FIELD SCREENING OF SELECTED *Coffea arabica* L. GENOTYPES AGAINST COFFEE LEAF RUST

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Abstract

Coffee Leaf Rust (CLR) is a fungal disease caused by *Hemileia vastatrix* Berk et Br. and is one of the major diseases of coffee. It causes premature leaf fall, yield loss and even death of the tree in severe cases. Coffee genotypes respond differently to biotic factors. This study was aimed at identifying potential sources of resistance genes to the disease. Forty five *Coffea arabica* L accessions were evaluated for their response to CLR under field conditions. CLR infection was assessed from the 45 genotypes subjected to similar field conditions in June 2010 when disease pressure was at peak. The experimental plot was laid out in a Randomized Complete Block Design with three replications. Each of the genotypes was represented by fifteen trees consisting of five trees per replication. Significant variation in tolerance to CLR was observed among the genotypes and some tolerant genotypes identified. HDT, Rumesudan, Barbuk Sudan, Ennareta, Geisha12, Babbaka Ghimira, Boma plateau and Tafari Kela were the most tolerant to CLR (recording a score of 0) while Drought Resistant II [DRII]) was the most susceptible recording a score of 8. Most of the accessions that demonstrated high phenotypic resistance have not been utilized as sources of resistance to CLR in coffee breeding programmes except HDT. Such genotypes could represent a highly valuable resource for *C. arabica* breeding against CLR if their reaction is confirmed.

Key Words: *Hemileia vastatrix*, Resistance, SH genes, Kenya**.**

Introduction

Coffee Leaf Rust (CLR) caused by the obligate parasitic fungus *Hemileia vastatrix* (Berkeley and Broome) is a major disease which greatly limits arabica coffee (*Coffea arabica* L) production in almost all growing countries around the world (Prakash *et al*., 2004). Although it is rather difficult to estimate precisely the global impact of this disease, the economic damage to world Arabica coffee production has been estimated to be between $(US)1 billion and 2 billion per year (Van der Vossen, 2001) due to crop losses of 20–25% (Prakash *et al*., 2004). Chemical control of CLR by use of fungicides is expensive leading to high production costs and is not safe to humans and environment (Gichuru *et al*., 2008). In view of the economics and to minimise the chemical input for disease management, the development and cultivation of tolerant cultivars is the most effective and viable option. Therefore, the development of coffee varieties resistant to CLR has been one of the major breeding objectives in many countries (Prakash *et al*., 2004).

C. arabica L. is the most important species of the *Coffea* genus, followed by *Coffea canephora* Pierri (Silveira *et al*., 2003). Much of the world coffee is still produced by traditional cultivars of *C. arabica* (66%) and *C. canephora* (34%), released some 50-80 years ago from relatively simple selection and breeding programmes and generally multiplied by seed. Most of these traditional cultivars are susceptible to CLR (Van der Vossen, 2009). Over 90% of the total Kenya coffee acreage is under Arabica coffee while the rest is occupied by Robusta coffee. The main coffee growing areas in Kenya are found in three altitude zones; the low altitude (1200 M –1580 M above sea level), the medium altitude (between 1580 M and 1700 M above sea level) and the high altitude (over 1700 M above sea level) (Mwangi, 1983). However,

production in low to medium altitudes is seriously constrained by CLR (Omondi *et al*., 2001).

Nine major dominant resistance factors to *H. vastatrix* have been so far inferred on the basis of the gene-for-gene concept. The resistance to leaf rust of coffee plants therefore appears to be conditioned by at least nine resistance genes designated as SH1–SH9, either singly or in combination, while the corresponding virulences have been indicated as V1–V9 (Bettencourt and Rodrigues 1988). This allows coffee genotypes to be classified in resistant groups according to the physiological races of the rust pathogen (Herrera *et al*., 2009). For instance the A-group expresses host resistance to all races of the CLR pathogen identified so far, while the E group includes cultivars with susceptibility to most races (Bettencourt and Rodrigues 1988). Of the 9 resistance factors, SH1, SH2, SH4 and SH5 have been found in *C. arabica*. The other genes, SH6, SH7, SH8 and SH9, have been introgressed from the diploid species *C. canephora*, while SH3 probably originates from another diploid species, *C. liberica*. To date more than 40 physiological races are known to be infecting different coffee genotypes in various coffee-growing countries. The resistance genes identified in *C. arabica*, used either singly or in combination, have not provided durable resistance to most of the races of rust fungus. In contrast, the SH3 gene from *C. liberica* as well as certain genes from *C. canephora* has provided long-lived protection under field conditions (Van der Vossen, 2005).

Coffee breeding programs are limited by the narrow genetic base of coffee, especially for pest and disease resistance improvement (Van der Vossen, 1985). The exploitation of genetic resources from the wild coffee is essential for the development of inbred lines, which can be adapted to new production systems (Silveira *et al*., 2003). *C. canephora* provides the main source for resistance genes not found in *C. arabica*, including CLR, CBD, and resistance to root-knot nematode (*Meloidogyne sp*) (Lashermes *et al.*, 2000). Natural and artificial hybrids derived from crosses between *C. arabica* and *C. canephora* have been intensively used in breeding programs. A good example is the Timor Hybrid, which has been exploited as a bridge to transfer rust resistance genes from *C. canephora* to cultivars of *C. arabica* (Fazuoli *et al.*, 1996). The Timor Hybrid was identified in a *C. arabica* field on the island of Timor (Bettencourt, 1973) and is believed to have derived from a spontaneous interspecific cross between *C. arabica* and *C. canephora* (Lashermes *et al.*, 2000).

The longevity of coffee seed is insufficient to use in the preservation of genetic resources of coffee. Consequently, coffee germplasm is conserved in field genebanks (Van der Vossen, 1985). The repeated appearance of new virulent races of CLR and break-down of resistance in Timor Hybrid (Prakash *et al*., 2010) and cv. Cauvery (Catimor lines) (Van der Vossen, 2009) in India, is challenging the breeders to keep searching for other sources of resistance to CLR. The coffee germplasm bank maintained at Coffee Research Station (CRS) Ruiru has many *C. arabica* accessions from Ethiopia, Sudan, Angola, India, Reunion, Portugal, Brazil, South and Central America and Kenya. The study was conducted to evaluate the response of selected Coffee accessions to CLR under field conditions. The study was also aimed at classifying the genotypes on the basis of susceptibility or tolerance to CLR and identifying potential sources of resistance genes to the disease.

Materials and Methods

Study Site: The study was carried out at Coffee Research Station (CRS), Ruiru, Kenya. The site lies within the Upper Midland 2 agro-ecological zone (UM 2) at latitude 1^{o} 06'S and longitude 36^{o} 45'E and is approximately 1620m above sea level (Kimemia *et al*., 2001). The area receives a mean annual rainfall of 1063mm and the mean annual temperature is 19^{o}C (minimum

12.8°C and maximum 25.2°C). The soils are classified as a humic nitisols and plinthic ferrasols. They are well drained, deep reddish brown, slightly friable clays with murram sections occassionally interrupting. The soil pH ranges between 5 and 6 (Jaetzold and Schmidt, 1983).

Test Materials: A total of forty five (45) *C. arabica* genotypes obtained from CRS germplasm conservation site were used in this study (Table 1). Some of them are elite genotypes that are being grown as commercial varieties (K7, Blue Mountain and Geisha) or have been used in breeding programs (HDT, Rume Sudan, Bourbon, N39, SL4) either as resistance donors or to improve quality. The experimental plot was laid out in a Randomized Complete Block Design with three replications. Each of the genotypes was represented by fifteen trees consisting of five trees per replication.

Disease assessment: Disease severity was scored using a 0-9 scale (Eskes and Toma-Braghini, 1981). Scale value 0 indicated absence of visible symptoms, 1 to 3 variation within tolerant reaction types (small flecks and tumefactions to large chlorotic areas without sporulation), 4 to 7 heterogeneous reaction types with increasing sporulation intensity and percentage of sporulating lesions, and 8 and 9 susceptible reaction types ranging from moderate (8) to high (9) sporulation intensity. Reaction types indicated by T (tolerant) represents scale values 0 to 3, MT (moderately tolerant) 4 and 5, MS (moderately susceptible) 6 and 7, and S (susceptible) 8 and 9. Scoring was done when defoliation started with more than 50% of the leaves of the most susceptible genotypes having sporulating lesions.

Data Analysis: The data was subjected to analysis of variance (ANOVA) using COSTAT software and effects declared significant at 5% level. Students-Newman-Keuls ($SNK_{5\%}$) was used to separate the means. In order to classify the genotypes on the basis of susceptibility or resistance to CLR, the data was organized into a matrix and subjected to cluster analysis using XLSTAT version 2010 Software and a dendrogram constructed using the unweighted pair-group method with arithmetic average (UPGMA).

Results and Discussion

Analysis of variance indicated highly significant ($p<0.0001$) variation amongst the genotypes in response to CLR. Out of the 45 genotypes evaluated, twenty four (24) fell in the tolerant (≤3) class, six (6) in the moderately tolerant (>3≤5) class, eight (8) in moderately susceptible (>5≤7) class and seven (7) in the susceptible (>7≤9) class (Fig. 1 and Table 2). HDT, Rumesudan, Barbuk Sudan, Ennareta, Geisha12, Babbaka Ghimira, Boma plateau and Tafari Kela were the most tolerant to CLR (recording a score of 0) while Drought Resistant II [DRII]) was the most susceptible recording a score of 8 (Fig. 1). Among these most tolerant genotypes, only three (HDT, Rumesudan and Geisha 12) has been utilized as sources of resistance in coffee breeding programmes. The rest have not been exploited as sources of resistance to CLR and probably they have not been characterized.

HDT (also known as Timor Hybrid), has been exploited world wide in transfering rust resistance genes from *C. canephora* into *C. arabica* cultivars (Silveira *et al*., 2003). HDT and its derivatives belong to resistant group A and are known to contain resistance genes, SH6-SH9 supposedly coming from C. canephora (Silva *et al*., 2006). Rume Sudan and Tafari kela, which are known to contain resistance gene SH5 (Silva *et al*., 2006), were among the eight genotypes that portrayed high field resistance in this study. This confirmed the report by Varzea and Marques (2005) that the two show high field resistance to CLR.

Table 1: List of *C. arabica* genotypes evaluated (Millot, 1969; Kathurima *et al*., 2009)

	Genotypes	Status	Source
1.	63	Museum Accession	Kitale, Kenya
2.	1255	Museum Accession	Ethiopia
3.	Angustifola	Museum Accession	NAL, Kenya
4.	Arousi	Museum Accession	Ethiopia
5.	Dalle	Museum Accession	Ethiopia
6.	Dilla	Museum Accession	Ethiopia
7.	Dilla Alghe	Museum Accession	Ethiopia
8.	Drought Resistant 1 (DR1)	Museum Accession	French Mission Selection
9.	Drought Resistant II (DRII)	Museum Accession	French Mission Selection
10.	Babbaka Ghimira	Museum Accession	Ethiopia
11.	Barbuk Sudan	Museum Accession	Boma Plateau, Sudan
12.	Blue Mountain	Elite Genotype	Jamaica
13.	Boma Plateau	Museum Accession	Boma Plateau, Sudan
14.	Bourbon	Elite Genotype	Reunion and Latin America
15.	Ennareta	Museum Accession	Ethiopia
16.	Erecta	Museum Accession	NAL, Kenya
17.	Eritrean Moca	Museum Accession	Ethiopia
18.	F53	Museum Accession	Kitale, Kenya
19.	G53	Museum Accession	Kitale, Kenya
20.	Geisha 9	Elite Genotype	Kitale, Kenya
21.	Geisha 10	Elite Genotype	Kitale, Kenya
22.	Geisha 11	Elite Genotype	Kitale, Kenya
23.	Geisha 12	Elite Genotype	Kitale, Kenya
24.	Gimma Galla	Museum Accession	Ethiopia
25.	Gimma Galla Sidamo	Museum Accession	Ethiopia
26.	Gimma Mbuni	Museum Accession	Ethiopia
27.	Grafts	Museum Accession	Not Known
28.	Guatemala	Museum Accession	Guatemala
29.	Hibrido De Timor (HDT)	Elite Genotype	Timor
30.	K7	Elite Genotype	Kenya
31.	Mocha (Series D)	Museum Accession	NAL, Kenya
32.	Mokka Cramers	Museum Accession	NAL, Kenya
33.	Murta	Museum Accession	Guatemala
34.	Mysore	Museum Accession	India
35.	N39	Elite Genotype	Tanzania
36.	Padang	Museum Accession	Puerto Rico
37.	Plateau Bronze	Museum Accession	NAL, Kenya
38.	Polysperma	Museum Accession	Lyamungu, Tanzania
39.	Pretoria	Museum Accession	Guatemala
40.	Rume Sudan	Elite Genotype	Boma Plateau, Sudan
41.	SeriesC	Museum Accession	NAL, Kenya
42.	SeriesL	Museum Accession	NAL, Kenya
43.	SL4	Elite Genotype	NAL, Kenya
44.	Tafari Kela	Museum Accession	Ethiopia
45.	Tanganyika Drought Resistant (TDR)	Museum Accession	Tanzania

NB: NAL= National Agricultural Laboratories

Rume sudan is a semi-wild accession with small beans and poor yields and has been used in coffee breeding programs in Kenya as a source of resistance to both CBD and leaf rust (Agwanda *et al*., 1997). Among the Geisha varieties, Geisha 12 was the most tolerant but Geisha 9 and 10 also recorded good tolerance. Geisha varieties have also been utilized in development of resistant hybrids in Tanzania (Nyange *et al*., 2000). They are have been found to contain resistance genes SH1 and SH5 (Teixeira-Cabral *et al*., 2004). Like Rume Sudan, the varieties Barbuk Sudan and Boma plateau originated from the Boma plateau in Sudan and therefore their genetic makeup could be somehow similar.

Other varieties which portrayed good field tolerance include Gimma Galla Sidamo, Dilla Alghe, Gimma Mbuni, Dilla, Grafts, Arousi, Polysperma, Guatemala, Bourbon, 1255, Gimma Galla, K7, Blue Mountain and Mysore. Among these, only Bourbon, Blue Mountain and K7 have been utilized in breeding programmes in Kenya. Bourbon, Blue Mountain and Padang belong to susceptible group E with resistance gene SH5 (Teixeira-Cabral *et al*., 2004). The good field tolerance observed in this study among these three genotypes would require further laboratory screening to confirm whether they were actually tolerant or escapes. K7 is a Kent type commercial variety (Agwanda *et al*., 1997) with resistance genes SH2 and SH5 while Dilla Alghe contains SH1 gene (Teixeira-Cabral *et al*., 2004). The two are considered to have partial resistance to CLR which is often pathogen nonspecific and involves both constitutive and induced defense mechanisms (Van der Vossen, 2005). The genotypes SL4 and N39 which fell under moderately susceptible and susceptible classes respectively have also been utilized in coffee breeding programmes in Kenya and Tanzania but there was no information about the resistance genes they contain. There was also no information about the rest of the genotypes and probably they have not been characterized. However, most of the Ethiopian Arabica coffees contain SH1, SH2, SH4 and SH5 genes and they are known to confer resistance to some races of *H. vastatrix* (Sera *et al*., 2007).

The 45 genotypes were clustered into three (3) main clusters at dissimilarity level of about 0.8 as shown by the broken line (Fig. 2). This confirmed the narrow genetic base reported in C. arabica (Gichimu and Omondi, 2010; Anthony *et al*., 2001; Kathurima *et al*., 2012). The first cluster (C1) contained the first 22 tolerant genotypes. The second cluster (C2) contained 12 members comprising of moderately susceptible and susceptible genotypes. The third cluster (C3) contained 11 members with a reaction type ranging from tolerant (Blue Mountain and Mysore), moderately tolerant (Dalle, Murta, Geisha 11, Pretoria, Padang and Angustifolia) to moderately susceptible (63, Eritrean Moca and Plateau Bronze) (Fig. 2 and Table 2). The cluster analysis was therefore successful in separating the genotypes according to reaction types though slightly different from the numerical classification of Eskes and Toma-Braghini, 1981. With the exception of cluster one which contained only tolerant genotypes, the other two clusters contained genotypes with varying reaction types. This was an indication that most of these genotypes contained quantitative rather than qualitative kind of resistance/susceptibility. The term 'quantitative' is used when differences between genotypes are not easily distinguishable while 'qualitative' is used when different genotypes show easily distinguishable phenotypes (Eskes, 1983).

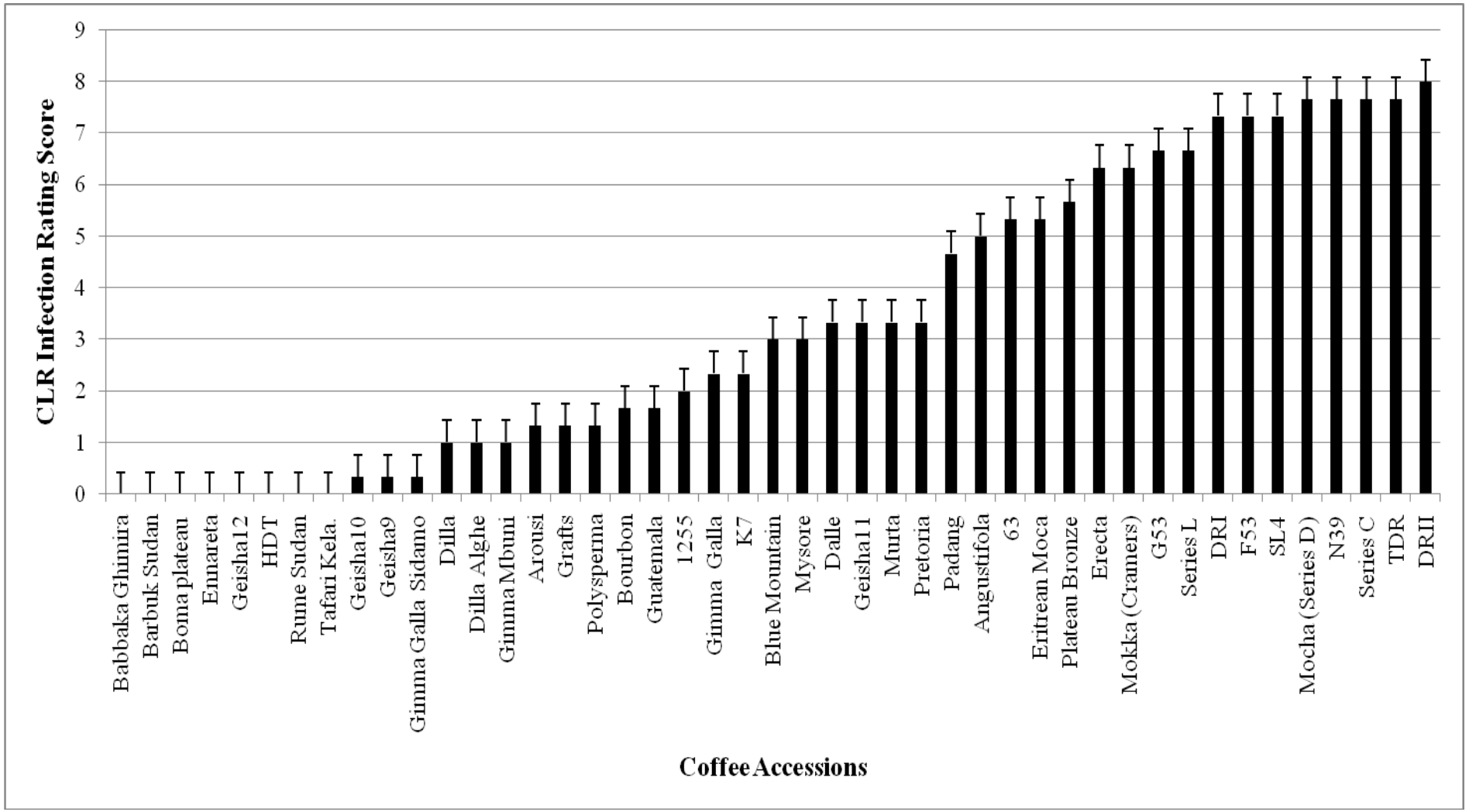

Fig. 1: Response of the coffee genotypes to CLR

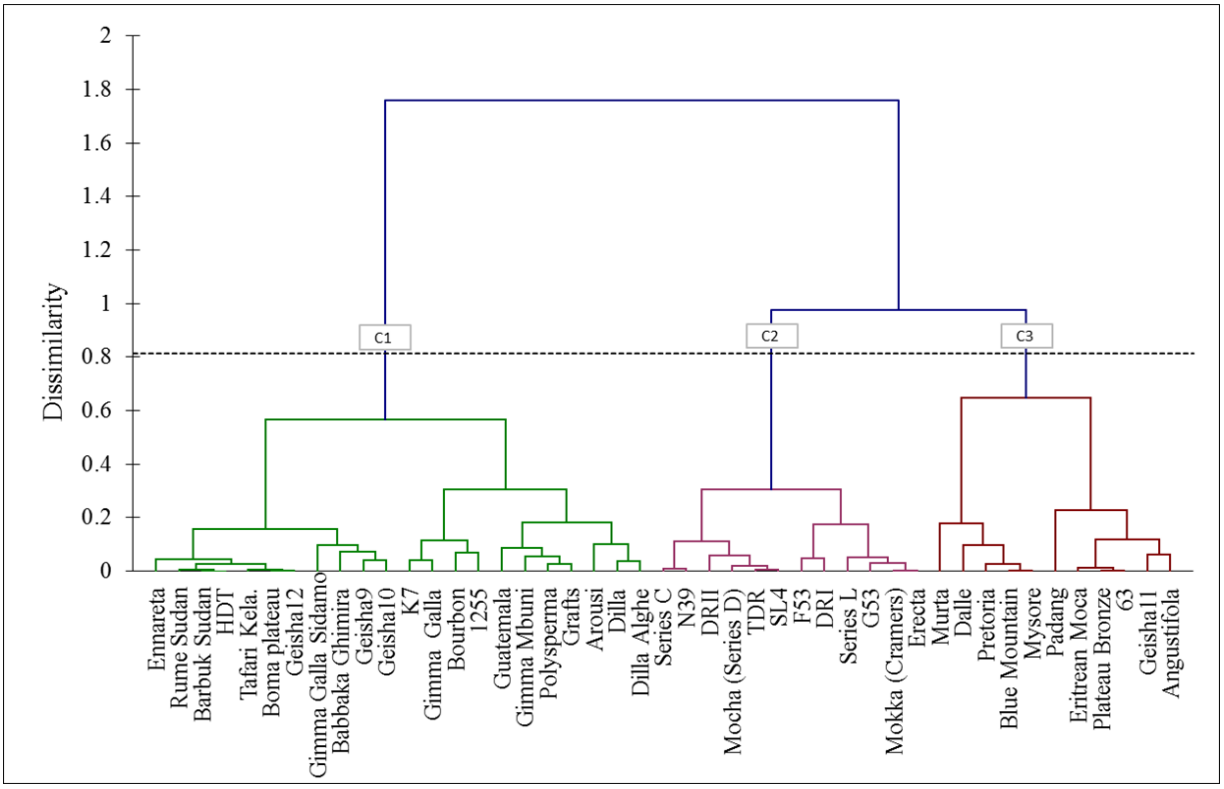

Fig. 2: Similarity of the coffee genotypes in response to CLR

Conclusion and Recommendations

This study was the first attempt to evaluate the response of various museum accessions available in Kenya to CLR under field conditions. The study successfully identified some genotypes with high field tolerance to CLR. Apart from HDT, Rume Sudan and Geisha, other genotypes that demonstrated high phenotypic tolerance such as Barbuk Sudan, Ennareta, Babbaka Ghimira, Boma plateau and Tafari Kela are little known and have not been utilized as sources of resistance to CLR in coffee breeding programmes. Other potential sources of resistance include Gimma Galla Sidamo, Dilla Alghe, Gimma Mbuni, Dilla, Grafts, Arousi, Polysperma, Guatemala, Bourbon, 1255 and Gimma Galla. Such genotypes could represent a highly valuable resource for *C. arabica* breeding against CLR if their reaction is confirmed. Laboratory screening of these genotypes is therefore recommended to confirm their reaction to CLR and to find out whether there were any escapes. The genotypes whose resistance genes are not yet known should also be characterized.

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Table 2: Classification of genotypes according to reaction type

	Genotype	Reaction Type	Clustering
1.	HDT	Tolerant	Cluster 1
2.	Rumesudan	Tolerant	Cluster 1
3.	Barbuk Sudan	Tolerant	Cluster 1
4.	Ennareta	Tolerant	Cluster 1
5.	Geisha12	Tolerant	Cluster 1
6.	Babbaka Ghimira	Tolerant	Cluster 1
7.	Boma plateau	Tolerant	Cluster 1
8.	Tafari Kela	Tolerant	Cluster 1
9.	Geisha10	Tolerant	Cluster 1
10.	Geisha9	Tolerant	Cluster 1
11.	Gimma Galla Sidamo	Tolerant	Cluster 1
12.	Dilla Alghe	Tolerant	Cluster 1
13.	Gimma Mbuni	Tolerant	Cluster 1
14.	Dilla,	Tolerant	Cluster 1
15.	Grafts	Tolerant	Cluster 1
16.	Arousi	Tolerant	Cluster 1
17.	Polysperma	Tolerant	Cluster 1
18.	Guatemala	Tolerant	Cluster 1
19.	Bourbon	Tolerant	Cluster 1
20.	1255	Tolerant	Cluster 1
21.	Gimma Galla	Tolerant	Cluster 1
22.	K7	Tolerant	Cluster 1
23.	Blue Mountain	Tolerant	Cluster 2
24.	Mysore	Tolerant	Cluster 2
25.	Dalle	Moderately Tolerant	Cluster 2
26.	Murta	Moderately Tolerant	Cluster 2
27.	Geisha 11	Moderately Tolerant	Cluster 2
28.	Pretoria	Moderately Tolerant	Cluster 2
29.	Padang	Moderately Tolerant	Cluster 2
30.	Angustifola	Moderately Tolerant	Cluster 2
31.	63	Moderately Susceptible	Cluster 2
32.	Eritrean Moca	Moderately Susceptible	Cluster 2
33.	Plateau Bronze	Moderately Susceptible	Cluster 2
34.	Mokka-Cramers	Moderately Susceptible	Cluster 3
35.	Erecta	Moderately Susceptible	Cluster 3
36.	Series L	Moderately Susceptible	Cluster 3
37.	G53	Moderately Susceptible	Cluster 3
38.	SL4	Moderately Susceptible	Cluster 3
39.	Drought Resistant I [DRI]	Susceptible	Cluster 3
40.	F53	Susceptible	Cluster 3
41.	N39	Susceptible	Cluster 3
42.	Mocha Series D	Susceptible	Cluster 3
43.	Series C	Susceptible	Cluster 3
44.	Tanganyika Drought Resistant [TDR]	Susceptible	Cluster 3
45.	Drought Resistant II [DRII])	Susceptible	Cluster 3

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