International Journal of Agricultural Sciences ISSN: 2167-0447 Vol. 2 (11), pp. 296–300, 2012. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

Variation in resistance to coffee leaf rust (*Hemileia vastatrix*, Berk. and Broome) among germplasm progenitors at the Tanzanian Coffee Research Institute (TACRI)

S. O. W. M. Reuben[1] and D. J. I. Mtenga[2]*

[1]Sokoine University of Agriculture, Department of Crop Science and Production, P. O. Box 3005, Morogoro, Tanzania.
[2]Tanzania Coffee Research Institute, P. O. Box 3004, Moshi, Tanzania.

Received 23 February, 2012; Accepted 16 November, 2012

Trials were carried out at the Tanzania Coffee Research Institute (TaCRI) from September 2006 to April 2007 and at the Coffee Research Center (CIFC), in Portugal, from March 2007 to June 2007, in order to evaluate the variation in resistance to Coffee Leaf Rust (CLR), *Hemileia vastatrix*, within and among four *Coffea arabica* varieties. The varieties tested were 'Bourbon', 'Compacts' ('Catimor'), 'Rume Sudan' and 'Hibrido de Timor' obtained from TaCRI germplasm. Local CLR isolates were used in the trials at TaCRI, while at CIFC, four CLR isolates 1126, 1427, 2191 and 5A, were used. There was no significant (P≤0.05) variation among accessions of Hibrido de Timor and Compacts, whereas significant variation was observed among accessions of Rume Sudan and Bourbon against CLR isolates at CIFC. Significant variation was observed in Rume sudan and Compacts varieties against the local isolate. There was significant (P≤0.05) variation in resistance to CLR among the varieties against all isolates. Accessions PNI086, PNI088 and CR127, of Compacts variety, and VCE 1594 and VCE 1587, of Hibrido de Timor, showed exceptionally high resistance. It is possible to select within the accessions of a coffee variety and among the varieties tested to obtain better genotypes.

Key words: Coffee leaf rust, breeding, resistance sources, genetic resources.

INTRODUCTION

Coffee (*Coffea arabica* L.) is Tanzania's largest export crop. It contributes approximately 115 million USD in annual export income and provides employment to some 400000 families (ICO, 2006). About 95% of coffee is grown by smallholders on average holdings of 1 to 2 ha, and 5% is grown on estates and only a quarter of small holders use purchased inputs (Baffes, 2003; Winter-Nelson and Temu, 2005). Numerous constraints exist for coffee production, the most serious being Coffee Leaf Rust (CLR) caused by *Hemileia vastatrix*, Berk. and Broome and Coffee Berry Disease (CBD) caused by *Colletotrichum kahawae,* Waller and Bridge. (Wrigley, 1988). Coffee leaf rust is a fungal disease that attacks leaves of both arabica and robusta coffee. Serious attack on susceptible coffee plants causes serious defoliation resulting in low yields and poor grain quality due to reduced photosynthesis and die-back, leading to total death of the tree. Coffee leaf rust can cause losses of up to 30% in production in susceptible varieties with no chemical control (Cristancho and Escobar, 2008). Together, the two diseases account for 40% of total production costs for small-scale coffee producers in Tanzania (Teri et al., 2004). Coffee leaf rust can effectively be controlled by intensive protective fungicide

*Corresponding author. E-mail: mtenga2003@yahoo.com.

Table 1. Varieties and accessions studied.

Variety	Accessions	Pedigree
Rume sudan	VC 298, VC 299, VC 506, VC509, and VC510	Semi-wild *C. arabica*
Hibrido de Timor	RRC70, RRC72, VCE 1594, VCE 1587and VCE1589	Hybrid *C.arabica x C. canephora*
Catimor/Compacts	PNI 088, PNI 086, PNI 087, CR 124 and CR127	Hybrid Caturra x Hibrido de Timor
Bourbon	N5, N39, N100, N197 and N218	Commercial variety pure line

application whose costs are prohibitive and beyond the capability of small holders. The most sustainable option is host resistance, which offers the added advantage of being eco-friendly. The main strategy that producing countries have adopted for the control of the fungus is the development of resistant varieties. However the variability in pathogenicity of *H. vastatrix* races is great and by 2005 about 45 physiological races of this species had been identified (Va´rzea and Marques, 2005).

Hibrido de Timor (HDT) a spontaneous hybrid between *C. arabica* and *C. canephora* showing a broad spectrum of resistance have been used as resistance donors in coffee breeding programs in different countries. Within the genus *Coffea*, at least nine dominant genes (S_H1–S_H9) confer resistance to *H. vastatrix.* Hibrido de Timor contain the resistance genes S_H5, S_H6, S_H7, S_H8 and S_H9. However the resistance spectrum conferred by these genes can be totally or partially annulled by the combination of virulence genes (v5–v9) present in different races of the fungus (Bettencourt and Rodrigues, 1988).

The Arabica coffee improvement programme at TaCRI has achieved a considerable success in developing CLR resistant varieties. However, inconsistencies on the levels of resistance have been observed in some of the progenitors used in the development of the varieties (Walyaro, 2004). Determination of the resistance levels within and among the progenitors will lead to identification of superior ones with consistent resistance, allowing the development of improved varieties with consistent, stable and durable resistance. The current investigation was, therefore, designed to establish the resistance levels against CLR within and among the main progenitors used at the coffee research institute in Tanzania.

MATERIALS AND METHODS

The plant material used in this work consisted of three coffee varieties which are Rume sudan, a semi wild arabica coffee variety; Hibrido de Timor a spontaneous hybrid between *C. arabica* and *C. canephora;* and Hibrido Catimor/Compacts a compact hybrid between Caturra de Timor from Tanzania Coffee Research Institute germplasm, which are the main progenitors for CLR resistance.

The commercial variety Bourbon was included as a control (Table 1). Coffee Leaf Rust races which were used at CIFC-Portugal for the progenitors evaluation were 1126, 1427, 2191 and 5A while for the experiment carried out at TaCRI in Tanzania a ‘local’ one was used. The races at CIFIC were preserved samples while the ‘local’ race used in Tanzania was directly collected in the field from infected leaves of a susceptible variety Bourbon (N39).

Fully matured, red-ripe coffee berries were hand-picked from trees in the centre rows of each accession from the germplasm plot. These were pulped using a small hand-pulper in individual lots for each accession. The pulper hopper was washed thoroughly using clean tap-water under high-pressure to ensure the complete removal of cherries from the previous lot before pulping the next. This procedure was followed for all lots. Pulped cherries from each accession were placed in individual containers and kept under room temperature for 72 h to ferment. After 72 h of fermentation, the cherries were washed and dried in the shade, separately in partitioned wire-mesh trays, for one week. Subsequently, they were pre-germinated and seedlings were raised in the green house.

Fully-expanded tender and succulent terminal-node leaves of six month-old plants were used for inoculation, which was done by spreading dry rust uredospores on the lower surface of the leaves using a sterilized camel-hair brush. Each leaf of the pair was inoculated with 0.5 mg of inoculum. After inoculation, the leaves were sprayed with sterile distilled water and the plants were placed in a moist chamber for 48 h before they were taken to the nursery terrace. Evaluation of inoculated leaves was done 30 to 40 days after inoculation, using a qualitative scale by D’Oliveira (1954-57). Consideration of resistance levels was done as follows: plants displaying lesions of type 0 and 1 are considered to be resistant; type 2 lesions, are considered to be moderate resistant (MR); type 3, lesions correspond to moderate-susceptible and are classified as (MS); type 4 lesions correspond to susceptible plants (S).

Table 2. Variation in severity of coffee leaf rust symptoms within varieties against the local isolate at TaCRI, Tanzania. Disease was scored by a numeric scale ranging from 0 (resistant) to 4 (susceptible). CV, variation coefficient; SEx, Standard error; LSD, least significant difference.

Variety	Accession	Mean score (0-4)
Rume sudan	VC 298	2.925
	VC 299	2.375
	VC 506	3.325
	VC 509	3.425
	VC 510	1.625
	Mean	2.735
	CV (%)	20.610
	Sex(±)	0.282
	$LSD_{0.05}$	0.869
Hibrido de Timor	VCE 1587	0.075
	VCE 1589	0.200
	VCE 1594	0.075
	RRC 70	0.200
	RRC 72	0.250
	Mean	0.160
	CV (%)	22.425
	Sex(±)	0.359
	$LSD_{0.05}$	NS
Compacts	PNI 086	0.000
	PNI 087	0.175
	PNI 088	0.000
	CR 124	0.250
	CR 127	0.000
	Mean	0.085
	CV (%)	42.960
	SEx(±)	0.016
	$LSD_{0.05}$	0.049
Bourbon	N5	3.675
	N39	3.250
	N100	3.425
	N197	3.600
	N218	3.850
	Mean	3.560
	CV (%)	11.880
	SEx(±)	0.103
	$LSD_{0.05}$	NS

Mean scores calculations

The trial was arranged in a split plot design with four replicates, five varieties and five accessions in each variety and each accession had 10 seedlings. A pair of inoculated leaves was assessed and scored using a of scale of 0 to 4. Each seedling was assigned a score, that is, 0, 1, 2, 3 or 4. The mean value of an accession was computed by adding individual values of seedlings within and across replicates and divided by 40. Calculated score means were subjected to analysis of variance and mean separation employing least significance difference (LSD).

RESULTS

Significant (P≤ 0.05) variation in the severity of coffee leaf rust was observed within and between varieties Rume sudan and compacts against local isolate of CLR at TaCRI Tanzania (Table 2). Varieties Hibrido de Timor and Bourbon did not show significant variation among accessions against this isolate. Varieties Hibrido de Timor and Compacts showed high resistance to this isolate, with mean scores of 0.160 and 0.085, respectively. Rume sudan and Bourbon varieties exhibited susceptibility with mean scores of 2.735 and 3.560, respectively. However, within Rume sudan variety, accession VC510 displayed resistance to local CLR isolate at TaCRI. There was significant (P≤ 0.05) variation among varieties on severity of CLR local isolates (Table 3). Overall mean scores among the varieties was 1.635. Two varieties, viz. Compacts and Hibrido de Timor, had mean scores lower than the overall mean and were resistant to the tested isolate. At CIFC, in Portugal, four isolates 1126, 1427, 2191 and 4A were used to evaluate variation in resistance. Significant (P≤0.05) variation within varieties for the severity of the CLR isolates was observed in Rume sudan and the control variety Bourbon (Table 4). Two varieties, viz. Hybrid de Timor and Compacts, showed high resistance, with mean scores of 0.016 and 0, respectively, against the four isolates. In contrast, Rume sudan and Bourbon varieties displayed susceptibility to all CLR isolates at CIFC. Three of the isolates used at CIFC, 1427, 2191 and 4A showed high aggressiveness compared with the local Isolate at TaCRI Tanzania on the varieties Bourbon and Rume sudan. Isolate 1126 showed reaction comparable to the local isolate used in Tanzania. Significant (P≤0.05) variation in severity in response to the four isolates was observed among varieties (Table 5). Hibrido de Timor and compacts displayed high levels of resistance against the four CLR isolates at CIFC, in Portugal. In contrast, Rume sudan and Bourbon were susceptible to all CLR isolates tested at CIFC.

DISCUSSION

In order to increase efficiency, reduce time and costs in breeding programmes, breeders have developed screening methods to expose plants as young as

Table 3. Variation in severity of coffee leaf rust among varieties against the local isolate at TaCRI, Tanzania. disease was scored by a numeric scale ranging from 0 (resistant) to 4 (susceptible). CV, variation coefficient; SEx, Standard error; LSD, least significant difference.

Varieties	Mean score (0-4)
Rume sudan	2.735
Hibrido de Timor	0.160
Compacts	0.085
Bourbon	3.560
Mean	1.635
CV(%)	25.190
SEx(±)	0.184
$LSD_{0.05}$	0.568

Table 4. Variation in severity of coffee leaf rust within varieties against the four CLR isolates at CIFC, in Portugal. disease was scored by a numeric scale ranging from 0 (resistant) to 4 (susceptible). CV, variation coefficient; SEx, Standard error; LSD, least significant difference.

Variety	Accession	Isolates				Mean score (0-4)
		1126	1427	2191	5A	
Rume sudan	VC 298	2.100	3.900	4.000	3.800	3.450
	VC 299	1.600	3.100	3.500	3.700	2.975
	VC 506	0.900	2.800	3.600	3.600	2.725
	VC 509	2.900	2.200	3.900	4.000	3.250
	VC 510	1.100	3.300	3.600	3.800	2.950
	Mean score	1.720	3.060	3.720	3.780	3.070
	CV(%)					16.770
	SEx(±)					0.257
	$LSD_{0.05}$					0.793
Hibrido de Timor	VCE 1587	0	0	0	0	0.000
	VCE 1589	0	0	0	0	0.000
	VCE 1594	0	0	0	0	0.000
	RRC 70	0	0	0	0	0.000
	RRC 72	0	0	0	0.080	0.080
	Mean score	0	0	0	0.016	0.016
	CV (%)					0.000
	SEx(±)					0.000
	$LSD_{0.05}$					NS
Compacts	PNI 086	0	0	0	0	0.000
	PNI 087	0	0	0	0	0.000
	PNI 088	0	0	0	0	0.000
	CR 124	0	0	0	0	0.000
	CR 127	0	0	0	0	0.000
	Mean score	0	0	0	0	0.000
	SEx(±)					0.000
	LSD0.05					NS

Table 4 Contd.

Bourbon	N 5	3.500	4.000	4.000	4.000	3.875
	N 39	4.000	4.000	4.000	4.000	4.000
	N 100	2.100	3.900	4.000	3.900	3.475
	N 197	1.800	3.700	4.000	3.800	3.325
	N 218	2.200	3.700	4.000	3.920	3.455
	Mean score	2.720	3.860	4.000	3.924	3.626
	CV (%)					12.560
	SEx(±)					0.228
	LSD0.05					0.701

Table 5. Variation in resistance to coffee leaf rust among varieties against CLR isolates at CIFC, in Portugal. Disease was scored by a numeric scale ranging from 0 (resistant) to 4 (susceptible). CV, variation coefficient; SEx, Standard error; LSD, least significant difference.

Varieties	Isolates				Mean scores (0-4)
	1126	1427	2191	5A	
Rume sudan	1.720	3.060	3.720	3.780	3.070
Hibrido de Timor	0.000	0.000	0.080	0.000	0.020
Compacts	0.000	0.000	0.000	0.000	0.000
Bourbon	2.600	3.840	4.000	3.920	3.590
Mean	1.080	1.725	1.950	1.925	1.670
CV (%)	52.5	17.780	7.560	4.770	
SEx(±)	0.253	0.137	0.066	0.40	
$LSD_{0.05}$	0.781	0.423	0.204	0.123	

possible to high concentrations of specific inocula, in order to identify resistant plants or lines in segregating populations (Van der Vossen et al., 1976; Ribeiro et al., 2001). In order for these methods to serve their purposes, they must be reliable, simple, rapid and feasible allowing large plant populations to be handle with minimal chance for escapes (FAO, 1984). Generally, the varieties Hibrido de Timor and Compacts showed high levels of resistance to all CLR isolates, whereas Rume sudan showed moderate susceptibility, and Bourbon was highly susceptible. A similar observation was recorded elsewhere on these varieties using laboratory and field evaluations (Varzea et al., 1985; Agwanda et al., 1997; Rodrigues et al., 2000; Silva et al., 2006). Thus, there is variation for selection and genetic improvement of resistance against CLR in these varieties. Resistance to coffee leaf rust is controlled by S_H major genes, and probably some minor genes that have not yet been identified with certainty (Prakash et al., 2004; Silva et al., 2006). These progenitors carries the following resistance genes; Hibrido de Timor S_H5, 6, 7, 8 and 9, Catimor S_H5, 6, 7, 8 and 9+; Rume Sudan S_H5 (Varzea, Marques, 2005). These accessions are, therefore, good sources of combined resistance to, CLR. The variety Rume sudan showed moderate resistance to CLR; however previous work has reported a good field resistance of Rume sudan against CLR (Varzea et al., 1985; Silva et al., 2006), probably due to variations in pathogen strains and or environments.

ACKNOWLEDGEMENTS

This work was financed by the European Union and TaCRI stakeholder's fund. Assistance from field staff at TaCRI and technical staff at CIFC is highly appreciated. This work is published with the permission of the Chief Executive Director, TaCRI, Tanzania.

REFERENCES

Agwanda CO, Lashermes P, Combes MC, Charrier ,A (1997). Identification of RAPD markers for resistance

to coffee berry disease, *Colletotrichum kahawae* in Arabica coffee. Euphy. 97:241-248.

Baffes J (2003). Tanzania's Coffee Sector: Constraints and Challenges in Global Environment. J.Intern. Dev. 17 (1): 21-43.

Bettencourt AJ, Rodrigues CJ (1988). Principles and practice of coffee breeding for resistance to rust and other diseases. In: Clarke RJ, Macrae R (eds) Coffee: agronomy. Elsevier Applied Science 3, London, pp 199–234

Cristancho M, Escobar C (2008). Transferability of SSR markers from related Uredinales species to the coffee rust *Hemileia vastatrix* .Genet.Mol. Res. 7 (4):1186-1192

D'Oliveira B (1954-57). As ferrugens do cafeeiro. Revista do Café Portugues (Coffee leaf rusts. J.Portuguese Coffee) 1(4): 5-13; 2(5): 5-12; 2(6): 5-15; 2(7): 9-18; 2(8): 5-22; 4(16) 5-15.

FAO (1984). Breeding for durable disease and pest resistance. Plant production and protection paper (55) 167pp.

ICO (2006). International Coffee Organization: Statistics [http://www.ico.org] site visited 22July 2007.

Prakash NS, Marques DV, Varzea VMP, Silva MC, Combes MC, Lashermes P (2004). Introgression molecular analysis of a leaf rust resistance gene from *Coffea liberica* into *Coffea arabica* L. Theor.Appl. Gen.109:1311-1317.

Ribeiro do Vale FX, Parlevliet JE, Zambolin L (2001). Concepts in plant disease resistance. Fitopat. Brasileira (Braz. J. Phytopath.). 26 (3): 577-589.

Rodrigues Jr CJ, Varzea VMP, Silva MC, Guerra-Guimaraes L, Rocheta M Marques DV (2000). Recent advances on coffee leaf rust. In*:* Proceedings International Scientific Symposium on Coffee*.* Bangalore, India 179-193pp.

Silva MC, Varzea VMP, Guerra-Guimaraes L, Azinheira HG, Fernandes D, Pettiot AS, Bernard B, Lashermes P, Nicole M. (2006). Coffee resistance to the main disease: leaf rust and coffee berry disease. Brazil. J. Plant Physiol.18 (1):119-147.

Teri JM, Kilambo DL, Mtenga DJ, Nyange NE, Nzallawahe TS, Chipungahelo GS, Kipokola TP, Kullaya IK (2004). Improved Arabica varieties for the benefit of Tanzania coffee producers. In: Proceedings of the 20th International conference on coffee science (ASCI), Bangalore, India, p.1187-1191.

Van der Vossen HAM (2001). Coffee breeding practices. In: Coffee. Recent developments. Edited by Clarke, R.J. and Vitzthum, O.G. Blackwell Science Ltd., London, UK. p.184-201.

Van der Vossen HAM and Walyaro DJ (1980). Breeding for resistance to coffee berry disease in *Coffea arabica* L. II Inheritance of resistance. Euphy. 29:777-791.

Van der Vossen HAM, Cook RT, MurakaruGNW (1976). Breeding for resistance to coffee berry disease caused by *Colletotrichum caffeanum* Noack (sensu Hindorf) in *Coffea arabica* L. I Methods of preselection for resistance. Euphy.. 25:733-745.

Varzea V, Marques DV (2005) Population variability of *Hemileia vastatrix* vs Coffee durable resistance. In Zambolim L Zambolim E Várzea VMP Eds Durable resistance to coffee leaf rust Universidade Federal de Viçosa Viçosa Brasil. pp 53-74.

Varzea VMP, Rodrigues Jr CJ and Mexia JT. (1985). Evaluation of the level of horizontal resistance to *Hemileia vastatrix* of some Arabica plants of different physiological groups when confronted with virulent races. In*:* Proceedings of the 11th International Scientific Conference on Coffee Science (ASCI) 11-15th February, 1985, Lome, Togo, p. 625-633.

Waller JM, Bridge PD, Black R, Hakiza G (1993). Characterization of coffee berry pathogen, *Colletotrichum kahawae* sp.nov. Mycol.Res.97:989-994.

Walyaro DJ (2004). Consultant report on coffee improvement at TaCRI. First mission 8-30 June EU-EDF contract, SER/STAB/FMO/TaCRI, Moshi, Tanzania. 34pp.

Winter-Nelson A and Temu C (2005). Impacts of price and transactions on input usage in a liberalizing economy: evidence from Tanzania coffee growers. Agric*.* Econ*.* 33 (3):243-253.

Wrigley G (1988). Coffee*.* Tropical Agriculture. Longman Scientific and Technical, Harlow, UK. 639pp.