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ASSESSMENT OF ROBUSTA COFFEE GENOTYPES ON THEIR REACTION TO COFFEE WILT DISEASE AND BREEDING FOR RESISTANCE

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ABSTRACT

Coffee wilt is the most destructive disease affecting robusta coffee in Tanzania. Since its outbreak in 1997 the disease has spread throughout the robusta coffee growing districts in Kagera region causing a loss of 1% of robusta coffee produced in Tanzania. A study was conducted to assess the performance of the hybrids of *C. canephora* against coffee wilt disease (CWD) and to screen germplasm of cultivated and wild coffee from Bushenyi and Minziro forests. A total of 7,320 individual seedlings from 124 genotypes were artificially inoculated with *Fusarium xylarioides*, a pathogen that causes coffee wilt disease. Resistant genotypes 13/61 and ML2 were crossed to two susceptible commercial varieties MS1 and MS2. The F_1 progenies and their parents were inoculated with *F. xylarioides* pathogen. The results showed that 93% of screened *C. canephora* seedlings were susceptible, while 7% were resistant to CWD at various levels. About 3.3% of resistant genotypes demonstrated complete resistant to the pathogen. The complete resistant genotypes were wild coffee accessions FB1, FB2, FB3 and FB4 from Bushenyi forest. Significant differences in the response of F_1 hybrids to CWD pathogen were observed at $P \leq 0.001$. High survival rates were recorded on the resistant parents (13/61 and ML2), while the lowest survival rates were observed on the susceptible parents (MS1 and MS2). A total of 78 out of 240 tested F_1 hybrids did not succumb to CWD. The overall results showed that transfer of resistant genes from the donor to the recipient parents is very low. All cultivated *C. canephora* which have exhibited resistant or tolerant together with wild genotypes which are completely resistant to CWD should be evaluated for advanced development of coffee varieties.

KEY WORDS: *Coffea canephora, Fusarium xylarioides*, Kagera region of Tanzania.

INTRODUCTION

In Tanzania coffee is one of important traditional cash crops that contributes USD 117 millions to export earnings annually (Baffes, 2003) and provides employment to 420 000 families. Out of 420 000 families, 250 000 are from Kagera region. The major coffee species grown in Tanzania are *Coffea arabica* and *Coffea canephora*. *Coffea arabica* is grown in the northern and southern highlands regions while *C. canephora* is grown in the western part mainly Kagera region. In recent years, *C. canephora* contributed 50% of the total coffee production and 25- 30% of the total export earnings in Tanzania. Production of robusta coffee has several constraints such as: small seeds with poor quality, low productivity due to ageing of coffee trees, poor coffee husbandry practices and invasion of coffee wilt disease (CWD) (Kilambo *et al.*, 1997) which currently is the most destructive. Coffee wilt disease attacks plants at all growth stages and infected plants dry up completely.

Coffee wilt disease was observed for the first time in 1927 in the Central African Republic (CAR) on *Coffea liberica* var. formerly *C. liberica* type *excelsa* (Figueres, 1940; Davis *et al.*, 2006). Between 1930s and 1950s the disease caused a serious crop loss in other central African countries in particular in Cameroon (Guillemat, 1946; Fraselle, 1950; Saccas, 1951; Muller, 1997). During that period, the disease was also reported destroying C. *canephora* in the Ivory Coast and caused serious loss of the crop (Delassus, 1954). Although this disease was the most destructive, yet some varieties of *C. canephora* imported from the Democratic Republic of Congo (DRC) between 1914 and 1933 showed level of resistance and this was confirmed through artificial inoculations (Meiffren, 1961). However, the same author in 1961 reported difference responses to CWD in the different countries, the varieties that showed resistance in Ivory Coast were completely susceptible in Central African Republic (CAR) probably due to the differences in the environmental conditions or physiological variations of the pathogen in different localities in the region.

In 1948 CWD was noted on *C. canephora* at Yangambi in DRC (Fraselle, 1950), and later on it became a major problem on *C. canephora*. In 1957, similar symptoms were observed on *Coffea arabica* in Ethiopia (Lejeune, 1958), and later on the disease was confirmed by Kranz and Mogk (1973). In 1980s CWD was reported in all coffee growing regions of Ethiopia (Pieters and Van der Graaff, 1980). Van der Graaff and Pieters (1978) reported resistance in some *C. arabica* cultivars. The presence of resistant *C. arabica* cultivars were confirmed through artificial inoculation (Pieters and Van der Graaff, 1980).

After the threat of CWD in African coffee growing countries**,** a number of strategies were laid down to eliminate the disease. These strategies included elimination of infected coffee trees over a large areas and search for resistant varieties from both wild and cultivated coffee. The first resistant varieties were identified in DRC (Musoli *et al.*, 2009). These varieties were used for replanting programme within DRC and Ivory Coast (Saccas, 1956; Meiffren, 1961). In Cameroon the disease was eliminated by massively uprooting infected coffee trees (Muller, 1997). These strategies were successful because the disease was managed from being a major to the minor one in the end 1950 and it eventually disappeared from DRC, Ivory Coast, Cameroon and CAR (Musoli *et al*., 2009).

In 1986, the new outbreak of CWD was reported on *C. canephora* in the North–East of DRC (Flood and Brayford, 1997). The disease rapidly spread to Uganda in 1993 and north-west Tanzania in Kagera region in 1997 (Kilambo *et al.*, 1997; Kilambo *et al.*, 2012; Musoli *et al.*, 2009). Since its appearance in Tanzania in 1997; CWD has spread to almost all robusta coffee growing areas in Kagera region (CABI, 2003, Kilambo *et al*., 1997; Kilambo *et al*., 2012). Yields loss caused by CWD is estimated to be 162 400 kg of clean coffee (1%) annually after a total of 54 400 coffee trees being killed by the disease (Kilambo *et al*., 2012). The monetary loss caused by CWD in Tanzania is estimated to be USD 316 200 annually for over 10 years (Kilambo *et al*., 2012).

In Tanzania, the disease is managed through eradication by uprooting and burning off all infected coffee bushes, planting disease free seedlings and prevention of movement of planting materials from infected to non-infected areas. However, these methods are impractical and ineffective. Use of resistant varieties is the most effective and cost effective control measures of the disease.

Following the successful use of resistant varieties to manage CWD in Ivory Coast, Cameroon and CAR, breeding programme were initiated in Uganda, DRC and Tanzania (Musoli *et al*., 2009; Kilambo *et al*., 2012). In all three countries Uganda, DRC and Tanzania assessment of CWD resistance varieties were done through artificial inoculation of *C. canephora* varieties. In Uganda, eight clones of *C. canephora* were reported to resist CWD while in Tanzania 201 lines from old robusta germplasm established in 1988 at Maruku had level of resistance against CWD and in 2011 four robusta varieties were officially released for commercial production. Following these works, the study was conducted to assess the reaction of cultivated *C. canephora* and wild genotypes with aim of identifying the resistant materials and use them in the breeding programme to develop resistant coffee varieties.

MATERIALS AND METHODS

Collection and isolation of *Fusarium xylarioides*

Fusarium xylarioides, a pathogen causing CWD was used to screen robusta coffee materials. The pathogen was isolated from tissues of infected coffee trees. Stems and branches showing typical CWD symptoms were collected from farmers' fields in four major robusta coffee growing districts of Bukoba, Karagwe, Misenyi and Muleba, and five samples were collected per district. Isolation, purification and identification of *F. xylarioides* were done as described by Rutherford *et al*. (2003). Samples of infected coffee stems/ braches were cut into small pieces of 15- 20 cm long and placed in envelopes for easy transportation to the laboratory at TaCRI substation at Maruku Agricultural Research Institute (ARI-Maruku). Isolation of the pathogen was initiated within 7 days of samples collection. Samples of 20 mm long from the margins of infected tissues were split lengthwise, surface sterilized in 2% sodium hypochlorite solution for 3 minutes. Thereafter samples were rinsed three times in sterile distilled water. The margins of sterilized infected tissues were placed in the Petri dishes containing the tap water agar (TWA) prepared by dissolving 20 g of technical agar number 3 in 1 litre of tap water and autoclaved at 121^{o}C for 15 minutes.

The isolates in the TWA were placed in the laboratory bench for three days under 12 hours alternating cycles of light and darkness. After incubation, the pathogens developed on each piece of wood were picked by sharp wire placed on the slide; thereafter examined and identified using different magnifications of a compound microscope. The single spore isolates were picked using the loop wire and sub cultured in the potato dextrose agar (PDA) medium that was prepared by dissolving 39 g of potato dextrose agar in 1 litre of distilled water and heated in a boiling water bath for 10 minutes. Thereafter, the medium was autoclaved for 15 minutes at 121^{o}C and then allowed to cool in the lamina hood. Cool medium was poured into sterilized Petri dishes and allowed to solidify. All samples of isolates were placed on solidified PDA in the Petri dishes and incubated for 7 days at 22^{o}C under 12 hours with alternating cycles of light and darkness until they grew.

Purification and identification of *F. xylarioides*

Cultures were purified by transferring 1 cm^2 plug from the growing edges of PDA media and then were purified by single spore isolation. Pure isolates of *F. xylarioides*

were re-grown on PDA in the Petri dishes under black and white fluorescent lights with a 12 hour photoperiod. Identification of developed fungi was done based on the morphological and microscopic characteristics. The morphological characters of fruiting bodies and conidia were observed using a compound microscope (Rutherford *et al*., 2003).

Culture and maintenance of isolates

Single spores of the experimental isolates were isolated from young colonies of micro conidia on the PDA by using a tungsten wire and stroke onto TWA. Isolated single spores were incubated overnight to allow germination. Under a x 40 objective, germinating spores for each single spore isolate were marked and picked off by using a tungsten wire needle and transferred onto Spezieller Nährstoffarmer Agar (SNA) (Nirenberg, 1976) prepared by dissolving 1.0 g KH_2PO_4, 1.0 g KNO_3, 0.5 g $MgSO_4.7H_20$, 0.5 g KCl, 0.2 g glucose, 0.2 g sucrose and 20 g agar in a litre of distilled water and autoclaved at 121^oC for 20 minutes. All single spore isolates were stored on the SNA on a slant at 4^oC in the refrigerator for further use (Rutherford *et al*., 2003).

Pathogenicity study

Pathogenicity study was conducted in the screen house at TaCRI substation at ARI-Maruku to assess the virulence of the pathogen isolates. One thousand two hundred (1200), 6 month-old robusta coffee cuttings of Maruku selection (MS1), the most susceptible robusta cultivars, were inoculated with 20 isolates of *F. xylarioides* at a concentration of 1.3 x 10^6 conidia/ml using a root dipping technique (Girma, 2004; Hakiza *et al.*, 2004). All potted experimental coffee cuttings were first removed from their former polythene tubes. Roots were washed with distilled water to remove soils. Bare root cuttings were dipped into the conidia suspension and distilled water (check) respectively for 10 minutes.

Each *F. xylarioides* isolate was inoculated to 60 robusta coffee cuttings and also 60 coffee cuttings were dipped into distilled water that was used as checks. After inoculation, cuttings were potted into sterilized polythene tubes (10 cm diameter, 15 cm height) containing sterilized soils. The cuttings were arranged in a Complete Randomized Design (CRD) with three replicates where control experiment (check) was placed 3 m away from other treatments to avoid contamination.

Disease incidence was recorded by counting coffee seedlings showing disease symptoms at 2 days interval for four months consecutively starting three weeks after inoculation. This experiment was repeated three times and the experimental materials were left in the screen house for one year to assess the mortality of the cuttings.

Screening robusta cultivars against CWD

Rooting boxes were constructed in the vegetative propagation structure covered with shade nets that allow 30% of sunlight. Cuttings from 104 cultivated *C. canephora* genotypes and seedlings of 20 genotypes of wild robusta coffee were rooted in sterilized soils in the rooting boxes covered with transparent plastic sheeting 750 gauges. Cuttings in the rooting boxes were maintained by spraying mist of cold water 3-5 times a day until roots formed. Under good management, coffee cuttings produced roots within the period of 3 to 4 months.

After three months rooted robusta cuttings were transplanted into the polythene tubes with diameter and height of 10 cm and 15 cm, respectively filled with sterilized soils and kept in the nursery for 2 - 3 months. After this period, 30 health seedlings for each selected coffee tree were used for screening programme. Two known susceptible robusta accessions MS1 and MS2 were used as checks. The experiment was conducted in the screen house at TaCRI substation at ARI- Maruku using a Completely Randomized Design (CRD) with three replicates. All cuttings including checks were inoculated with a 1.3 x 10^6 conidia of *F. xylarioides* / ml of the most virulent isolate (CWD 20 MI) obtained from the pathogenicity study using the root dipping technique (Girma, 2004; Hakiza *et al*., 2004). The disease incidence was recorded at an interval of 2 days for four months consecutively by scoring 1 for observed symptoms and 0 for none developed symptoms starting three weeks after inoculation. The proportion of dead coffee seedlings caused by CWD was calculated. The experiment was repeated three times.

Breeding for resistance to CWD

The breeding strategy of this study was to develop robusta varieties with durable resistance to CWD by introducing resistant genes to the susceptible robusta using markers assisted selection (MAS). The resistant parents used were identified from studies on pathogenicity test, genetic distance analysis and morphological characterizations of different accessions. Crosses were made between two robusta accessions resistant to CWD and other two robusta accessions susceptible to CWD. The resistant robusta accessions 13/61 and ML2 were used as male parents (Kilambo *et al*., 2012). The susceptible robusta accessions MS1 and MS2 were used as female parents.

The experiment was laid out on the existing robusta germplasm at ARI Maruku. All experimental plots were well managed using appropriate agronomic practices such as proper weeding, pruning and recommended fertilizers application. Prior to crossing, all experimental parents were tagged and primary branches with many flower buds were selected and marked. After the first rain, the flowers on the selected primary branches were removed and then experimental branches were covered with white bags made up of white clothes. The experimental branches were inspected daily to monitor flowers. After flowering, pollens were harvested from the male parents by cutting off covered branches. These branches were carefully inserted into the covered

branches of the female parents and shook gently to enable the pollens drop on the stigma of open flowers. Pollination activity was done from 06.00 to 10.00 AM in the morning. After crossing, all pollinated flowers were firmly covered with the white bags until all pollinated flowers withered. Thereafter, the cover bags were removed and branches with pollinated flowers were labelled. On experimental branches all new flower buds were removed daily for the period of 10-11 months when crossed berries were ripe and ready to be harvested. Ripe berries were harvested and processed to get F_1 seeds. The F_1 seeds together with those from the parents were labelled and raised in the nursery to obtain sufficient seedlings. A total of 240 F1 progy seedlings from crosses of cultivars 13/61 x MS1, 13/61 x MS2, Muleba No, 2 x MS1 and Muleba No 2 x MS2 and 240 seedlings of their respective parents were tested against CWD. The experiment was conducted in the screen house at TaCRI substation at ARI- Maruku using a Completely Randomized Design (CRD) with three replicates. All F_1 progenies and seedlings from their respective parents cultivars were inoculated with a 1.3 x 10^6 conidia of *F. xylarioides* /ml of the most virulent isolate (CWD 20 MI) obtained from the pathogenicity study using the root dipping technique (Girma, 2004; Hakiza *et al.*, 2004). The disease incidence was recorded at an interval of 2 days for four months consecutively by scoring 1 for observed symptoms and 0 for none developed symptoms starting three weeks after inoculation.

Data Analysis

Data for pathogenicity screening for resistance and breeding experiments were analyzed by using ANOVA using GENSTAT statistical software package. Means were separated by using Fisher's Least Significant Differences Test (P= 0.05). Heritability analysis in a narrow sense (h^2) for traits related to resistance to CWD was analyzed using the model; h^2 = Var (A)/Var (P); where Var (P) is phenotypic variance in the trait due to the sum of genetic effects (Var (A) + Var (D) + Var (I) + Var (E), Var (A) the additive genetic variance due to the average effects of the alleles, (Var D and Var I) are non additive genetic variances and Var (E) is environment variance. Expected gene gain of F1 progenies were estimated by using the following formula: Expected selection gain = Selection differential x single tree heritability. Where selection differential = Mean of selected trees - Mean of all trees.

RESULTS

Mortality of artificially inoculated susceptible *C. canephora* with 20 isolates of CWD pathogen

Percent mortality of susceptible *C. canephora* cultivar, MS1 inoculated with 20 isolates of CWD pathogen varied significantly ($P \leq 0.001$) among tested isolates (Table 1). The overall mean mortality of susceptible robusta cultivar was 38.4% at 79 days after inoculation. The highest mortality rates (83.3%) was recorded 79 days after inoculation in isolates CWD 16 BK (83.3%), and CWD 20 MI (83.3%) collected from Bukoba and Missenyi districts followed by isolates: CWD 40BK (75%), CWD (17) MI (68.3%), CWD 6(2) MI (68.3%), CWD 9(1) ML (53.3%) from Bukoba, Missenyi and Muleba districts respectively (Table 1). The results also showed that after 79 days of inoculation there were no increased percent mortality of inoculated coffee cuttings among tested CWD isolates. Coffee cuttings dipped into isolate CWD 10 ML and distilled water did not show any disease symptom demonstrated by less than 7% mortality (Table 1).

Table 1: Mortality of MS 1 *C. canephora* (genotype) artificially inoculated with 20 CWD isolates from various locations in farmers' fields during pathogenicity test.

Isolate Code	Source of isolates Location (Districts)	Altitudes m.a.s.l	Coordinate		Mortality (%) 79 days after inoculations
Un inoculated seedlings					5.0^{h}
CWD 10 ML	Muleba	1523m	S 01.78068°	E031.57043°	6.7^{h}
CWD 16 BK	Bukoba	1252m	S 01.24221°	E031.85309°	83.3^{a}
CWD 17 MI	Misenyi	1180m	S 01.27713°	E031.73098°	68.3^{ab}
CWD 19 MI	Misenyi	1180m	S 01.27712°	E031.73097°	35.0^{cde}
CWD 20 MI	Misenyi	1181m	S 01.27711°	E031.73096°	83.3^{a}
CWD 29 KR	Karagwe	1246m	S 01.62478°	E031.46283°	35.0^{cde}
CWD 30 KR	Karagwe	1247m	S 01.62479°	E031.46284°	38.3^{cde}
CWD 33 KR	Karagwe	1336m	S 01.24911°	E031.96346°	48.3^{cde}
CWD 35 BK	Bukoba	1252m	S 01.21989°	E031.84458°	43.3^{cde}
CWD 36 BK	Bukoba	1252m	S 01.24221°	E031.85309°	8.3^{gh}
CWD 38 KR	Karagwe	1352m	S 01.25963°	E031.95629°	33.3^{de}
CWD 40 BK	Bukoba	1252m	S 01.24321°	E031.85309°	75.0^{a}
CWD6(2) MI	Misenyi	1182m	S 01.27713°	E031.73098°	68.3^{ab}
CWD6(1) MI	Misenyi	1182m	S 01.27713°	E031.73098°	10.0^{gh}
CWD7(3)ML	Muleba	1549m	S 01.78104°	E031.57533°	13.3^{fgh}
CWD7(2)ML	Muleba	1513m	S 01.76502°	E031.59168°	30.0^{def}

CWD8(1)BK	Bukoba	1251m	S 01.21989°	E031.84458°	31.7^{def}
CWD15KR	Karagwe	1246m	S 01.24881°	E030.46304°	10.0^{gh}
CWD7(1)ML	Muleba	1508m	S 01.76564°	E031.59176°	26.7^{efg}
CWD9(1)ML	Muleba	1511m	S 01.76564°	E031.59176°	53.3^{bc}
Mean					38.4
CV%					29.7
d.f.					20
P-Value					≤ 0.001
L.S.D(P= 0.05)					18.8

Means with the same letter (s) in the column are not significantly different according to Fisher's protect least significant difference test (P= 0.05).

Screening robusta genotypes against CWD

The results showed significant variations (P ≤ 0.001) in the mortality percent of cultivated *C. canephora* and wild coffee genotypes artificially inoculated with *F. xylarioides* pathogen 79 days after inoculation (Table 2). The overall mean mortality of cultivated *C. canephora* and wild coffee genotypes was 57.65%. The mortality of the most susceptible genotypes ranged from 65 to 100% (Table 2). The percent mortality for moderate susceptible genotypes ranged from 24 to 64% followed by the least susceptible (14-23%) and resistant (0-13%) (Table 2). Out of 124 robusta coffee cultivars tested against CWD, 61 of them survived. These include; 49 cultivated robusta coffee, 8 and 4 wild coffee genotypes from Minziro and Bushenyi forests respectively.

Table 2: Reaction of cultivated *C. canephora* and wild coffee genotypes against *F. xylarioides.*

Cluster	Genotype code	Origin source	Plant form	% mortality
I	054KR 16	Karagwe	Bending	100^{a}
	086ML15	Muleba	Erect	100^{a}
	344 MI 19	Missenyi	Erect	100^{a}
	337 MI 21	Missenyi	Bending	100^{a}
	125 BK 11	Bukoba	Erect	100^{a}
	294 KR 8	Karagwe	Bending	96.33^{ab}
	320 KR 12	Karagwe	Bending	95.00^{abc}
	005 MI 5	Missenyi	Bending	93.67^{a-d}
	308 ML 20	Muleba	Erect	93.33^{a-d}
	346 MI 18	Missenyi	Semi erect	93.33^{a-d}
	091 KR 23	Karagwe	Semi erect	91.67^{a-d}
	FM 12- wild	Minziro forest	Erect	90.00^{a-e}
	FM 17- wild	Minziro forest	Erect	90.00^{a-e}
	109 BK 5	Bukoba	Erect	89.33^{a-f}
	181 ML 5	Muleba	Bending	89.33^{a-f}
	323 ML 24	Muleba	Semi erect	87.33^{a-g}
	009 MI 9	Missenyi	Erect	86.67^{a-g}
	112 BK 6	Bukoba	Semi erect	85.00^{a-g}
	158 MI 12	Missenyi	Semi erect	85.00^{a-g}
	030 KR 18	Karagwe	Semi erect	84.67^{a-g}
	108 BK 4	Bukoba	Erect	83.33^{a-h}
	293 KR 7	Karagwe	Semi erect	82.33^{a-h}
	295 BK 23	Bukoba	Semi erect	82.33^{a-h}
	055 KR 15	Karagwe	Erect	82.00^{a-h}
	139 MI 11	Missenyi	Semi erect	81.67^{a-h}
	333 MI 22	Missenyi	Semi erect	81.67^{a-h}
	079 ML 17	Muleba	Semi erect	81.67^{a-h}
	118 (1/61) BK	Bukoba	Erect	80.67^{a-i}
II	FM 9 – wild	Minziro forest	Erect	80.33^{a-i}
	057 BK 2	Bukoba	Erect	80.00^{a-i}
	060 KR 13	Karagwe	Semi erect	79.00^{a-i}
	049KR 21	Karagwe	Erect	78.33^{a-j}
	292 KR 6	Karagwe		78.00^{a-j}
	047MS2 BK (1)	Bukoba	Bending	77.33^{a-k}
	284 KR 3	Karagwe	Erect	76.67^{a-k}

330 MI 24	Missenyi	Erect	75.67 $^{b-l}$
347 MR 10	Bukoba	Erect	75.33 $^{b-l}$
120 ML 13	Muleba	Semi erect	73.67 $^{b-l}$
007 MI 7	Missenyi	Semi erect	73.33^{b-m}
FM 10 – wild	Minziro forest	Erect	73.33^{b-m}
FM 8 - wild	Minziro forest	Erect	70.00^{c-n}
283 KR 2	Karagwe	Semi erect	69.67^{d-o}
311 KR 9	Karagwe	Bending	69.67^{d-o}
006 MI 6	Missenyi	Bending	68.67^{e-p}
316 ML 22	Muleba	Semi erect	68.67^{e-p}
240 BK 14	Bukoba	Bending	68.33^{e-q}
Mean			57.65
CV%			27.30
d.f			244
P-value			≤ 0.001
LSD			19.46

Table 2: Continued

Cluster	Genotype code	Origin source	Plant form	% mortality
	023 KR 20	Karagwe	Semi erect	68.33^{e-q}
	002 MI 2	Missenyi	Semi erect	67.67^{e-q}
	306 ML 20	Muleba	Erect	67.00^{e-r}
	FM 20 – wild	Minziro forest	Erect	66.67^{e-s}
	092 KR 24	Karagwe	Erect	65.33^{f-t}
	324 ML 25	Muleba	Bending	64.67^{g-t}
	257 BK 18	Bukoba	Semi erect	63.33^{g-t}
	123 BK 10	Bukoba	Erect	60.00^{h-u}
	142 BK 13	Bukoba	Bending	59.67^{h-u}
III	062 KR 14	Karagwe	Semi erect	59.33^{h-v}
	059 BK 3	Bukoba	Bending	58.33^{h-w}
	080 ML 16	Muleba	Bending	56.67^{i-w}
	004 MI 4	Missenyi	Semi erect	56.67^{i-w}
	008 MI 8	Missenyi	Bending	56.67^{i-w}
	011MI 11	Missenyi	Semi erect	56.33^{i-w}
	170 ML 10	Muleba	Erect	54.33^{j-x}
	269 BK 22	Bukoba	Bending	53.00^{k-y}
	FM 16 – wild	Minziro forest	Erect	51.67^{l-y}
	115 BK 8	Bukoba	Erect	51.67^{l-y}
	160 ML 11	Muleba	Erect	51.67^{l-y}
	FM 18 – wild	Missenyi	Erect	51.67^{l-y}
	010 MI 10	Missenyi	Semi erect	51.33^{l-y}
	332 MI 23	Missenyi	Bending	50.33^{m-y}
	087 ML 12	Missenyi	Erect	49.67^{m-z}
	310 MI 25	Missenyi	Erect	49.3l^{m-z}
	167 MI 17	Missenyi	Erect	46.67^{n-n}
	185 ML 4	Muleba	Erect	44.33^{o-z}
	192 ML 1	Muleba	Bending	44.00^{o-z}
	175 ML 8	Muleba	Bending	43.33^{o-z}
	077 ML 18	Muleba	Erect	42.33^{p-z}
	162 MI 14	Missenyi	Erect	41.67^{p-z}
	026 BK 26	Bukoba	Semi erect	41.67^{p-z}
	255 BK 16	Bukoba	Semi erect	40.00^{q-z}
	FM 14 – wild	Minziro forest	Erect	40.00^{q-z}
IV	259 BK 19	Bukoba	Semi erect	39.33^{r-z}
	194 ML 3	Muleba	Semi erect	39.00^{r-z}
	268 BK 21	Bukoba	Bending	38.33^{r-z}
	131 MS 1 BK 2	Bukoba	Bending	38.33^{r-z}

	172 ML 9	Muleba	Erect	37.67^{r-z}
	036 KR 12	Karagwe	Semi erect	37.00^{r-z}
	003 MI 3	Missenyi	Erect	36.67^{r-z}
	179 ML 6	Muleba	Bending	35.00^{s-z}
	349 ML 2	Muleba	Erect	35.00^{s-z}
	037 ML 19	Muleba	Erect	34.67^{t-z}
	FM 5 – wild	Minziro forest	Erect	33.33^{t-z}
	127 ML 12	Muleba	Bending	33.00^{t-z}
	046 KR 22	Karagwe	Erect	31.67^{u-z}
	Mean			57.65
	CV%			27.30
	d.f			244
	P-value			≤ 0.001
	LSD			19.46

Table 2 Continued

Cluster	Genotype code	Origin source	Plant form	% mortality
	FM 6 – wild	Minziro forest	Erect	30.00^{u-z}
	FM 13 –wild	Minziro forest	Erect	29.67^{v-z}
	165 MI 16	Missenyi	Erect	26.33^{v-z}
	263 BK 20	Bukoba	Erect	24.67^{v-z}
	020 BK MS 5	Bukoba	Bending	24.33^{v-z}
	114 BK 4	Bukoba	Bending	24.33^{v-z}
	FM 19 – wild	Minziro forest	Erect	23.33^{v-z}
	FM 14 – wild	Minziro forest	Erect	23.33^{v-z}
	288 KR 5	Karagwe	Bending	23.33^{v-z}
	FM 11 – wild	Minziro forest	Erect	19.00^{w-z}
	348 (13/61)	Bukoba – variety	Bending	13.67^{w-z}
	287 KR 4	Karagwe	Bending	13.67^{w-z}
V	012 MI 12	Missenyi	Bending	13.00^{w-z}
	FM 7 – wild	Minziro forest	Erect	12.00^{w-z}
	001 MI 1	Missenyi	Erect	9.33^{yz}
	Uninoculated seedlings			1.60^{yz}
	FB 1 – wild	Bushenyi forest	Bending	0.00^{z}
	FB 4 – wild	Bushenyi forest	Bending	0.00^{z}
	FB 2 –wild	Bushenyi forest	Bending	0.00^{z}
	FB 3 – wild	Bushenyi forest	Bending	0.00^{z}
	Mean			57.65
	CV%			27.30
	d.f			244
	P-value			≤ 0.001
	LSD			19.46

Means followed by the same letter(s) in the column are not significantly different according to Fisher's protected least significant difference test (P = 0.05)

Initiating a breeding programme for resistance to CWD

Significant differences (P ≤ 0.001) in the survivors of *C. canephora* artificially inoculated with CWD pathogen were observed 79 days after inoculation (Table 3). The overall mean survival was 36.6%. The highest survival rate were recorded in the resistant parents, Maruku 1 (13/61) (78.6%) and Muleba 1 (76.2%) (Table 3). Seventy eight (78) hybrid lines were developed through crossing resistant robusta cultivars ML2 and 13/61 to susceptible robusta cultivars MS1 and MS2. These lines include the crosses of ML2 x MS1 (30), ML2 x MS2 (16), 13/61 x MS2 (20), and 13/61 x MS1 (12).

The lowest survival rates of 2.4% and 4.8% were observed on robusta cultivars Maruku selections 1(MS1) and 2(MS2), respectively and the survival percent of *C. canephora* hybrids were less than 4 5% (Table 3).

Table 3: Survival of robusta hybrids artificially inoculated with *Fusarium xylarioides* isolate CWD 20 MI.

Robusta hybrids/cultivars	Survivor (%)	(h^2)	Gain of resistant gene (%)
Cultivar 13/61	78.6[a]	62.4	0.0
Cross of cultivars: 13/61 x Maruku selection 1 (MS1)	21.4[bcd]	17.0	24.4
Cross of cultivars: 13/61 x Maruku selection 2 (MS2)	42.8[b]	34.0	48.4
Cultivar: Muleba Number 2 (ML2)	76.2[a]	60.5	0.0
Cross of cultivars: Muleba no 2 x Maruku selection 1 (MS1)	35.7[b]	28.3	43.5
Cross of cultivars: Muleba no 2 x Maruku selection 2 (MS2)	30.9[bc]	24.6	34.4
Cultivar: Maruku selection 1 (MS1)	2.4[d]	1.9	0.0
Cultivar: Maruku selection 2 (MS2)	4.8[cd]	3.8	0.0
Mean	36.6	29.1	37.6
CV%	41.6		
d.f.	7		
P-Value	≤ 0.001		
L.S.D (P = 0.05)	26.3		

Note: h^2 represents narrow sense heritability; Means for the survival rates of robusta hybrids and their parents followed by the same letter (s) in the column are not significantly different (P = 0.05) according to Fisher's protected least significance difference test (P = 0.05).

DISCUSSION

The pathogenicity study showed that the aggressiveness of the pathogen causing the typical symptoms of coffee wilt disease on robusta coffee varied among collected isolates. Out of 20 isolates tested five of them from Bukoba (2) and Missenyi (3) districts were most aggressive. The most aggressive isolates included CWD 20 MI, CWD 16 BK, CWD 40 BK, CWD 6 (1) MI and CWD 17 MI. The most aggressive isolates caused seedlings death rates of 68– 83.3% were recorded in susceptible robusta coffee seedlings inoculated with isolates collected from Missenyi and Bukoba. Other isolates from Bukoba, Karagwe, Missenyi and Muleba districts were moderate to least aggressive in infecting the susceptible robusta coffee cultivars. The moderate aggressive CWD isolates caused death rates of 31.7 to 53.3% to susceptible robusta seedlings. The least aggressive CWD isolates caused the death rates of 6.7 to 30%). The results of this study are consistent to results reported by Girma (2004) who observed variations on the aggressiveness of *F. xylarioides* in causing coffee wilt disease symptoms on arabica coffee. According to Girma (2004) the low coffee seedlings death of 38.0, 33.2 and 35.6% were caused by moderate aggressive isolates Gx3, Gx5 and Gx8 respectively in arabica coffee while the most aggressive isolates Gx1, Gx4 and Gx11 caused the death rates of 58.1, 57.3 and 67.8%, respectively. The variations in the aggressiveness of the isolates *F. xylarioides* pathogen tested could be attributed to the presence of different strains infecting robusta coffee and cause CWD.

The interaction of *C. canephora* with *F. xylarioides* has been studied and reported by several authors. In many studies the mortality of artificially inoculated *C. canephora* with *F. xylarioides* varied between genotypes ranging from most susceptible to resistant genotypes (Girma *et al.*, 2009; Hakiza *et al*., 2009; Van de Graaff and Pieters, 1978; Merdassa, 1986; Girma 1997; Girma and Hindorf, 2001; Girma *et al*., 2001; Girma, 2004; Kalonji-Mbuyi *et al.*, 2009; Kilambo *et al*., 2012). The results of this study revealed differential variations on the percent mortality of all 124 screened genotypes of cultivated *C. canephora* and wild coffee tested using the most aggressive isolate CWD 20. The observed variability in the mortality of tested genotypes showed variability on resistance or susceptibility to CWD. The overall results indicated that 93% of artificially inoculated coffee genotypes with *F. xylarioides* pathogens were susceptible at varying levels from the highest to least susceptible. The results showed that out of 124 investigated accessions 57 genotypes demonstrated resistance to CWD at varying levels. These included 42 cultivated robusta cultivars, 4 wild coffee from Bushenyi forest and 11 wild coffee genotypes from Minziro forest. The results showed that nine (9) which are 7% of tested coffee genotypes demonstrated the highest resistance to coffee wilt disease and 4 genotypes (3.2 %) had complete resistance. The genotypes FB 1, FB 2, FB 3 and FB 4 which were collected from Bushenyi forest had complete resistance to CWD. These findings are supported by Girma (2004) who reported that coffee genotypes with less than 20 % of infections were most resistant to CWD. However, the overall results showed that the genotypes of many cultivated *C. canephora* had low levels of resistance to CWD. These results imply that more work is needed to improve the resistance of cultivated *C. canephora* against CWD and at the same time to maintain other agronomic traits which control yields and quality. Furthermore, the results of this study showed that cultivated *C. canephora* and wild coffee can be grouped into 5 sub-groups based on their reaction to CWD attacks. The results of this study are in lines with the findings carried out in DRC and Uganda that revealed differential reactions to CWD among genotypes from different localities (Kalonji-Mbuyi *et al*. 2009; Musoli *et al*., 2008). Musoli *et al.* (2009) identified eight CWD resistant clones through exploitations of intra specific variability in *C. canephora*.

Furthermore, Kalonji-Mbuyi *et al*. (2009) classified *C. canephora* genotypes into four different groups based on their reactions to CWD. This study therefore, revealed five groups that are closely related to those reported by Kalonji-Mbuyi *et al*. (2009). The high variations on the response of genotypes to CWD were probably attributed to the genomic variations among cultivated *C. canephora* and wild coffee genotypes resulted through natural hybridization among coffee species in the population.

Furthermore, the mortality rates for the moderate resistant genotypes were highly diverse showing the significant variations within closely related *C. canephora*. According to Musoli *et al*. (2009) genetically *C. canephora* populations are very diverse even among the genotypes from the same locality or the member of the same progenies. The variations on the mortality rates among genotypes could be attributed by genetic diversity of cultivated *C. canephora* and wild coffee that resulted through natural hybridizations. The findings of this study further supported the previous reports on the variations of the resistance of coffee genotypes to CWD (Fraselle, 1950; Delassus, 1954; Bouriquest, 1959; Porteres, 1959; Girma, 2004; Musoli *et al*., 2008; Girma *et al*., 2009; Phiri *et al*., 2009; Kilambo *et al*., 2012). The variations on the mortality within related individuals in some identified genotype groups is probably attributed to heterozygosity of *C. canephora* resulting from out crossing of individuals within the population (Musoli, 2007). The overall results showed the genetic difference between the populations of cultivated *C. canephora* and wild coffee genotypes to CWD resistance. The observed high proportions of robusta genotypes tolerant to CWD infections indicate that there is a high potential of getting resistant cultivars. These findings should be explored further to identify more resistant robusta cultivars needed for developing robusta varieties for replanting programme in Kagera region. These findings are supported by Musoli *et al*. (2009) who reported that the genetic diversity of C. *canephora* noted in Uganda is high enough to be exploited for resistance against CWD. Musoli *et al*. (2009) and Saccas (1956) reported that CWD in the DRC and Ivory Coast was effectively managed by planting resistant robusta varieties. Therefore, the valuable information generated from this study should be the basis for planning control measures for management of CWD in Tanzania. Furthermore, the resistant cultivars identified in this study should be evaluated in the hot spot areas for the disease under field conditions in different agro- ecologies to verify their resistance and determine their performance for other agronomic traits.

Coffee hybridization is aimed at improvement of its productivity, quality and reduces disease susceptibility (Kathurima et *al*., 2012; Walyaro, 1983, Kilambo *et al*, 2013 a & b). In Tanzania, for instance, 19 varieties of *Coffea arabica* which are high yielding, good quality and resistance to both coffee berry and coffee leaf rust diseases were developed through hybridization (TaCRI, 2005; TaCRI, 2006; TaCRI, 2011; TaCRI, 2012). However, this study showed low heritability of CWD disease resistance traits. These findings revealed by the low survival percentage of all hybrids compared to the parents that donated traits for resistance. These findings are in line with those of Musoli *et al*. (2009) who reported 33% of resistant genes can be transferred from resistant to susceptible parents. Furthermore, Musoli *et al*. (2013) studying the heritability of resistance to coffee wilt disease (*F. xylarioides* Steyaert) reported significant quantitative genetic variations among the robusta clones and progenies and suggested polygenic control of the resistance. On their findings, Musoli *et al*. (2013) observed moderate heritability of 0.333 from clones in the field. The authors further reported the means for heritability of 0.183 and 0.369 of half-sibling progenies and parents in the field and screen house respectively. These findings are in agreement with the findings in this study that the genetic heritability for the four progenies resulted from crossing the two susceptible to two resistant cultivars were significantly low.

The low heritability of traits for resistance to CWD observed in this study poses a big challenge for improving *C. canephora* through back crossing the F_1 progenies to the susceptible parents possessing good traits such as high yielding ability, large bean sizes, good quality, resistance to other diseases and other agronomic traits that can easily result into developing breeding lines which can have a negative attributes to targeted varieties. Musoli *et al*. (2013) reported that improvement of robusta against CWD is possible for selecting tolerant parents in breeding programme. Furthermore, C. *canephora* is open pollinated crop, use of seeds from hybrid lines can easily result into segregation and developing varieties that are true to their original parents (Marandu *et al*., 2004). The problem of segregation can be avoided through propagation techniques.

Since robusta coffee is cross pollinated species, it is recommended to release a set comprises of at least five or more individual varieties (Wrigley, 1988; Tshilenge *et al*., 2009). This aims to enhance fertilization of flowers of non-related individuals, the process called modified backcrossing commonly used in vegetative propagated crops with high inbreed depression. This set of variety should be vegetatively propagated through cuttings of *C. canephora* varieties with good traits' attributes (Wrigley, 1988).

CONCLUSION

The results showed aggressiveness of *F. xyalorioides* to cause CWD on susceptible *C. canephora* genotypes vary among isolates. The resistance to CWD is very low within cultivated *C. canephora* and their related genotypes from Minziro forest. However, genotypes of wild coffee from Bushenyi forest had complete resistance to CWD. Most of cultivated *C. canephora* and their closely related wild coffee from Minziro forest were tolerant to CWD.

The presence of tolerant *C. canephora* in the fields probably contribute to the existence of *C. canephora* in Tanzania, Uganda, DRC and other countries where CWD has been reported. The genetic diversity observed in screening showed the potential of exploiting resistance against CWD. The results on the response of robusta showed that 34 hybrids developed from crossing resistant cultivars ML2 and 13/61 to the susceptible cultivars MS1 and MS2 survived against CWD.

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