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Full Length Research Paper

Variation in resistance to coffee berry disease (*colletotrichum kahawae)* among germplasm progenitors at Tanzania coffee research institute (tacri)

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Four trials were executed at the Tanzania Coffee Research Institute (TaCRI) from September 2006 to April 2007 and at the Coffee Rust Research Center (CIFC), Portugal from March 2007 to June 2007 to evaluate variation in resistance to Coffee Berry Disease (CBD) caused by *Colletotrichum kahawae* within and among four *Coffea arabica* varieties. The varieties tested were Bourbon, Compacts (Catimor), Rume sudan and Hybrid de Timor obtained from TaCRI germplasm. Local CBD isolate was used in the trials at TaCRI while at CIFC Portugal, four CBD isolates T3, Ca1, Z9 and Q2 were used in the resistance evaluation. There was significant (P ≤ 0.05) variation against CBD within and among varieties against most isolates. Accessions PNI086, VCE1589 and VC299 in varieties Compacts, Hybrid de Timor and Rume sudan respectively showed high resistance while variety Bourbon a control, showed high levels of susceptibility. It is therefore necessary in breeding programmes to select the best accessions within varieties for development of superior coffee varieties with resistance to CBD.

Key words: Coffee berry disease; breeding; *Coffea arabica*; resistance sources

INTRODUCTION

Coffee (*Coffea*) is one of the most important beverages in the world with a current estimated value of 10 billion US$ and it is ranked second largest trade commodity next to petroleum (Luiz et al., 2005). Coffee is also an extremely important source of national export revenue and internal cash income for farmers in many poor countries from Africa, Asia and Latin America (ICO, 2005). The two most economically important species of the genus *Coffea* are *Coffea arabica* L. and *Coffea canephora* Pierre ex Froehner. In the world coffee trade, Arabica coffee accounts for about 70% while Robusta coffee contributes about 30% (ICO, 2006). Coffee is Tanzania's largest export crop. It contributes approximately $115 million to export earning, and provides employment to some 400,000 families. About 95 percent of coffee is grown by smallholders on average holdings of 1–2 hectares, and 5 percent is grown on estates. Almost all of Tanzania's coffee production is exported (Baffes, 2003).

Numerous production constraints have been mentioned. However, the most serious one is diseases, mainly coffee berry disease (CBD) (*Colletotrichum kahawae*, Waller

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Table 1: Varieties and accessions studied

Variety	Accessions
Rume sudan	VC 298, VC 299, VC 506, VC509, and VC510
Hybrid de Timor	RRC70, RRC72, VCE 1594, VCE 1587and VCE1589
Catimor/Compacts	PNI 088, PNI 086, PNI 087, CR 124 and CR127
Bourbon	N5, N39, N100, N197 and N218

Note: Bourbon is a commercial (susceptible) variety, used as a check.

and Bridge) and coffee leaf rust (CLR) *(Hemileia vastatrix*, Berk et Br). Coffee Berry Disease is an anthracnose of the green and ripening berries while Coffee Leaf Rust is a fungal disease which attacks leaves of both *Arabica* and *Canephora* coffee (Wrigley, 1988). Together, the two diseases viz CBD and CLR control account for 40% of total production cost for small scale coffee producers in the country (Teri et al., 2004; Mtenga et al., 2006).

The Arabica coffee improvement programme at the Tanzania Coffee Research Institute (TaCRI) has achieved a considerable success in developing CBD and CLR resistant varieties. However some inconsistencies on the levels of resistance have been observed in some of the progenitors used in the development of the varieties (Walyaro, 2004). Establishment of levels of resistance in the progenitors of resistance will lead to identification of superior which will be used for development of improved varieties with consistent, stable and durable resistance to the diseases. The objective of the current study was to evaluate the resistance levels within and among the main progenitors of CBD resistance used at TaCRI.

MATERIALS AND METHODS

Varieties and accessions studied

The materials which were used in this work are three coffee varieties from Tanzania Coffee Research Institute germplasm which are the main progenitors for CBD resistance. In each variety, five accessions were evaluated as shown in Table 1.

CBD isolates tested

Four Coffee Berry Disease isolates from Tanzania (T3), Kenya (Q2), Zimbabwe (Z9) and Cameroon (Ca1) were used for the experiments done at CIFC- Portugal while a local isolate extracted from freshly infected berries was used for the experiment done in Tanzania.

Experimental procedure

Fully matured, red-ripe coffee berries were handpicked from the trees from centre rows of each accession involved from the germplasm plot. These were pulped using a small hand pulper in separate lots for each accession. The pulper hopper was washed thoroughly using clean tap water under high pressure to ensure all the cherries from previous lot is cleaned out of the pulper before pulping the next lot. This procedure was followed for all lots. Pulped cherries from each accession were put in separate containers, placed under room temperature for 72 hours for fermentation. Then the cherries were washed and dried under shed separately in partitioned wire-mesh trays for one week before they were pre-germinated to raise hypocotyls.

Sources of inoculums

According to Cook (1973b) green infected berries with active lesions are the best source of inoculum for initial isolation because of low contamination with other non pathogenic *Colletotrichum* spp. and optimum pathogenicity of the isolates. Initial isolation of the CBD pathogen was achieved by incubating green infected berries from unsprayed susceptible variety Bourbon (N39) at 20^0C-24^0C on moist sterilized sand in closed but somewhat ventilated plastic boxes for 10 days (Cook, 1973b). This was followed by planting the conidia on

Table 2: Variation in resistance to coffee berry disease (CBD) 0-4 score within varieties against Q2, Ca1, T3, Z9 CBD isolates at CIFC Portugal and Local one in Tanzania

Varieties	Accessions	Isolates	Mean	Scores		
Rume sudan		Q2	Ca1	T3	Z9	Local
	VC 298	0.350	2.550	1.000	0.733	0.160
	VC 299	0.817	1.133	1.067	0.500	0.040
	VC 506	0.683	2.233	0.900	0.983	0.040
	VC 509	0.767	3.900	1.217	0.833	0.060
	VC 510	0.633	3.650	0.850	0.750	0.060
	mean	0.650	2.693	1.007	0.760	0.072
	CV (%)	29.620	8.790	32.950	20.650	89.580
	SE±	0.000	0.137	0.000	0.091	0.000
	$LSD_{0.05}$	ns	0.446	ns	0.298	ns
Hybrid de Timor	VCE 1587	0.883	2.583	1.967	1.833	0.010
	VCE 1589	0.783	2.400	0.633	0.950	0.050
	VCE 1594	3.717	3.883	1.933	2.183	0.340
	RRC 70	2.283	3.933	1.417	2.183	0.440
	RRC 72	1.300	3.617	2.150	1.600	0.050
	mean	1.793	3.283	1.620	1.750	0.178
	CV (%)	15.260	6.360	19.500	18.980	70.300
	SE±	0.158	0.121	0.183	0.192	0.061
	$LSD_{0.05}$	0.516	0.395	0.595	0.625	0.189
Compacts	PNI 086	0.567	1.850	0.950	0.717	0.000
	PNI 087	2.033	3.800	1.600	1.783	0.010
	PNI 088	0.050	3.85	0.167	0.283	0.000
	CR 124	1.350	3.900	2.317	2.367	0.470
	CR 127	0.850	3.967	1.817	0.750	0.040
	mean	0.970	3.473	1.370	1.180	0.104
	CV (%)	23.030	5.590	12.830	20.140	35.630
	SE±	0.129	0.113	0.102	0.153	0.158
	$LSD_{0.05}$	0.421	0.367	0.332	0.498	0.487
Bourbon	N 5	4.000	4.000	4.000	4.000	3.590
	N 39	4.000	4.000	4.000	4.000	3.510
	N 100	4.000	4.000	4.000	4.000	1.980
	N 197	4.000	4.000	4.000	4.000	1.430
	N 218	4.000	4.000	4.000	4.000	3.390
	mean	4.000	4.000	4.000	4.000	2.780
	CV (%)	0.000	0.000	0.000	0.000	14.620
	SE±	0.000	0.000	0.000	0.000	0.211
	$LSD_{0.05}$	ns	ns	ns	ns	0.650

3.4% malt extract agar containing 0.04% streptomycin. A spore suspension containing 2 $x10^6$ spores/ml was prepared from these pure cultures. The suspension was tested and proved spore viability in excess of 80%.This procedure was followed in extraction and preparation of inoculum of the local CBD isolate used in the experiment conducted in Tanzania. For the experiments carried out in Portugal; isolates were maintained in malt extract agar (MEA) and preserved for long periods in agar slants.

Test for resistance

One hundred seeds of each variety were sown out, with the parchment removed, in moist sterilized media of sand and top forest soil placed in plastic boxes with closely fitting transparent lids, keeping the boxes at normal room temperatures (20-24^{0}C). The seedlings were ready for inoculation when they had hypocotyl stem of 3-5cm long, usually within 5-6 weeks after sowing out the seeds. At this stage the cotyledons are still enclosed in the testa. Just before inoculation the lid was removed from the plastic box, the seedlings sprayed with the standard CBD inoculum (2 x 10^6 spores/ml) by means of a small atomizer and the box was immediately closed again. A repeat inoculation was applied after 48 hours. The temperature of 22-24^{0}C was maintained for four days for successful infection, at the same time relative humidity in the boxes was maintained at 100%. This was followed by incubation period at a lower temperature of (19-20^{0}C) with the lids removed from the boxes to allow for normal humidity. The first symptoms became noticeable within one week after the first inoculation, but full expression of the disease susceptibility was attained at two weeks' time. At the end of the incubation period seedlings were individually scored for disease symptoms developed on the hypocotyl stem using a scale with range 0-4 (Van der Graaf, 1981).

Data analysis

A mean grade of infection obtained from the scores (G) were calculated for accessions in each replication as

$$G = 1/N \sum_{i=1}^{4} in_i$$

Where, i is the disease class, n_i is the number of seedlings in class i and N is the total number of seedlings scored (Omondi et al., 2001). Calculated score means were subjected to analysis of variance, and where a significant variation was found; means were separated using Duncan's New Multiple Range Test.

RESULTS

There was significant (P≤0.05) variation in resistance to coffee berry disease (CBD) within varieties Hybrid de Timor, Compacts and Bourbon against a local isolate at TaCRI Tanzania (Table 2). No significant variation was observed between accessions in Rume sudan variety. Rume sudan, Hybrid de Timor and Compacts showed high levels of resistance with mean scores of 0.072, 0.178 and 0.104 respectively. The control variety Bourbon showed susceptibility with mean score of 2.780. At CIFC Portugal when coffee berry disease isolate T3 was used, significant (P≤ 0.05) variation in resistance was observed within varieties in two varieties of Hybrid de Timor and Compacts while the variety Rume sudan and Bourbon did not show significant variation in resistance within the varieties (Table 2).

High level of resistance was observed in varieties Rume sudan, Hybrid de Timor and Compacts with mean scores 1.007, 1.620 and 1.370 respectively. However, some accessions within varieties Hybrid de Timor (RRC 72) and Compacts (CR 124) had exceptional high susceptibility scores of 2.150 and 2.317 respectively. Bourbon variety showed highest susceptibility with mean score of 4.000. Accessions within varieties Rume sudan, Hybrid de Timor and Compacts showed significant (P≤ 0.05) variation in resistance to CBD isolate Ca1 (Table 2). All the varieties showed susceptibility to this isolate. Mean scores for varieties against this isolate were 2.693, 3.283, 3.473 and 4.000 respectively for varieties Rume sudan, Hybrid de Timor, Compacts and Bourbon a control variety. However, accession VC299 within variety Rume sudan and PNI 086 within Compacts showed good levels of resistance with scores of 1.133 and 1.850 respectively. Three varieties, Rume sudan, Hybrid de Timor and Compacts showed significant (P≤0.05) variation in resistance to CBD within varieties against CBD isolate Z9 at CIFC Portugal (Table 2). There was no significant variation in resistance within variety Bourbon which was highly susceptible to this isolate.

The varieties Rume sudan, Hybrid de Timor and Compacts showed resistance with mean scores of 0.760, 1.750 and 1.180 respectively. Bourbon variety showed susceptibility with mean score of 4.000. However, within Hybrid de Timor, accessions VCE 1594 (2.183), RRC 70 (2.183); Compacts accession CR 124 (2.367) showed susceptibility to the Z9 CBD isolate at CIFC, Portugal. Rume sudan, Hybrid de Timor and compacts though differed significantly showed good levels of resistance against this isolate while Bourbon the control was highly susceptible. Observation on the reaction of CBD isolate Q2 showed that there was a significant variation (P≤0.05) in resistance to this isolate within varieties in varieties Hybrid de Timor and Compacts while there was no

Table 3: Variation in resistance to coffee berry disease (CBD) 0-4 score among varieties against Q2, Ca1, T3, Z9 CBD isolates at CIFC Portugal and a 'Local' one in Tanzania

Variety	Isolates Mean scores				
	Q2	Ca1	T3	Z9	Local
Rume sudan	0.650	2.693	1.007	0.760	0.072
Hybrid de Timor	1.793	3.283	1.620	1.750	0.178
Compacts	0.970	3.473	1.370	1.180	0.104
Bourbon	4.000	4.000	4.000	4.000	2.780
mean	1.853	3.362	1.999	1.922	0.784
CV (%)	41.770	20.510	29.260	31.170	66.790
SE±	0.346	0.308	0.522	0.273	0.233
$LSD_{0.05}$	1.067	0.949	0.777	0.840	0.719

significant variation in resistance to this isolate in varieties Rume sudan and Bourbon (Table 2).

Significant (P ≤ 0.05) variation was observed among varieties against a local isolate (Table 3). Three varieties Rume sudan, Hybrid de Timor and Compacts did not differ in their resistance level among themselves but showed significant difference with the control variety Bourbon which showed susceptibility. There was significant (P≤0.05) variation among varieties against the isolate T3 (Table 3). Rume sudan, Hybrid de Timor and Compacts were relatively resistant to this isolate while Bourbon displayed significant susceptibility to the T3 isolate. There was significant (P≤0.05) variation in resistance among varieties against Ca1 isolate at CIFC, Portugal (Table 3). All varieties were susceptible with mean scores ranging from 2.693 for Rume sudan to 4.000 for the susceptible control Bourbon. On the overall, there was a significant variation (P≤0.05) in resistance to CBD isolate Z9 among varieties (Table 3). Significant (P≤0.05) variation in resistance to CBD among varieties was observed against the isolate Q2 (Table 3). Varieties Rume sudan, Hybrid de Timor and Compacts showed high resistance to isolate Q2 with mean scores 0.650, 1.793 and 0.970 respectively. The variety Bourbon showed consistently high susceptibility among varieties.

DISCUSSION

In order to increase efficiency, reduce time and cost in breeding programmes, breeders have developed screening methods in which plants as young as possible are exposed to high concentrations of specific inoculum in order to identify resistant plants or lines in segregating populations (Van der Vossen et al., 1976; Ribeiro et al., 2001). These methods in order to serve the purpose must be reliable, simple, rapid and feasible to allow the handling of large plant populations with minimal chance for escapes (FAO, 1984).

Coffee breeding programmes are limited in progress due to the narrow genetic base of cultivated varieties especially for pest and disease resistance (Van der Vossen, 1985). However, nowadays natural and artificial hybrids derived from *Coffea arabica* x *Coffea canephora* are intensively used as source of resistance to coffee berry disease caused by *Colletotrichum kahawae* (Lashermes et al., 1996b). A natural hybrid, Hybrid de Timor (Timor hybrid) and the artificial hybrid Catimor (Compacts) and other derivatives such as Sarchimor are some of the most widely utilized materials both as released varieties and breeding lines especially in central America (Silveira et al., 2003).

Variation in resistance to CBD within varieties in Rume sudan and Compacts (Catimor) have been reported when large populations of these varieties from diverse geographic origins were worked on (Agwanda et al., 1997). The unique resistance observed on accessions VC 299 (Rume sudan) and PNI 086 (Compacts) against Ca1 isolate which is the most aggressive of all could be that for this particular isolate, there are no gene sequences in the other accessions that give gene products to confer resistance while these sequences are available in accessions VC 299 and PNI 086 in Rume sudan and Compacts respectively. The varieties Rume sudan, a semi wild arabica coffee variety, Hybrid de Timor a spontaneous hybrid between *C. arabica* x *C. canephora* and Catimor (Compacts) a hybrid derived from Hybrid de Timor x Catuai are reputed for their resistance to coffee berry disease. The resistance in

Rume sudan is controlled by two major genes *R.R* while in Hybrid de Timor it is controlled by a single major gene *T* (Van der Vossen and Walyaro, 1980; Agwanda et al., 1997; Omondi et al., 2001). Resistance in Catimor (Compacts) is controlled by one gene easily transferable *Ck-1* identified recently (Gichuru et al., 2006).Variation in resistance to CBD within varieties can be explained basing on two possible reasons. The first possibility may be due to natural out cross in pollination with susceptible varieties in neighbouring plots since even though *Coffea arabica* is a self fertile species, there is chance outcross of up to 10% (Van der Vossen, 1985). The second possibility may be due to segregation especially in hybrid varieties Hybrid de Timor and Compact (Catimor) but less so in pure lines Bourbon and Rume sudan. Thus, there is scope for further selection and genetic advancement within the varieties for resistance against the CBD isolates.

Many authors have found out that the aggressiveness of the CBD isolate population shows variability between isolates from the same or different geographic origins (Van der Vossen, 1985; Omondi et al., 2001). A study by Bieysse et al. (unpublished data INCO- project ICA4-CT-2001-10008) on isolates from Cameroon, Kenya, Burundi, Tanzania, Rwanda, Malawi, Angola and Ethiopia concluded that the strains might be classified in two groups- East Africa and Cameroon, each geographical population showing a strong homogeneity between the isolates (Silva et al., 2006). These isolates plus several others are preserved at CIFC Portugal the International center for Coffee leaf rust research for world-wide use in research for resistance to (CBD) and (CLR) (Silva et al., 2006). Cultivated arabica coffee has very narrow genetic base. However, low variations have been reported through DNA marker studies such as AFLP (Steiger et al., 2002) and RAPD (Masumbuko et al., 2003; Chaparro et al., 2004). Host plant genetic resistance is the most cost effective and sustainable approach to the control of plant disease (Richardson et al., 2006). Major gene resistance is relatively easier and the most frequently used in plant breeding. However, it is considered as non-durable compared with polygenic and horizontal resistance which is relatively difficult to identify and deploy to cultivated varieties (Ribeiro do Vale et al., 2001; Silva et al., 2006). Other alternatives for acquisition of resistance to diseases which are available and can be exploited include induced resistance (Aldwinckle and Alvaro, 2002, 2005; Fernandez et al., 2004) and genetic manipulation as reported in other works (Ribas et al*.,* 2006; Silva et al., 2006).

CONCLUSION

General levels of resistance to CBD disease in the evaluated progenitors of Rume sudan, Hybrid de Timor and Compacts against isolates T3, Z9, Q2 at CIFC Portugal and the local isolate at TaCRI-Tanzania proved that there is good resistance against the current CBD isolates in Tanzania. However if varieties developed in Tanzania are introduced to West Africa or the isolate Ca1 from Cameroon is introduced to Tanzania; there is a possibility of a threat against which all the progenitors fall susceptible when evaluated at CIFC-Portugal. When selecting parents for particular crossing in developing new improved hybrids for CBD resistance, selection within varieties is important.

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