune to all coffee rust races
CVT11	i	i	Genotype immune to all coffee rust races
CVT12	i	i	Genotype immune to all coffee rust races
CVT13	i	i	Genotype immune to all coffee rust races
CVT14	i	i	Genotype immune to all coffee rust races
CVT15	2	I	Genotype infected with all possible coffee rust race (s)
CVT16	2	I	Genotype infected with all possible coffee rust race (s)
SC9	2	I	Genotype infected with all possible coffee rust race (s)
SC13	2	I	Genotype infected with all possible coffee rust race (s)
Ctr 088	I	i	Genotype immune to all coffee rust races
N39	X	X	Genotype susceptible to all coffee rust races

Key: N39 is susceptible to all available rust races.

Descriptive scale: i = immune, without any sign of infection; fl = flecks, reaction of hypersensitivity; = necrotic spots; t = small tumefactions at the penetration site; 0 = chlorosisi; 1 = rare sporulating sori, 2= small or medium sized pustules; 3 = medium sized or large pustules surrounded by chlorosis; 4 = large sporulating pustules; X = highly variable size of sporulating pustules. Categories: Resistant: I, fl, t, 0; Moderately Resistant: 1 and 2; Moderately Susceptible: 3 and 4; Susceptible: X.

4. Discussion

The presence of appropriate plant organ, conducive weather conditions and a pathogen helps in assessing the response of genotype for its resistance, partial resistance, moderate susceptible and susceptibility. From flowering, which occurs shortly after the end of the dry season, coffee berry takes about nine months to maturity (harvest). 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). The expanding berry (4-16 weeks after flowering) is the most susceptible stage; this is unlike fully expanded green berries, which are resistant (Mulinge, 1970). The presence of free water (rain, mist or dew) on berry surfaces and temperatures between 21 and 23 ºC are favourable condition for infection and development of epidemics (Nutman & Roberts, 1960; Bock, 1956). Incubation period varies between 3 days with soft green berries (Bock, 1956) to 3 weeks with hard green berries (Mulinge, 1970). Conidia are only released and dispersed by rain between and within coffee trees.

Epidemiology for CLR requires optimum temperatures of between 21 and 25 ºC for the development of *H. vastatrix* (Guggenheim & Harr, 1978). Rain is an important vector for short range distribution of the uredospores, creating suitable conditions for germination (Rayner, 1961). When these conditions prevail across sites they may allow infection and thereby a separation between susceptible and resistant genotypes among the compact coffee. Rainfall and temperature conditions at Lyamungu for 2009/10 were conducive for the development and infection of CBD and CLR (Figures 6 and 7), N39 the susceptible variety succumbed to CBD and CLR, but compact genotypes were resistant.

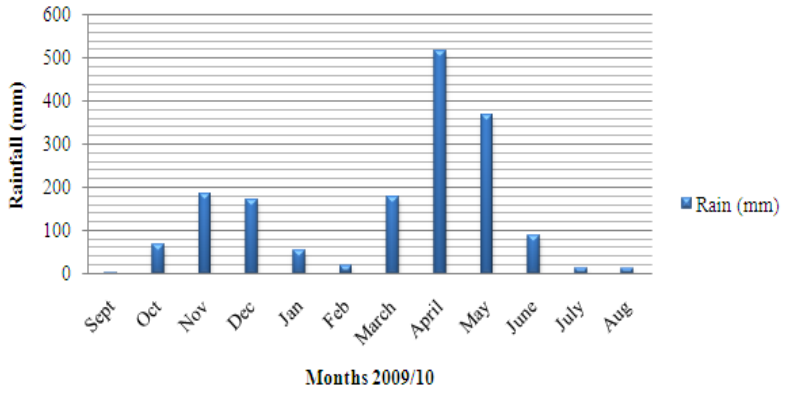

Figure 6. Rainfall Lyamungu 2009/10

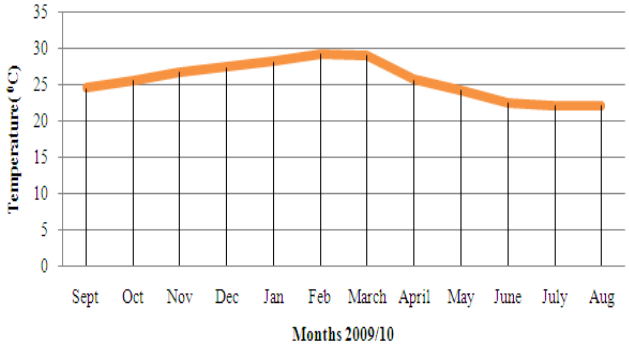

Figure 7. Temperature Lyamungu 2009/10

Differential interaction was shown between *C. kahawae* strains 2010/1, 2010/2, 2006/7 and 2006/14 and *C. arabica* genotypes. While genotype CVT9 (PRO 127 x (Rume Sudan x Catuai) showed resistance to strains 2010/1 and 2006/14, it reacted to strains 2006/7 and 2010/2. *C. kahawae* strain 2006/14 is reported to be the most pathogenic (Kilambo, 2008). Gichimu and Phiri (2012), Gladys et al. (2009) and Varzea *et al.* (2002a) reported the occurrence of differential interaction among the coffee cultivars and *Colletotrichum* spp. used in their studies. The resistance shown by these genotypes may possibly be genetically as one of the parents in the cross is Rume Sudan which carries R-gene (van der Vossen & Walyaro, 2009; van der Vossen & Walyaro, 1980). Variety N39 shows susceptibility to the four *C. kahawae* strains. As Akinsanmi et al. (2006) report, resistance in coffee is possibly made up of more than one mechanism; chemical and mechanical. Genotype CVT14 (Ctr086 x (N39 x Rume Sudan Selfed F_2), showed resistance to almost all *C. kahawae* strains. When studying the genetic diversity among commercial coffee cultivars Kathurima et al. (2012) emphasized that Arabica varieties with widen genetic diversity have added advantages over those with narrow genetic base.

Although van der Vossen et al. (1976) found possible correlation between hypocotyls test and reaction of CBD on expanding berries was not the case in this study. Genotypes CVT10 (Ctr127 x (N39 x Rume Sudan selfed F_2) and CVT14 (Ctr086 x (N39 x Rume Sudan Selfed F_2) showed CBD resistance when green expanding berries were artificially inoculated, but the hypocotyls did succumb to the disease. As Gustavo et al. (2007) document, different resistance in reactions was found in hypocotyls test in relation with fruits in coffee inoculated with

Colletotrichum gloeosporioides isolates. Van der Graff (1982) observed different resistance reaction to *C. kahawae* in the types of CBD evaluation methods. The coffee genotypes CVT2 (Ctr088 x (SL34 x HdT) x Kent x Rume Sudan), CVT4 (Ctr088 x (SL34 x HdT) x Rume Sudan), CVT5 (Ctr088 x (Rume Sudan x Catuai), CVT7 (Ctr088 x (HdT x N39) x SL28) x (N39 x Rume Sudan)), CVT8 (Ctr 088 x (N39 x HdT) x (N39 x HdT) x Rume Sudan) and CVT13 (Ctr127 x (Blue Mountain Jamaica x Cioccie) x Rume Sudan) showed resistance to the four *C. kahawae* strains used in the study. Variety N39 showed high susceptibility to the four *C. kahawae* strains when CBD hypocotyl test was applied. The overall observation here is that expression of resistance varied with the test *C. kahawae* strains and the hybrid crosses. These results are in agreement with the findings of van der Graff (1982) and Omondi et al. (2001) which also revealed variations on the effect of the pathogen and resistance of the coffee genotypes.

Studies done previously on *C. arabica* genotypes variety x *C. kahawae* strains reported existence of interaction, but found to be less significant to suggest conclusively that differential interaction exists (Omondi et al., 2000). Further studies within *C. kahawae* at the DNA level have also revealed limited polymorphism (Beynon et al., 1995). The formation of hyphal fusions during infection of *C. kahawae* is a likely mechanism creating pathogen variations as there is involvement of an exchange of genetic materials (Mwang'ombe et al., 1992).

Out of sixteen (16), nine compact genotypes showed immune reaction from the coffee rust inoculum collected from N39 and KP423. Immune reaction may possibly be resulted from HdT and Catimors which provides genes for resistance to CLR. HdT has been utilized in developing resistant coffee hybrids to CLR in Kenya (Gichimu, 2012). Variety N39 was the most susceptible genotype to CLR. The pathogen causing CLR, *H. vastatrix* Berk et Br., breaks the coffee resistance genes of the most of the cultivars. This is because the physiologic races of the pathogen causing CLR are increasing. Varzea et al. (2002b; 2002c) reported 40 physiological races. In Tanzania, Rodrigues Jr. et al. (1975) reported the existence of seven races but by 2007, additional coffee rust races were reported (TaCRI, 2009; CIFC, 2007). The available races might form part of the CLR inoculum collected from N39 used to test the compact genotypes. While N39 is reported to be attacked by all possible coffee rust races, KP423 is attacked by the most virulent coffee rust race I (Kushalapa & Eskes, 1989). In spite this situation, the host resistance of the compact genotypes which has genes S_H6 and S_H9 provide adequate protection against CLR epidemics (van der Vossen, 2005; Rodrigues Jr. et al., 1975). It can therefore be deduced that the nine identified compact genotypes showed resistance to CLR

5. Conclusion

In this study the results indicate that there is a significant magnitude of resistance of the compact genotypes when tested across coffee growing regions and under controlled conditions. However, there are compact genotypes which are resistant to CBD and CLR and therefore, can be adapted across the prevailing coffee disease strains. These coffee genotypes are potential sources of resistance to CBD and CLR in breeding programmes for developing future commercial coffee cultivars. This study also indicates that *C. kahawae* strains are likely to have adapted to the coffee genotypes. However, further investigations are needed to establish the possibility of the existence of physiologic races of the *C. kahawae* strains.

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