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GENETIC DIVERSITY IN COFFEA CANEPHORA BASED ON THEIR REACTIONS TO RACES OF HEMILEIA VASTATRIX (BERK AND BROOME)

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ABSTRACT

Coffee leaf rust (CLR) has been persistently causing serious yield reduction on *Coffea arabica* coffee in Tanzania. For several decades now there has been no information on the response of *Coffea canephora* to different races of *Hemileia vastatrix* in Tanzania. In recent years variations on the reactions of *Coffea canephora* to coffee leaf rust disease was observed in the robusta coffee germplasm at TaCRI Maruku. An experiment was conducted at Maruku coffee research institution to investigate various races of *H. vastatrix* infecting cultivated *C. canephora* and wild coffee genotypes. Assessment on the reactions to the pathogen of *H. vastatrix* was conducted by using 114 cultivated *C. canephora* and 23 wild coffee genotypes. Two main groups of *C. canephora* with complete and susceptible genotypes were identified. The investigations revealed that 41.2% of assessed genotypes demonstrated complete resistance to coffee leaf rust disease. The remaining 58.8% of evaluated were susceptible to the disease at varied levels of severity ranging from 100 to10%. Susceptible genotypes were subdivided into eleven subgroups corresponding to the response to specific races. *Variations* in the response of *C. canephora* genotypes to different physiological races of *H. vastatrix* revealed genetic diversity among the genotypes of cultivated and wild *C.canephora* in Tanzania.

KEYWORDS: Genotypes of Robusta Coffee, Leaf rust races.

INTRODUCTION

Coffee leaf rust (CLR) caused by *Hemileia vastatrix* (Berk. & Broome) for several decades has been a major cause of yield loss of Arabica coffee in Tanzania (Kilambo *et al*., 2013a). In Tanzania CLR is the second important disease infecting Arabica coffee after coffee berry disease (CBD) caused by *Colletrotrichum kahawae* (Kilambo *et al*., 2013b, Kilambo *et al*., 2015). Coffee leaf rust disease was noted for the first time in 1861 around Lake Victoria in East Africa (Rayner, 1960). In 1894 CLR was reported for the first time infecting cultivated coffee in Tanganyika (Mainland Tanzania (Rayner, 1960). Worldwide, about 49 physiological races of *H. vastatrix* have been reported as causative agents of coffee leaf rust disease of which 21 exist in Tanzania (Rodrigues Jr. *et al*., 1975., CIFC, 2007., Kilambo *et al*., 2013a, Gichuru *et al*., 2012).

For several decades, CLR disease has been controlled by application of copper-based fungicides (Gichuru *et al*., 2012; Kilambo *et al*., 2013a). However, in recent years efforts have been done by coffee research institutions to develop coffee varieties which are resistant to various physiologic races of *H. vastatrix* infecting Arabica coffee (Gichuru *et al*. 2012, Kilambo *et al*., 2013a). In East Africa (Kenya and Tanzania), breeding programmes had been undertaken by coffee research institutes to develop Arabica varieties which are resistant to CLR disease using the resistance genes existing from pure Arabica varieties and that of Robusta origin (derived through hybridization of the Timor Hybrid) with Arabica coffee (van der Vossen and Walyaro, 1981, Kilambo *et al*., 2013a). These programme resulted into the release of two Arabica varieties in Kenya and 19 varieties in Tanzania (Gichuru *et al*., 2010, Gichuru *et al*., 2012, Kilambo *et al*., 2013a, Kilambo *et al*., 2015). Since the outbreak of CLR worldwide, several research works has been conducted focusing on the interactions of the physiologic races of *H. vastatrix* with Arabica genotypes. It has been reported that Robusta coffee genotypes have been considered as the main source of resistance genes (van der Vossen and Walyaro, 1981, Kilambo *et al*., 2013a). However, in recent years it has been noted that the newly discovered races of *H. vastatrix* have virulence genes v6–v9 that breakdown the resistant gene S_H6-S_H9 present in Hibrido de Timor derivatives (Varzea and

Marques, 2005, Sera *et al.*, 2007). In recent years; Robusta coffee germplasm materials established at TaCRI Maruku- sub- station in 1988, which were known to be resistant to CLR, have shown be infected by the CLR disease. Based on these observations, a study was conducted to investigate the reactions of Robusta coffee genotypes to coffee leaf rust disease and identify the physiologic races of *H. vastatrix* infecting Robusta coffee.

MATERIALS AND METHODS

An experiment was conducted to investigate the diversity *of C. canephora* genotypes in Kagera region based on their reactions to different coffee leaf rust (*H. vastatrix*) races. Field sampling of leaves *of C. canephora* genotypes with *H. vastatrix* pathogen was done on114 cultivated *C. canephora* and 23 wild trees from robusta coffee germplasm at TaCRI Maruku Coffee Research Institute. Out of 114 assessed cultivated robusta genotypes, 110 and 4 genotypes originated from Tanzania and Uganda, respectively. Among the wild coffee genotypes, 19 accessions were collected from Minziro forest and the other four from Bushenyi forests in Missenyi district bordering Uganda. This experiment was divided into two parts. The first experiment involved general assessment of reactions of cultivated Robusta coffee and wild coffee genotypes to coffee leaf rust disease. The second experiment involved investigation of different physiological races of *H. vastatrix* infecting Robusta and wild coffee genotype.

Experiment 1: Reaction of genotypes to coffee leaf rust disease

In this experiment, an investigation was conducted to assess the reactions of 114 cultivated Robusta and 23 wild coffee genotypes. Three coffee trees per accession picked at random were used to investigate the coffee leaf rust disease reaction to genotypes. Infection levels and severity scores of coffee leaf rust disease were assessed based on the rating scales of 1-6 as described by Ngulu *et al*.(1998); 1 nil sporulating leaf rust lesions on whole tree, 2 few sporulating leaf rust lesions per branch (< 10%), 3 scattered sporulating leaf rust lesions (10-25%), 4 moderately sporulating leaf rust lesions (25-50%), 5 moderately severely sporulating leaf rust lesions (50-75%) and 6 heavy sporulating leaf rust lesions (> 75%).

Experiment 2: Investigation of different physiological races of *H. vastatrix* infecting cultivated *C. canephora* and wild coffee

This experiment was conducted as a biotic descriptor of the diversity *of C. canephora* genotypes in Kagera region. Field sampling of leaves of *C. canephora* genotypes with *H. vastatrix* pathogen was done on 114 cultivated *C. canephora* and 23 wild coffees from coffee germplasm at Maruku Coffee Research Institute. The Leaves with lesions of coffee leaf rust disease were collected from 53 *C. canephora* genotypes infected with *H. vastatrix* pathogen. Four infected leaves were picked from each infected genotype, labelled, pressed in the tissue papers and packed in the envelopes to keep the isolates alive. The samples were shipped to the laboratory at Lyamungu Coffee Research Institute in Moshi Tanzania for laboratory studies. In the laboratory, the four infected leaves per genotype were pressed between news papers and left to dry without affecting the lesions of rust. The uredospores from lesions were harvested by gentle scrapping off into conical flasks contained sterilized distilled water. In the conical flasks containing the suspensions of uredospores, a drop of tween 80 was added per flask to allow uniform dispersion of uredospores. The concentrations of uredospores per conical flask were standardized at 1 x 10^6 spores / ml. Inoculation was done by dipping camel brushes into the suspension of uredospores, rubbed on the undersides of twelve (12) leaves of each of fourteen CLR differentials (Eskes and Tom-Braghini, 1981) per isolate and labelled. Fourteen coffee leaf rust differential plants were used to differentiate the reaction of coffee leaf rust races. The inoculated leaves of CLR differential plants and 2 un-inoculated healthy leaves per each CLR differential plants used as control were placed in a labelled plastic box of 30 cm length, 15 cm width and 10 cm height covered with black polythene. The black polythene provides dark conditions that stimulate the formation of the germ tubes followed by appresioum which later, initiate the infection processes. Inoculated leaves of CLR differential plants and their respective control were left in the box for 45 days to allow development of visible lesions of CLR. Assessment of CLR on differential plants was concluded 45 days after inoculation. Disease symptoms observed on differential plants were scored by using the rating scales of 1 to 9 by Eskes and Tom-Braghini (1981); whereby 0 describes absence of lesions and 9 intense lesions. The presence of different CLR races was determined according to Rodrigues *et al.* (1975) and Varzea and Marques (2005) who collected samples of coffee leaves infected with *H. vastatrix* from different coffee growing areas and artificially inoculated the leaves of CLR differentials to establish physiological rust races.

RESULTS

Reaction of genotypes to coffee leaf rust

Results showed significant ($P \leq 0.001$) variations in the reaction of 137 genotypes to coffee leaf rust disease (CLR) infection (Table 1). The variations of CLR disease severity scores showed high genetic variability among cultivated C. canephora and wild coffee genotypes (Table 1). The overall mean disease severity score was 2.2 on a disease score scale of 1-6. The disease scores for the most susceptible genotypes were 4 to 6 (Table 1), and these comprised 16.9% of investigated genotypes. The most susceptible genotypes were from cultivated Robusta coffee (C. canephora). The least susceptible genotypes had disease scores of 2 to 3 (Table 1) while the mean disease score for resistant genotypes was 1. The results showed that least susceptible and resistant genotypes comprise 32.4 and 41.8 % of assessed coffee genotypes, respectively. The overall results indicated that

the highest proportions of genotypes within the experimental population were susceptible to CLR (58.8 %) with varying levels of susceptibilities (Table 1). The 41.2 % of experimental C. canephora and wild coffee evaluated comprised the genotypes which were completely resistant to CLR.

Table 1: Coffee leaf rust disease (CLR) severity on C. canephora and wild coffee genotypes from germplasm at TaCRI-Maruku substation.

Genotype code	Origin	Severity (1-6)
287KR 4	Karagwe	6.0
139MI11	Missenyi	6.0
036KR12	Karagwe	6.0
292KR6	Karagwe	6.0
005MI5	Misenyi	6.0
006MI6	Misenyi	6.0
240BK14	Bukoba	6.0
MS 3	Bukoba	6.0
MS 5	Bukoba	6.0
Robusta hybrid	Bukoba	6.0
MS 2	Bukoba	6.0
Robusta ex – coffee nursery	Bukoba	6.0
268BK21	Bukoba	5.0
131MS1BK12	Bukoba	5.0
115BK8	Bukoba	5.0
023KR20	Karagwe	5.0
026BK26	Bukoba	5.0
030KR18	Karagwe	5.0
FM 3 ex – Minziro forest	Minziro forest – Missenyi	5.0
Uganda 3	Uganda	5.0
280KR1	Karagwe	4.0
060KR13	Karagwe	4.0
179ML6	Muleba	4.0
323ML24	Muleba	4.0
283KR2	Karagwe	4.0
087ML12	Muleba	4.0
046KR22	Karagwe	4.0
086ML15	Muleba	4.0
158MI12	Misenyi	4.0
MS 1	Bukoba	4.0
Uganda 1	Uganda	4.0
Uganda 1	Uganda	4.0
FM 2, ex –Minziro	Minziro forest- Missenyi	4.0
324ML25	Muleba	3.0
Uganda 4	Uganda	3.0
FM 1, ex Minziro forest	Minziro forest Missenyi	3.0
091KR23	Karagwe	2.0
008MI8	Misenyi	2.0
009MI9	Misenyi	2.0
003MI3	Misenyi	2.0
007MI7	Misenyi	2.0
293KR7	Karagwe	2.0
308MI21	Misenyi	2.0
344MI19	Misenyi	2.0
306ML20	Muleba	2.0
288KR5	Karagwe	2.0
320KR12	Karagwe	2.0
295BK23	Bukoba	2.0
057BK2	Bukoba	2.0
257BK18	Bukoba	2.0

004MI4	Misenyi	2.0
079ML17	Muleba	2.0
002MI2	Misenyi	2.0
059BK3	Bukoba	2.0
172ML9	Muleba	2.0
123BK10	Bukoba	2.0
062KR14	Karagwe	2.0
114BK4	Bukoba	2.0
259BK19	Bukoba	2.0
010MI10	Misenyi	2.0
269BK22	Bukoba	2.0
037ML19	Muleba	2.0
092KR24	Karagwe	2.0
108BK4	Bukoba	2.0
255BK16	Bukoba	2.0
109BK5	Bukoba	2.0
181ML5	Muleba	2.0
118(1/61)	Bukoba	2.0
011MI11	Misenyi	2.0
315KR11	Karagwe	2.0
175ML8	Muleba	2.0
177MI7	Misenyi	2.0
192ML1	Muleba	2.0
263BK20	Bukoba	2.0
167MI17	Misenyi	2.0
020BKMS5	Bukoba	2.0
047MS2BK1	Bukoba	2.0
055KR15	Karagwe	1.0
194ML3	Muleba	1.0
164MI15	Misenyi	1.0
193ML2	Muleba	1.0
112BK6	Bukoba	1.0
165MI16	Misenyi	1.0
025KR19	Karagwe	1.0
170MI10	Misenyi	1.0
120ML2	Muleba	1.0
160MI13	Misenyi	1.0
125BK11	Bukoba	1.0
127ML12	Muleba	1.0
001MI1	Misenyi	1.0
185ML4	Muleba	1.0
142BK13	Bukoba	1.0
147KR25	Karagwe	1.0
294KR8	Karagwe	1.0
162MI14	Karagwe	1.0
054KR16	Karagwe	1.0
310MI25	Misenyi	1.0
311KR9	Karagwe	1.0
312KR10	Karagwe	1.0
012MI12	Misenyi	1.0
316ML22	Misenyi	1.0
077ML18	Muleba	1.0
284KR3	Karagwe	1.0
080ML16	Muleba	1.0
330MI24	Misenyi	1.0
332MI23	Misenyi	1.0
333MI22	Misenyi	1.0
337MI21	Misenyi	1.0

342 MI20	Missenyi	1,0
049KR21	Karagwe	1.0
346MI18	Missenyi	1.0
347MR10 - variety (control)	Bukoba	1.0
348(13/61) – variety (control)	Bukoba	1.0
349ML2 – variety (control)	Muleba	1.0
FB1	Bushenyi forest	1.0
FB2	Bushenyi forest	1.0
FB3	Bushenyi forest	1.0
FB4	Bushenyi	1.0
FM5	Minziro forest	1.0
FM6	Minziro forest	1.0
FM7	Minziro forest	1.0
FM8	Minziro forest	1.0
FM9	Minziro forest	1.0
FM10	Minziro forest	1.0
FM11	Minziro forest	1.0
FM12	Minziro forest	1.0
FM13	Minziro forest	1.0
FM14	Minziro forest	1.0
FM15	Minziro forest	1.0
FM16	Minziro forest	1.0
FM17	Minziro forest	1.0
FM18	Minziro forest	1.0
FM19	Minziro forest	1.0
FM20	Minziro forest	1.0
Mean		2,2
CV %		65.27
d.f		136
Observed P-Value		< 0.001

(b. i) Races of *H. vastatrix* infecting *C. canephora* genotypes

The results of this study showed that cultivated *C. canephora* and wild coffee genotypes are infected by fifteen different races of *H. vastatrix (*Table 2).The races were I, II, III, XIV, XVI, XX, XXII, XXIII, XXVIII, XXIX, XXX, XXXI, XXXIV, XXXIX and XLI. The results showed that each race was specific to genotypes of cultivated *C. canephora*. The results showed that race XLI was recorded frequently compared to the other fourteen races.

(b. ii). Reaction of physiological races of *H. vastatrix* to differential plants

The results of the reactions of different physiological races of *H. vastatrix* isolated from *C. canephora* genotypes to tested differential plants are summarized in Table 3. The differential plants tested were resistant, tolerant, susceptible or high susceptible to some physiological races causing coffee leaf rust disease. The results indicated that all differential plants tested were resistant to races I, III and XXIII except Matari and DK 16/1 which were susceptible to physiological race I (Table 3). Matari and DK 16/1 were susceptible to physiological races isolated from FM3 ex-Minziro forest, Uganda clone (3 & 4), Robusta ex – coffee nursery at Maruku research centre and FM3- ex- Minziro forest genotypes, respectively. Differential plants 63/1 bourbon, 681/7 *C. canephora* Uganda, 1621/ *C. congensis* Uganda, 168/12 *C. excelsa* Uganda, 32/1 DK 16/1, 849/1 Matari, 420/10 MN 1535 x HW 26/14, 33/1 S.288- 23, and 110/5 S4 Agro were susceptible to physiological race II isolated from different Robusta coffee genotypes (Table 3). The results showed that only differential plants 110/5 S4 Agro and S.288-23 were susceptible to races XIV and XVI (Table 3). The differential plant 681/7 *C. canephora* Uganda was only susceptible to race XX isolated from Robusta hybrid ex – hybrid trial, FM I Robusta ex – Minziro forest and Uganda clones (1 & 4). The differential coffee plants Hibrido de Timor coded 832/1 and 1343/269 were susceptible to unknown and XXII races. The unknown races infecting Hibrido de Timor 832/1 was isolated from Maruku selections (MSs 1, 2 & 5), FM 1, robusta ex- Minziro forest and Uganda clones (1, 2 & 4). Race XXII was isolated from Robusta hybrid ex- hybrid trial at Maruku coffee research centre. The differential plant 1621/13 *C. congensis* from Uganda was susceptible to race XXIII isolated from FM 3, robusta ex- Minziro forest, robusta hybrid ex – hybrid trial, robusta ex- coffee nursery, Uganda clone (1 & 2) (Table 3). Differential plant MN 1535/33 x 2614 was susceptible to race isolated from FM 2, robusta ex- Minziro forest and Uganda clone 4.

Table 2: Races of *H. vastatrix* infecting *C. canephora* and wild genotypes in Kagera region.

Identified races	Genotypes code	Origin of genotype	Location inocula collected	Proportion (% of race)
I	030 KR 18	Karagwe district	Robusta germplasm at Maruku	
	047 MS 2 – Maruku selection 2	Bukoba district	Robusta germplasm at Maruku	
	Uganda clone I	Uganda	Demo plot in Bukoba district	
	MS 3 – Maruku selection 3	Bukoba district	Robusta germplasm at Maruku	
	131 MS 1- derivative	Bukoba district	Robusta germplasm at Maruku	
	Uganda clone 3	Uganda	Demo plot in Bukoba district	9.8
	Uganda clone 4	Uganda	Demo plot in Bukoba district	
	FM 1- Ex- Minziro forest	Minziro- Missenyi district	Robusta germplasm at Maruku	
	FM 2- Ex -Minziro forest	Minziro- Missenyi district	Robusta germplasm at Maruku	
	FM 3- Ex- Minziro forest	Minziro- Missenyi district	Robusta germplasm at Maruku	
	Robusta hybrid	Bukoba district	Robusta hybrid trial at Maruku	
	Uganda clone 1	Uganda	Demo plot in Bukoba district	
	Robusta Ex- nursery	Bukoba district	Robusta germplasm at Maruku	
II	MS 3 -Maruku selection 3	Bukoba district	Robusta germplasm at Maruku	
	MS 5- Maruku selection 5	Bukoba district	Robusta germplasm at Maruku	
	047 MS 2- Maruku selection 2	Bukoba district	Robusta germplasm at Maruku	9.1
	131 MS 1- Maruku selection 1	Bukoba district	Robusta germplasm at Maruku	
	Uganda clone 3	Uganda	Demo plot in Bukoba district	
	Uganda clone 4	Uganda	Demo plot in Bukoba district	
	FM 1- Ex- Minziro forest	Minziro- Missenyi district	Robusta germplasm at Maruku	
	FM 2- Ex -Minziro forest	Minziro- Missenyi district	Robusta germplasm at Maruku	
	FM 3- Ex- Minziro forest	Minziro- Missenyi district	Robusta germplasm at Maruku	
	Robusta hybrid	Bukoba district	Robusta hybrid trial at Maruku	
	Uganda clone 1	Uganda	Demo plot in Bukoba district	
	Robusta Ex- nursery	Bukoba district	Robusta germplasm at Maruku	
III	Uganda clone I	Uganda	Demo plot in Bukoba district	
	MS 2- Maruku selection 2	Bukoba district	Robusta germplasm at Maruku	
	Uganda clone 4	Uganda	Demo plot in Bukoba district	3.8
	FM 2- Ex -Minziro forest	Minziro- Missenyi district	Robusta germplasm at Maruku	
	Uganda clone 2	Uganda	Demo plot in Bukoba district	
XIV	Uganda clone 1	Uganda	Demo plot in Bukoba district	
	Uganda clone 2	Uganda	Demo plot in Bukoba district	
	Uganda clone 3	Uganda	Demo plot in Bukoba district	
	Uganda clone 4	Uganda	Demo plot in Bukoba district	
	FM 1- Ex- Minziro forest	Minziro- Missenyi district	Robusta germplasm at Maruku	7.6
	FM 2- Ex -Minziro forest	Minziro- Missenyi district	Robusta germplasm at Maruku	
	FM 3- Ex- Minziro forest	Minziro- Missenyi district	Robusta germplasm at Maruku	
	MS5, Maruku selection 5	Bukoba district	Robusta germplasm at Maruku	

	Robusta hybrid	Bukoba district	Robusta hybrid trial at Maruku	
	Robusta Ex- nursery	Bukoba district	Robusta germplasm at Maruku	
XVI	Uganda clone 1	Uganda	Demo plot in Bukoba district	
	Uganda clone 2	Uganda	Demo plot in Bukoba district	
	Uganda clone 4	Uganda	Demo plot in Bukoba district	4.5
	FM 3- Ex- Minziro forest	Minziro- Missenyi district	Robusta germplasm at Maruku	
	Robusta hybrid	Bukoba district	Robusta hybrid trial at Maruku	
	Robusta Ex- nursery	Bukoba district	Robusta germplasm at Maruku	
	Uganda clone 1	Uganda	Demo plot in Bukoba district	
XX	Uganda clone 2	Uganda	Demo plot in Bukoba district	
	Uganda clone 3	Uganda	Demo plot in Bukoba district	
	Uganda clone 4	Uganda	Demo plot in Bukoba district	6.8
	FM 1- Ex- Minziro forest	Minziro- Missenyi district	Robusta germplasm at Maruku	
	FM 2- Ex -Minziro forest	Minziro- Missenyi district	Robusta germplasm at Maruku	
	FM 3- Ex- Minziro forest	Minziro- Missenyi district	Robusta germplasm at Maruku	
	Robusta hybrid	Bukoba district	Robusta hybrid trial at Maruku	
	Robusta Ex- nursery	Bukoba district	Robusta germplasm at Maruku	
XXII	Uganda clone I	Uganda	Demo plot in Bukoba district	
	Uganda clone 2	Uganda	Demo plot in Bukoba district	
	Uganda clone 3	Uganda	Demo plot in Bukoba district	
	MS 3 -Maruku selection 3	Bukoba district	Robusta germplasm at Maruku	6.1
	Uganda clone 4	Uganda	Demo plot in Bukoba district	
	FM 1- Ex- Minziro forest	Minziro- Missenyi district	Robusta germplasm at Maruku	
	Robusta hybrid	Bukoba district	Robusta hybrid trial at Maruku	
	Robusta Ex- nursery	Bukoba district	Robusta germplasm at Maruku	
XXIII	Uganda clone 1	Uganda	Demo plot in Bukoba district	
	Uganda clone 2	Uganda	Demo plot in Bukoba district	
	Uganda clone 4	Uganda	Demo plot in Bukoba district	
	FM 1- Ex- Minziro forest	Minziro- Missenyi district	Robusta germplasm at Maruku	6.1
	FM 2- Ex -Minziro forest	Minziro- Missenyi district	Robusta germplasm at Maruku	
	FM 3- Ex- Minziro forest	Minziro- Missenyi district	Robusta germplasm at Maruku	
	Robusta hybrid	Bukoba district	Robusta hybrid trial at Maruku	
	Robusta Ex- nursery	Bukoba district	Robusta germplasm at Maruku	
XXVIII	036 KR 12	Karagwe district	Robusta germplasm at Maruku	1.5
	008 MI 8	Missenyi district	Robusta germplasm at Maruku	
XXIX	Uganda clone 3	Uganda	Demo plot in Bukoba district	
	Uganda clone 4	Uganda	Demo plot in Bukoba district	
	Uganda clone 2	Uganda	Demo plot in Bukoba district	

	FM 1- Ex- Minziro forest	Minziro- Missenyi district	Robusta germplasm at Maruku	
	FM 2- Ex -Minziro forest	Minziro- Missenyi district	Robusta germplasm at Maruku	6.1
	FM 3- Ex- Minziro forest	Minziro- Missenyi district	Robusta germplasm at Maruku	
	Robusta hybrid	Bukoba district	Robusta hybrid trial at Maruku	
	Robusta Ex- nursery	Bukoba district	Robusta germplasm at Maruku	
XXX	Uganda clone I	Uganda	Demo plot in Bukoba district	3.8
	Uganda clone 2	Uganda	Demo plot in Bukoba district	
	Robusta hybrid	Bukoba district	Robusta hybrid trial at Maruku	
	Uganda clone 4	Uganda	Demo plot in Bukoba district	
	Robusta Ex- nursery	Bukoba district	Robusta germplasm at Maruku	
XXXI	306 ML	Muleba district	Robusta germplasm at Maruku	
	005 MI 5	Missenyi district	Robusta germplasm at Maruku	1.5
XXXIV	060 KR 13	Karagwe district	Robusta germplasm at Maruku	
	108 BK 4	Bukoba district	Robusta germplasm at Maruku	1.5
XXXIX	308MI 21	Missenyi district	Robusta germplasm at Maruku	
	109 BK 5	Bukoba district	Robusta germplasm at Maruku	1.5
XLI	006MI 6	Missenyi district	Robusta germplasm at Maruku	
	007MI 7	Missenyi district	Robusta germplasm at Maruku	
	010 MI 10	Missenyi district	Robusta germplasm at Maruku	
	037 ML 19	Muleba district	Robusta germplasm at Maruku	
	062 KR 14	Karagwe district	Robusta germplasm at Maruku	
	086 ML 15	Muleba district	Robusta germplasm at Maruku	
	087 ML 12	Muleba district	Robusta germplasm at Maruku	
	091 KR 23	Karagwe district	Robusta germplasm at Maruku	
	113 BK	Bukoba district	Robusta germplasm at Maruku	
	114 BK 2	Bukoba district	Robusta germplasm at Maruku	30.3
	115 BK 8	Bukoba district	Robusta germplasm at Maruku	
	123 BK 10	Bukoba district	Robusta germplasm at Maruku	
	125 BK 11	Bukoba district	Robusta germplasm at Maruku	
	131 MS I – Maruku selection 1	Bukoba district	Robusta germplasm at Maruku	
	139 ML 11	Muleba district	Robusta germplasm at Maruku	
	160 MI 13	Missenyi district	Robusta germplasm at Maruku	
	167 MI 17	Missenyi district	Robusta germplasm at Maruku	
	179 ML 6	Muleba district	Robusta germplasm at Maruku	
	193 ML 2	Muleba district	Robusta germplasm at Maruku	
	240 BK 14	Bukoba district	Robusta germplasm at Maruku	
	255 BK 16	Bukoba district	Robusta germplasm at Maruku	
	257 BK 18	Bukoba district	Robusta germplasm at Maruku	
	268 BK 21	Bukoba district	Robusta germplasm at Maruku	

	269 BK 22	Bukoba district	Robusta germplasm at Maruku	
	280 KR 1	Karagwe district	Robusta germplasm at Maruku	
	283 KR 2	Karagwe district	Robusta germplasm at Maruku	
	287 KR 4	Karagwe district	Robusta germplasm at Maruku	
	288 KR 5	Karagwe district	Robusta germplasm at Maruku	
	323 ML 23	Muleba district	Robusta germplasm at Maruku	
	324 ML 24	Muleba district	Robusta germplasm at Maruku	

Table 3: Reactions of differential plants to *H. vastatrix* races infecting C. *canephora* in Kagera region, Tanzania.

Code	Coffee designation	Scores of rust severity (1- 9)	Race	Source of inocula	Cultivar name	Resistance reaction to differential plants
63/1	Bourbon	1	I	FM 1, Ex- Minziro forest	*C. canephora*	Resistant
63/1	Bourbon	2	I	Robusta, ext – nursery	*C. canephora*	Tolerant
63/1	Bourbon	2	I	FM 3, Ex- Minziro forest	*C. canephora*	Tolerant
63/1	Bourbon	2	I	Robusta hybrid, ext- hybrid trials	*C. canephora*	Tolerant
63/1	Bourbon	2	I	Uganda clone 3	*C. canephora*	Tolerant
63/1	Bourbon	2	I	Uganda clone 1	*C. canephora*	Tolerant
63/1	Bourbon	2	I	Uganda clone 4	*C. canephora*	Tolerant
849/1*	Matari	2	1	Uganda clone 2	*C. canephora*	Tolerant
849/1*	Matari	2	1	Uganda clone 1	*C. canephora*	Tolerant
849/1*	Matari	4	1	FM 3, Ex- Minziro forest	*C. canephora*	Susceptible
849/1*	Matari	7	1	Uganda clone 3	*C. canephora*	High susceptible
849/1*	Matari	7	1	Uganda clone 4	*C. canephora*	High susceptible
849/1*	Matari	6	1	Robusta, ext – nursery	*C. canephora*	High susceptible
32/1*	DK 16/1	1	I	Robusta hybrid, ext- hybrid trials	*C. canephora*	Resistant
32/1*	DK 16/1	2	I	Uganda clone 1	*C. canephora*	Tolerant
32/1*	DK 16/1	2	I	Uganda clone 3	*C. canephora*	Tolerant
32/1*	DK 16/1	2	I	Uganda clone 4	*C. canephora*	Tolerant
32/1*	DK 16/1	7	I	FM 3, Ex- Minziro forest	*C. canephora*	Resistant
H419/20*	1535/20 Mundo *	1	II	MS 2, Maruku selection 2	*C. canephora*	Resistant
H419/20*	1535/20 Mundo *	1	II	MS 3, Maruku selection 3	*C. canephora*	Resistant
H419/20*	1535/20 Mundo *	2	II	MS 5, Maruku selection 5	*C. canephora*	Tolerant
H419/20*	1535/20 Mundo *	2	II	MS 1, Maruku selection 1	*C. canephora*	Tolerant
1343/269	Hybrido de Timor	2	II	MS 1, Maruku selection 1	*C. canephora*	Tolerant
1343/269	Hybrido de Timor	2	II	MS 5, Maruku selection 5	*C. canephora*	Tolerant
63/1	Bourbon	3	II	MS 2, Maruku selection 2	*C. canephora*	Resistant
63/1	Bourbon	6	II	MS 1, Maruku selection 1	*C. canephora*	High Susceptible
63/1	Bourbon	4	II	MS 3, Maruku selection 3	*C. canephora*	Susceptible
63/1	Bourbon	5	II	MS 5, Maruku selection 5	*C. canephora*	Susceptible
681/7	*C. canephora* Uganda	2	II	MS 1, Maruku selection 1	*C. canephora*	Tolerant

681/7	*C. canephora* Uganda	2	II	MS 3, Maruku selection 3	*C. canephora*	Tolerant
681/7	*C. canephora* Uganda	5	II	MS 5, Maruku selection 5	*C. canephora*	Susceptible
681/7	*C. canephora* Uganda	4	II	MS 2, Maruku selection 2	*C. canephora*	Susceptible
829/1	*C. canephora* Uganda	2	II	MS 1, Maruku selection 1	*C. canephora*	Tolerant
829/1	*C. canephora* Uganda	2	II	MS 5, Maruku selection 5	*C. canephora*	Tolerant
263/1	*C. congensis, Uganda*	2	II	MS 1, Maruku selection 1	*C. canephora*	Tolerant
263/1	*C. congensis, Uganda*	2	II	MS 2, Maruku selection 2	*C. canephora*	Tolerant
1621/13	*C. congensis, Uganda*	2	II	MS 5, Maruku selection 5	*C. canephora*	Tolerant
1621/13	*C. congensis, Uganda*	3	II	MS 2, Maruku selection 2	*C. canephora*	Susceptible
1621/13	*C. congensis, Uganda*	7	II	MS 3, Maruku selection 3	*C. canephora*	High susceptible
1621/13	*C. congensis, Uganda*	8	II	MS 1, Maruku selection 1	*C. canephora*	High susceptible
168/12	*C. excelsa* Longkoi	1	II	MS 1, Maruku selection 1	*C. canephora*	Resistant
168/12	*C. excelsa* Longkoi	2	II	FM 1, Ex – Minziro forest	*C. canephora*	Tolerant
168/12	*C. excelsa* Longkoi	3	II	FM 2, Ex – Minziro forest	*C. canephora*	Susceptible
168/12	*C. excelsa* Longkoi	4	II	Uganda clone 1	*C. canephora*	Susceptible
168/12	*C. excelsa* Longkoi	4	II	MS 2, Maruku selection 2	*C. canephora*	Susceptible
168/12	*C. excelsa* Longkoi	4	II	Robusta ex- nursery at Maruku	*C. canephora*	Susceptible
168/12	*C. excelsa* Longkoi	8	II	Robusta hybrid, ex – hybrid trial	*C. canephora*	High susceptible
32/1	DK 16/1	2	II	MS 1, Maruku selection 1	*C. canephora*	Tolerant
32/1	DK 16/1	2	II	MS 2, Maruku selection 2	*C. canephora*	Tolerant
32/1	DK 16/1	4	II	MS 3, Maruku selection 3	*C. canephora*	Susceptible
32/1	DK 16/1	7	II	MS 5, Maruku selection 5	*C. canephora*	High susceptible
849/1	Matari	1	II	MS 1, Maruku selection 1	*C. canephora*	Resistant
849/1	Matari	1	II	MS 2, Maruku selection 2	*C. canephora*	Resistant
849/1	Matari	4	II	MS 5, Maruku selection 5	*C. canephora*	Susceptible
420/10	MN1535 x HW26/14	2	II	MS 2, Maruku selection 2	*C. canephora*	Tolerant
420/10	MN1535 x HW26/14	2	II	MS 3, Maruku selection 3	*C. canephora*	Tolerant
420/10	MN1535 x HW26/14	2	II	MS 5, Maruku selection 5	*C. canephora*	Tolerant
420/10	MN1535 x HW26/14	3	II	MS 1, Maruku selection 1	*C. canephora*	Resistant
33/1	S.288-23	2	II	MS 2, Maruku selection 2	*C. canephora*	Tolerant
33/1	S.288-23	2	II	MS 5, Maruku selection 5	*C. canephora*	Tolerant
33/1	S.288-23	7	II	MS 3, Maruku selection 3	*C. canephora*	High susceptible
110/5	S4 Agro	2	II	MS 1, Maruku selection 1	*C. canephora*	Tolerant
110/5	S4 Agro	4	II	MS 2, Maruku selection 2	*C. canephora*	Susceptible
110/5	S4 Agro	5	II	MS 3, Maruku selection 3	*C. canephora*	Susceptible
128/2	Dilla and Alghae	1	III	FM 1 Robusta ex – Minziro forest	*C. canephora*	Resistant
128/2	Dilla and Alghae	1	III	Uganda clone 4	*C. canephora*	Resistant
128/2	Dilla and Alghae	1	III	Uganda clone 1	*C. canephora*	Resistant
128/2	Dilla and Alghae	1	III	Uganda clone 2	*C. canephora*	Resistant

110/5	S4 Agro	2	XIV	FM 2 Robusta ex – Minziro forest	*C. canephora*	Tolerant
110/5	S4 Agro	2	XIV	Uganda clone 2	*C. canephora*	Tolerant
110/5	S4 Agro	2	XIV	Uganda clone 3	*C. canephora*	Tolerant
110/5	S4 Agro	4	XIV	MS 5, Maruku selection 5	*C. canephora*	Susceptible
110/5	S4 Agro	4	XIV	FM 1 Robusta ex – Minziro forest	*C. canephora*	Susceptible
110/5	S4 Agro	4	XIV	Robusta ex- nursery at Maruku	*C. canephora*	Susceptible
110/5	S4 Agro	4	XIV	FM 3 Robusta ex – Minziro forest	*C. canephora*	Susceptible
110/5	S4 Agro	4	XIV	Uganda clone 4	*C. canephora*	Susceptible
110/5	S4 Agro	6	XIV	Robusta hybrid, ex – hybrid trial	*C. canephora*	High susceptible
110/5	S4 Agro	6	XIV	Uganda clone 1	*C. canephora*	High susceptible
33/1	S.288- 23	2	XVI	Uganda clone 4	*C. canephora*	Tolerant
33/1	S.288- 23	2	XVI	Robusta ex- nursery at Maruku	*C. canephora*	Tolerant
33/1	S.288- 23	2	XVI	Robusta hybrid, ex – hybrid trial	*C. canephora*	Tolerant
33/1	S.288- 23	3	XVI	FM 3 Robusta ex – Minziro forest	*C. canephora*	Susceptible
33/1	S.288- 23	4	XVI	Uganda clone 1	*C. canephora*	Susceptible
681/7	*C. canephora* Uganda	1	XX	FM 3 Robusta ex – Minziro forest	*C. canephora*	Tolerant
681/7	*C. canephora* Uganda	2	XX	Uganda clone 1	*C. canephora*	Tolerant
681/7	*C. canephora* Uganda	2	XX	Uganda clone 3	*C. canephora*	Tolerant
681/7	*C. canephora* Uganda	2	XX	Robusta ex- nursery at Maruku	*C. canephora*	Tolerant
681/7	*C. canephora* Uganda	2	XX	FM 2 Robusta ex – Minziro forest	*C. canephora*	Tolerant
681/7	*C. canephora* Uganda	3	XX	Robusta hybrid, ex – hybrid trial	*C. canephora*	Susceptible
681/7	*C. canephora* Uganda	4	XX	FM 1 Robusta ex – Minziro forest	*C. canephora*	Susceptible
681/7	*C. canephora* Uganda	4	XX	Uganda clone 4	*C. canephora*	Susceptible
681/7	*C. canephora* Uganda	6	XX	Uganda clone 2	*C. canephora*	High susceptible
832/1	Hibrido de Timor	2	Unknown	FM 2, Ex – Minziro forest	*C. canephora*	Tolerant
832/1	Hibrido de Timor	2	Unknown	FM 3, Ex – Minziro forest	*C. canephora*	Tolerant
832/1	Hibrido de Timor	4	Unknown	MS5, Maruku selection 5	*C. canephora*	Susceptible
832/1	Hibrido de Timor	4	Unknown	FM 1, Ex – Minziro forest	*C. canephora*	Susceptible
832/1	Hibrido de Timor	4	Unknown	Uganda clone 1	*C. canephora*	Susceptible
832/1	Hibrido de Timor	4	Unknown	Uganda clone 2	*C. canephora*	Susceptible
832/1	Hibrido de Timor	4	Unknown	Uganda clone 4	*C. canephora*	Susceptible
832/1	Hibrido de Timor	6	Unknown	MS 2, Maruku selection 2	*C. canephora*	High susceptible
832/1	Hibrido de Timor	6	Unknown	MS 3, Maruku selection 3	*C. canephora*	High susceptible
829/1	*C. canephora* Uganda	1	Unknown	Uganda clone 3	*C. canephora*	Resistant
829/1	*C. canephora* Uganda	2	Unknown	FM 1, Ex – Minziro forest	*C. canephora*	Tolerant
829/1	*C. canephora* Uganda	2	Unknown	Robusta ex- nursery at Maruku	*C. canephora*	Tolerant
829/1	*C. canephora* Uganda	2	Unknown	FM 2, Ex – Minziro forest	*C. canephora*	Tolerant
829/1	*C. canephora* Uganda	2	Unknown	Robusta hybrid, ex – hybrid trial	*C. canephora*	Tolerant
829/1	*C. canephora* Uganda	2	Unknown	Uganda clone 1	*C. canephora*	Tolerant

829/1	*C. canephora* Uganda	2	Unknown	Uganda clone 2	*C. canephora*	Tolerant
829/1	*C. canephora* Uganda	2	Unknown	Uganda clone 4	*C. canephora*	Tolerant
1343/269	Hibrido de Timor	2	XXII	FM 1, Ex – Minziro forest	*C. canephora*	Tolerant
1343/269	Hibrido de Timor	2	XXII	Robusta ex- nursery at Maruku	*C. canephora*	Tolerant
1343/269	Hibrido de Timor	2	XXII	FM 2, Ex – Minziro forest	*C. canephora*	Tolerant
1343/269	Hibrido de Timor	2	XXII	Uganda clone 1	*C. canephora*	Tolerant
1343/269	Hibrido de Timor	3	XXII	Robusta hybrid, ex – hybrid trial	*C. canephora*	Susceptible
1343/269	Hibrido de Timor	4	XXII	MS 3, Maruku selection 3	*C. canephora*	Susceptible
1343/269	Hibrido de Timor	4	XXII	Uganda clone 3	*C. canephora*	Susceptible
1343/269	Hibrido de Timor	6	XXII	Uganda clone 2	*C. canephora*	High susceptible
1343/269	Hibrido de Timor	6	XXII	Uganda clone 4	*C. canephora*	High susceptible
1621/13	*C. congensis* Uganda	2	XXIII	FM 1, Ex – Minziro forest	*C. canephora*	Tolerant
1621/13	*C. congensis* Uganda	2	XXIII	Uganda clone 4	*C. canephora*	Tolerant
1621/13	*C. congensis* Uganda	2	XXIII	FM 2, Ex – Minziro forest	*C. canephora*	Tolerant
1621/13	*C. congensis* Uganda	3	XXIII	FM 3, Ex – Minziro forest	*C. canephora*	Susceptible
1621/13	*C. congensis* Uganda	4	XXIII	Robusta hybrid, ex – hybrid trial	*C. canephora*	Susceptible
1621/13	*C. congensis* Uganda	4	XXIII	Uganda clone 1	*C. canephora*	Susceptible
1621/13	*C. congensis* Uganda	7	XXIII	Robusta ex- nursery at Maruku	*C. canephora*	High susceptible
1621/13	*C. congensis* Uganda	8	XXIII	Uganda clone 2	*C. canephora*	High susceptible
H 420/10	MN 1535/33 x HW 26/14	1	XXIX	FM 3, Ex – Minziro forest	*C. canephora*	Resistant
H 420/10	MN 1535/33 x HW 26/14	2	XXIX	FM 1, Ex – Minziro forest	*C. canephora*	Tolerant
H 420/10	MN 1535/33 x HW 26/14	2	XXIX	Robusta ex- nursery at Maruku	*C. canephora*	Tolerant
H 420/10	MN 1535/33 x HW 26/14	2	XXIX	Robusta hybrid, ex – hybrid trial	*C. canephora*	Tolerant
H 420/10	MN 1535/33 x HW 26/14	2	XXIX	Uganda clone 2	*C. canephora*	Tolerant
H 420/10	MN 1535/33 x HW 26/14	2	XXIX	Uganda clone 3	*C. canephora*	Tolerant
H 420/10	MN 1535/33 x HW 26/14	3	XXIX	FM 2, Ex – Minziro forest	*C. canephora*	Susceptible
H 420/10	MN 1535/33 x HW 26/14	4	XXIX	Uganda clone 4	*C. canephora*	Susceptible
H 419/20	MN 1535/33 x 26/13	1	XXX	Robusta hybrid, ex – hybrid trial	*C. canephora*	Resistant
H 419/20	MN 1535/33 x 26/13	1	XXX	Robusta ex- nursery at Maruku	*C. canephora*	Resistant
H 419/20	MN 1535/33 x 26/13	2	XXX	Uganda clone 1	*C. canephora*	Tolerant
H 419/20	MN 1535/33 x 26/13	2	XXX	Uganda clone 4	*C. canephora*	Tolerant

DISCUSSION

The results from this study revealed that races of *H. vastatrix* infecting *C. canephora* are very important tools of studying the genetic diversity of cultivated *C. canephora* and wild coffee. Genetic variations were observed among 53 *C. canephora* genotypes based on their reactions to *H. vastatrix* races. In the previous study conducted between 2012 and 2016 six races of *H. vastatrix* were recorded from 40 *C. canephora* genotypes established at Maruku coffee research centre in Bukoba district in Kagera region, Tanzania (Ng'homa, 2016).The races included: I, XXVIII, XXXI, XXXIV, XXXIX and XLI. These races were identified infecting specific genotypes of cultivated *C. canephora* and hence classified cultivated *C. canephora* into six groups. In the recent study conducted in 2017 involving other 13 cultivars grown in Kagera region, eleven races of *H. vastatrix* were discovered infecting genotypes of cultivated and wild *C. canephora*. The races included; I, II, III, XXI, XIV, XVI, XX, XXII, XXIII, XXIV, XXVIII, XXIX, XXX, XXXI, and XXXIV.

In both studies races XLI was observed frequently in many samples followed by races I, II, XIV, XXII, XXIII, XXIX, XXX, XVI and III, respectively. The least observed races were XXVIII, XXXI, XXXIV and XXXIX. The genotypes of cultivated and wild *C. canephora* are race specific. Some genotypes can be infected by several races while others are infected by a few races. The genotypes infected by several races include Uganda clone 1, Robusta ex- coffee nursery, Robusta hybrid, FM 1 Ex – Minziro forest, FM 2 Ex-Minziro forest, FM 3 Ex- Minziro forest, Uganda clone 2, Uganda clone 3 and Uganda clone 4. Other genotypes are either infected by three; two or one races. The variations in reactions of 15 physiological races of *H. vastatrix* to cultivated and wild C. *canephora* confirmed the genetic diversity of *C. canephora*.

The current study revealed that susceptible *C. canephora* has more genetic diversity groups than those reported by Ng'homa (2016). Among the coffee leaf rust physiological races, race XLI is the most dominating one infecting 30.3 % of susceptible *C. canephora* genotypes followed by races I (9.8%), II (9.1%), XIV (7.6%), XX (6.8%), XXII (6.1%), XXIII (6.1%), XXIX (6.1%), XVI (4.5%), III (3.8%) and races XXVIII, XXXI, XXXIV and XXXIX each 1.5%. The susceptible *C. canephora* genotypes infected by the same race(s) probably showed genetic similarities though there were variations on the infection levels from one genotype to another indicating the highest genetic variations within *C. canephora* genotypes.

On the other hand the reactions of different physiological races of *H. vastatrix* isolated from Robusta cultivars were also reported infecting Arabica coffee. Races I, II, III, XIV, XVI, XX, XXIII, XXVIII, XXIX, XXX, XXXI, XXXIV, XXXIX and XLI which observed infecting Robusta coffee in Kagera region in Tanzania also infect Arabica coffee in different countries (Gichuru *et al*., 2012; Kilambo *et al.*, 2013a; Sera *et al*., 2007). Races I, II, XXVIII and XXXI were among the known pathogen identified infecting *C. arabica* in Tanzania (Kilambo *et al*., 2013a). Races I, II and XX which infect *C. canephora* in Tanzania, are among the old races infected *C. arabica* in Kenya (Gichuru *et al*., 2012). In this study races XIV, XXIII, XXIX, XXX, XXXI, XXXIV, XXXIX and XLI which were recorded in *C. canephora* were also recorded in *C. arabica* in Tanzania. Only races III, XXIII and XLI which infect *C. canephora* in Tanzania also infect *C. arabica* in Kenya (Gichuru *et al*., 2012). Furthermore, the results showed that the aggressiveness and their abilities of identified 15 races to cause severe disease symptoms on tested differential plants vary among themselves. All 15 races could cause infection levels between 1 and 9 score depending on the genotypes and their virulence. Additionally results showed that some races caused severe symptoms to tested HDT derivatives. Races caused severe symptoms to the HDT derivatives included XXII and unknown races. Both races XXII and unknown had symptoms scores ranging from 3 to 6 scales indicating that HDT derivatives are susceptible to these races. The findings on the susceptibility of HDT derivatives to some races observed in this study are in line with those of Caicedo *et al*. (2013), Ligado *et al*.(2015), Varzea and Marques (2005) and Van der Vossen (2005) who reported the break of resistance in HDT derivatives.

The observation of 15 races of *H. vastatrix* infecting *C. canephora* in Tanzania has posed a big challenge to coffee breeders in Tanzania. This is because for several decades now the major disease of *C. canephora* was thought to be coffee wilt disease. But according to these findings, CLR is now becoming the second important disease of *C. canephora* in Tanzania that needs immediate attention to rescue Robusta coffee production in the country. Moreover, the identification and characterization of new races of *H. vastatrix* infecting both *C. arabica* and *C. canephora* is another challenge to breeders to identify the resistant genes from *C. canephora* that can be used in breeding programme to improve levels of resistance on both *C. arabica* and *C. canephora*.

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