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Assessment of genetic diversity among African cassava *Manihot esculenta* Grantz accessions resistant to the cassava mosaic virus disease using SSR markers

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Abstract

A study was conducted to determine the extent of genetic diversity among African cassava (*Manihot esculenta* Crantz) accessions resistant to the cassava mosaic virus disease (CMD), using simple sequence repeat (SSR) markers. The accessions included a breeding stock (clone 58308), five improved lines, 62 CMD resistant and 10 CMD susceptible landraces. Genetic diversity was assessed among accessions in five cluster groups derived from UPGMA analysis on data from 18 SSR primer pairs. Average gene diversity, He, was high in all cluster groups, with an average heterozygosity of 0.591 ± 0.061. The estimator of inbreeding Fis revealed a low level of inbreeding within groups and averaged −0.262 ± 0.142. Gene diversity among all accessions was 51.4% and gene diversity within cluster groups was 46.6%, while 4.8% was due to diversity between the different cluster groups. The amount of genetic differentiation measured by Gst and Fst were 9.6% and 12.1% respectively, indicating a weak genetic structure.

Introduction

The cassava mosaic virus disease (CMD) is an important constraint to cassava production in Africa. It is caused by any one or a combination of the white-fly-transmitted cassava mosaic geminiviruses, the African cassava mosaic virus (ACMV), the East African cassava mosaic virus (EACMV), the Ugandan variant of EACMV (UgV) and the South African cassava mosaic virus (SACMV) which exist on the continent. The disease is widespread in the cassava growing belt of Africa (Figure 1). The most severe effects of the disease is associated with UgV and in situations where mixed infection by ACMV and EACMV occur (Ogbe et al. 2003). In tackling the early disease epidemics of CMD in Africa, resistance was obtained from a cross between cassava and its wild relative *Manihot glaziovii* Müll. Arg (Nichols 1947). The clone 58308, which was selected from this hybridisation process, has been used as the main source of resistance in breeding programs across Africa for several decades. CMD, however, still remains a major economic constraint in Africa, causing severe yield losses. Estimated annual yield losses between 1992 and 1997 in Uganda, amounted to US $ 60 million (Otim-Nape et al. 1997), and on the continent over 2.2 billion tonnes

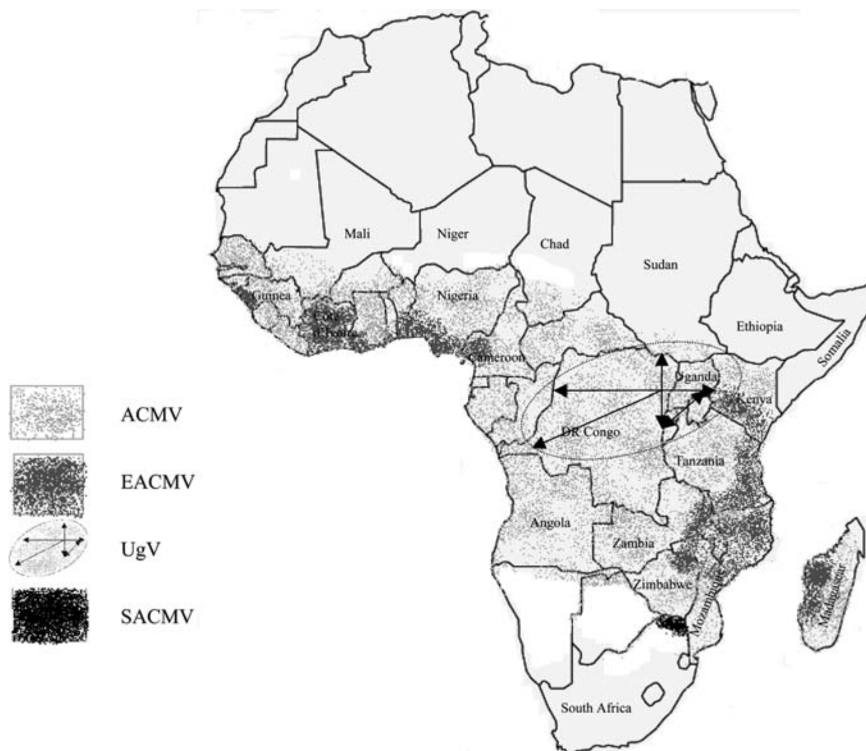

Figure 1. Map of Africa showing distribution of cassava mosaic gemini viruses causing CMD in cassava belt (adapted from Atiri et al. 2004 and modified with information from Legg and Fauquet 2004).

of storage root, at a cost of US $ 440 million is lost annually (Thresh et al. 1997).

Extensive use of closely related cultivars could result in vulnerability to pests and diseases. The use of diverse parental combinations in breeding may provide a greater supply of allelic variation that can be used to create new favourable gene combinations and increase in the levels of genetic variation, heterosis with marked increase in transgressive segregants in a population (Cowen and Frey 1987; Van Esbroeck and Bowman 1998). Durable resistance to CMD that would be difficult for the pathogens to circumvent in the long term is an important breeding objective to ensure the sustainability of cassava production in Africa. Magoon and Krishnan (1977) showed that for maximum efficiency and reliability of breeding programmes in cassava, parents should be genetically diverse.

Cassava is generally propagated with stem cuttings thereby maintaining the genetic constitution of the genotype. Nevertheless, in farmer's fields, a high level of inter and intra-varietal diversity is also known to exist due to farmer's occasional use of spontaneous seedlings from natural hybridisation, for subsequent planting and the exchange of material between farmers. These new genotypes, referred to as landraces, form part of the crop's genetic resources (Gulick et al. 1983; Hershey 1987), and provide a wealth of new genes for various traits including resistance to CMD (Hahn et al. 1977). Landraces have been considered as valuable initial material for plant breeding because they contain co-adapted gene complexes with tolerance and adaptation to diseases and specific ecological conditions respectively (Harlan 1975).

Relationships among genotypes can be assessed based on information of the geographic origin of genotypes, pedigree information, and information about plant characteristics. Geographic information is specifically useful when other information on the genotypes is either not available or is very sparse, while pedigree information may sometimes not be available or consist of erroneous or inadequate historical records (Ajmone-Marsan et al. 1992; Schut et al. 1997).

Pedigree information on African cassava landraces is not easily available. Furthermore, farmers are known to exchange and distribute material among themselves, and different ethnic groups assign different vernacular names to similar varieties or similar names to different varieties (Mignouna et al. 1998). This result in duplication of collected genotypes and makes the information on the geographic origin unreliable in assessing relationships.

Plant characteristics, which can be made available for any set of genotypes, to assess relationship among the genotypes (Schut et al. 1997), include agronomic and morphological characters (phenotypic), biochemical characters (e.g. storage proteins, isozymes) and molecular (DNA) markers. They are useful in studies of landraces and have been used to characterise genetic diversity in cassava (Rogers and Fleming 1973; Lefevre and Charrier 1993; Haysom et al. 1994). Molecular markers and biochemical markers provide a means to accurately estimate the genetic diversity and genetic structure of a species (Hamrick and Godt 1997). Genetic diversity studies among some cultivated cassava accessions have previously been studied using RFLPs (Beeching et al. 1993), RAPDs (Marmey et al. 1994; Mignouna and Dixon 1997; Asante and Offei 2003) and AFLP markers (Sanchez et al. 1999; Fregene et al. 2000).

Comparative studies in crop plants have shown that simple sequence repeat (SSR) or microsatellite markers, which are single locus markers with multiple alleles, are more variable than most other markers and provide an effective means for discriminating between genotypes (Morgante and Olivieri 1993; Powell et al. 1996). Recently, SSR loci have been developed into PCR based markers for cassava (Chavarriaga-Aguirre et al. 1998; Mba et al. 2001) and are presently the preferred markers because of their simplicity and production of multiple alleles, which enable efficient detection of polymorphism among accessions. Chavarriaga-Aguirre et al. (1998) identified 14 GA repeats in cassava, and used four of these to evaluate the genetic diversity of the cassava core collection at the Centro Internacional de Agricultura Tropical (CIAT, Colombia) (Chavarriaga-Aguirre et al. 1999). The results revealed high levels of heterozygosity, putative duplicates, and an unequal representation of cassava diversity, by country, in the core collection. SSRs have also been used to elucidate the genetic diversity, structure and differentiation in the crop's primary and secondary centres of diversity (Fregene et al. 2003), demonstrate the degree of relatedness between cassava and five of its relatives (Roa et al. 2000) and analyse the variation in natural populations of putative progenitors of cassava (Olsen and Schaal 2001).

To ensure that durable resistance is maintained within the African cassava germplasm there is a need to increase the levels of resistance within the genepool using additional sources of resistance with a wider genetic base. Thus, this study was conducted to determine the extent of genetic diversity among CMD resistant cassava accessions and their relationship with susceptible accessions using SSRs, and further extend the information to predict sources of resistance to CMD, for breeding of resistance to and management of CMD.

Materials and methods

Genetic Material

The cassava accessions for the study were selected from a collection of over 1235 landraces and 388 improved accessions, of the cassava breeding programme at the International Institute of Tropical Agriculture (IITA). These accessions have been grown in Ibadan and Ubiaja, Nigeria over several years and characterised for their CMD status over the years. Sixty-eight resistant accessions with mean CMD severity scores of 1 (no obvious symptoms) and 2 (mild chlorotic patterns on leaves or mild leaf distortion at the base of the leaves), including the main resistant genetic stock, clone 58308, five improved accessions and 62 resistant landraces were selected. Ten susceptible landraces with mean CMD severity scores of 4 (severe mosaic distortion, reductions of leaf lamina with about 2/3 of the plant affected) or 5 (severe mosaic, severe distortion of leaves, stunting of entire plant and about 4/5 of leaves affected) were also included (Table 1). Cuttings were made from mature plants, planted in nursery beds and watered twice a week to produce young leaves for DNA extraction.

Table 1. List of cassava accessions their pedigree/local name (or assigned code by country collectors), country of origin CMD status (R = resistant, S = susceptible) and assigned cluster group of genetic similarity based on UPGMA and PCA.

Accession	Pedigree/local name	Country/origin	CMD status	Cluster group	
				UPGMA	PCA
91/02324	TME1 OP	IITA	R	1	I
TME209	1254(880887)	Cameroon	R	1	I
58308	*M.esculenta* × *M. glaziovii*	IITA	R	1	III
TME258	25	Ghana	R	1	III
TME429	MAIN 4	Togo	R	1	III
TME431	MAIN 11	Togo	R	1	III
TME526	Ka13 (Kenya Ostrom)	Côte d'Ivoire	R	1	III
TME557	Lossakpleh	Côte d'Ivoire	S	1	III
TMS30572	58308 × Branca de Santa Caterina OP	IITA	R	1	III
M94/0583	9193 × Atu (TME4)	IITA	R	2	I
TME1	Antiota	Nigeria	R	2	I
TME12	Tokunbo	Nigeria	R	2	I
TME13	MS-20	Nigeria	R	2	I
TME240	Toma 75	Togo	R	2	I
TME242	Toma 76	Togo	R	2	I
TME279	Obasanjo	Nigeria	R	2	I
TME282	Alice Local	Nigeria	R	2	I
TME287	Power	Nigeria	R	2	I
TME288	Akano	Nigeria	R	2	I
TME3	2ND Agric	Nigeria	R	2	I
TME379	Ofegbe	Nigeria	R	2	I
TME4	Atu	Nigeria	R	2	I
TME435	RB92/0175	Benin	R	2	I
TME443	CAP94090	Benin	R	2	I
TME446	RB92/0204	Benin	R	2	I
TME449	RB92/0182	Benin	R	2	I
TME451	CAP94067	Benin	R	2	I
TME498	R.A 16	Nigeria	R	2	I
TME5	Bagi Wawa	Nigeria	R	2	I
TME581	Oke Local	Nigeria	R	2	I
TME6	Lapai-1	Nigeria	R	2	I
TME62	Yau Rogor	Nigeria	R	2	I
TME7	Oko-Iyawo	Nigeria	R	2	I
TME60	Darazo Rogor	Nigeria	S	2	II
TME228	Toma 97	Togo	R	2	III
TME455	RB92/0116	Benin	R	2	IV
TME278	Oko Warangbala	Nigeria	R	2	V
TME461	RB92/0188	Benin	R	3	I
TME11	Igueeba	Nigeria	R	3	II
TME241	Toma 136	Togo	R	3	II
TME631	SE 210	Ghana	R	3	II
TME8	Amala	Nigeria	R	3	II
TME9	Olekanga	Nigeria	R	3	II
TME199	RB89/59	Benin	R	3	III
TME204	RB98/0113	Benin	R	3	III
TME243	Toma 26	Togo	R	3	III
TME419	Gbazekoute	Togo	R	3	III
TME456	CAP94062	Benin	R	3	III
TME477	RB92/0104	Benin	R	3	III
TME479	Agric	Benin	R	3	III
TME480	RB92/0119	Benin	R	3	III

Table 1. Continued.

Accession	Pedigree/local name	Country/origin	CMD status	Cluster group	
				UPGMA	PCA
TME565	Prescose de Angola (ANG-4)	Angola	R	3	III
TMS30001	Lost Pedigree information	IITA	R	3	III
TME225	92/0099	Togo	R	3	IV
TME470	CAP94066	Benin	R	3	IV
TME104	Rogor-5	Nigeria	S	3	VI
TME478	RB92/0123	Benin	R	3	VI
TME568	Mundele Paco (ANG-3)	Angola	R	4	II
TME630	Amin	Ghana	R	4	II
TME635	MNN 55	Ghana	R	4	II
TME382	Suleja-5(92/0163)	Nigeria	S	4	III
TME107	Danwara	Nigeria	S	4	VI
TME117	Isunikankiyan	Nigeria	S	4	VI
TME123	Panya	Nigeria	S	4	VI
TME218	881260(882160)	Cameroon	S	4	VI
TME401	Toma 141	Togo	S	4	VI
TME546	SS4 (T8)	Uganda	R	4	VI
TME59	Dandualla-2	Nigeria	S	4	VI
TME638	EJ 79	Ghana	R	4	VI
M94/0121	9000137 S2 (SM)	IITA	R	5	III
TME230	Toma 36	Togo	R	5	III
TME437	RB92/0103	Benin	R	5	III
TME474	CAP94/064	Benin	R	5	III
TME229	RB92/0130	Benin	R	5	IV
TME236	Toma 37	Togo	R	5	IV
TME232	Toma 63	Togo	R	5	V
TME434	RB92/0155	Benin	R	5	V
TME572	Udoh Local	Nigeria	R	5	VI

DNA extraction and microsatellite analysis

About 3–5 g young leaves per accession were harvested for extraction of genomic DNA following the Dellaporta procedure (Dellaporta et al. 1983). The DNA obtained was stored in absolute ethanol and transported to the CIAT, where the analyses were carried out. The DNA samples were precipitated, and quantified using a fluorometer (TKO, 100 Hoefer) then standardised to 10 ng/μL working concentrations.

Thirty-six highly polymorphic SSR markers, which are widely distributed in the cassava genomic map and described by Chavarriaga-Aguirre et al. (1998) and Mba et al. (2001), were used in genotyping the DNA samples. Each 25 μL PCR reaction contained 25 ng genomic DNA, 0.2 μM of each forward and reverse primers, 10 mM Tris–HCl (pH 7.2), 50 mM KCl, 1.5 mM MgCl, 0.2 mM of each dNTP and 1 U *Taq* DNA polymerase. The PCR profile involved an initial denaturation for 5 min at 94 °C then 30 cycles at 94 °C for 60 s, 55 °C for 2 min, 72 °C for 2 min and a final extension at 72 °C for 5 min. About 3.5 μL of the PCR product was denatured and electrophoresed on a 4% polyacrylamide sequencing gels for 2 h at 100 W, and DNA was visualised by silver staining according to Promega's silver staining protocol.

Estimation of genetic diversity

SSR markers, which resulted in complex patterns (i.e. more than 2 alleles) were excluded to maintain a strict di-allelic model of inheritance (Fregene et al. 2003). Eighteen markers that gave distinct di-allelic patterns were chosen for gene diversity analysis (Table 3). To determine the relationship among accessions based on a hierarchical cluster analysis, the alleles were recorded as bands and scored as 1 or 0 for present and absent respec-

tively. The data in this form were used to calculate genetic distances between pairs of cassava accessions from comparisons of the band scores. Then using the unweighted pair-group mean average (UPGMA) cluster method of Nei's genetic distances (Sneath and Sokal 1973), a dendrogram of genetic similarity was generated. The genetic distances and dendrogram were computed with the NTSYS-PC computer programme, version. 2.02 (Rohlf 1997).

To estimate genetic diversity among the accessions, SSR loci were recorded as diploids with single bands taken to indicate the presence of two identical alleles. Genetic diversity among accessions partitioned into the cluster groups generated from the genetic similarity analysis was estimated using the software package GEN-SURVEY (Vekemans and Lefebvre 1997). Genetic diversity was estimated using five statistics averaged over loci; the percentage of polymorphic loci (P); the mean number of alleles per locus or allelic richness (A); the average observed heterozygosity (Ho); the average gene diversity (He); and average inbreeding coefficient (Fis), were computed, according to Nei (1978). For all loci and for all accessions the total heterozygosity, (Ht), and the proportion of among accession differentiation (Gst) were estimated, according to Nei (1978). Total heterozygosity within cluster groups (Hs) and between cluster groups (Dst), corrected for small samples (Nei and Chesser 1983) was also estimated. Standard deviations for each locus were estimated by jack-knifing over loci using 200 bootstraps (Weir 1990).

Genetic differentiation was quantified with the F statistic (Fst) described by Weir and Cockerham (1984) using FSTAT 2.9 (Goudet 2000). Fst gives the same estimate of genetic differentiation as Gst, but takes into account variation in sample sizes, as is the case in this study. The statistic was estimated per allele, per locus and overall. Confidence intervals were calculated per locus over samples, and over loci by jack-knifing, and the overall by bootstrapping 1000 times over loci, which provides a rigorous test of hypothesis for genetic differentiation.

Pairwise genetic distances between pairs of accessions in the population using Nei's distance corrected for small sample sizes (Nei 1978), generated by GEN-SURVEY, were subjected to principal component analysis (PCA) using the statistical analysis software (SAS) package (SAS 1999) to deduce multivariate relationships among the cassava genotypes. The first and second principal components were employed to reveal the multivariate relatedness of accessions in a three dimensional scatter plot.

Results

Genetic relationships amongst cassava accessions

The dendrogram of genetic similarity among accessions, with the coefficient of genetic similarity ranging from 0 to 0.50, is presented in Figure 2. At 0.39 similarity, the 78 accessions clustered into 4 main groups. The first group had nine members, including the resistant genetic stock 58308 and its progeny TMS30572, the improved accession 91/02324, and four resistant and one susceptible landraces. The next group, which was the largest, was made up of two improved accessions, M94/0583 at the extreme top of the group, followed by 47 accessions, including improved TMS30001 and two susceptible landraces (TME60 and TME104) and resistant landrace TME456 at the end of the group. All the resistant landraces from the Republic of Benin and the majority of resistant landraces from Nigeria and Togo were in this group. The group also included a resistant landrace from Angola and one from Ghana. Closer examination of the dendrogram revealed that this group could be further divided into two groups, making five genetic similarity groups in all. Thus, the second group of accessions from the top of the dendrogram was from M94/0583 to TME498 and the third group was from TME479 to TME456, including of the improved accession TMS30001, 17 resistant and one susceptible landraces. Group four, from TME638 to TME546, consisted of seven susceptible and five resistant landraces. The last group, group five, was a loose association between improved accession M94/0121 and eight other resistant landraces.

The dendrogram further revealed strong genetic similarity between the Nigerian landraces TME581 and TME12, between TME5 and TME3, between TME62, TME6 and TME4, between accessions TME242 and TME240 from Togo, and between TME479 and TME470 from Benin. Strong similarities were also detected between TME435 from Benin and TME288 from Nigeria, and between TME480 from Benin and TME225 from Togo.

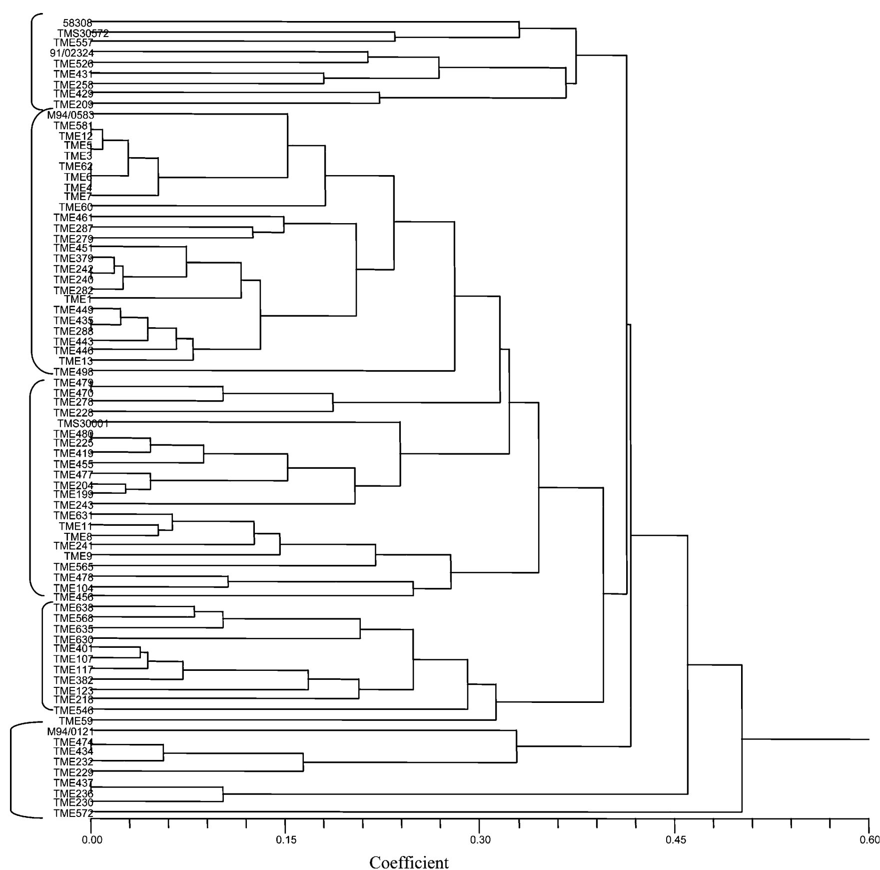

Figure 2. Dendrogram of Genetic distance showing the association between CMD resistant cassava accessions and a reference set of susceptible accessions based on SSR markers using UPGMA cluster analysis.

The first three principal components computed from the genetic distance matrix accounted for 27.17, 19.84 and 10.20% of the total variation respectively. The PCA scatter plot which gives the spatial representations of genetic distances among accessions, revealed six clusters groups along the second principal component axis (Figure 3). The first group on one extreme was made up entirely of 27 CMD resistant accessions, the second group comprised eight resistant and one susceptible accession, group III had 21 resistant and two susceptible, groups IV and V were made up of resistant accessions, while group VI on the other extreme of the axis was a loose association between four resistant and seven susceptible accessions. Within the first group, the first principal component axis assigned positive values to the accessions while the third axis assigned positive and negative values. Accessions in group II, were assigned positive values by both the first and third principal axes, while group IV and V were assigned positive values by the first principal component axis and negative values by the third principal axis.

Generally the PCA scatter plot, detected trends similar to the hierarchical clustering illustrated in the dendrogram. For instance, with the exception of TME228, TME278, and TME453, all CMD resistant accessions in group two of the dendrogram clustered in group I of the PCA. Resistant accessions in group II of the PCA scatter plot, were in either groups three or four of the dendrogram, while resistant accessions in group III of the scatter plot were in either groups one, three or five of the dendrogram. Furthermore, the two close associations between TME240 and TME242 and between TME4 and TME62 revealed by the dendrogram were also detected in cluster group I of the PCA, which suggest these could be duplicates in the collection.

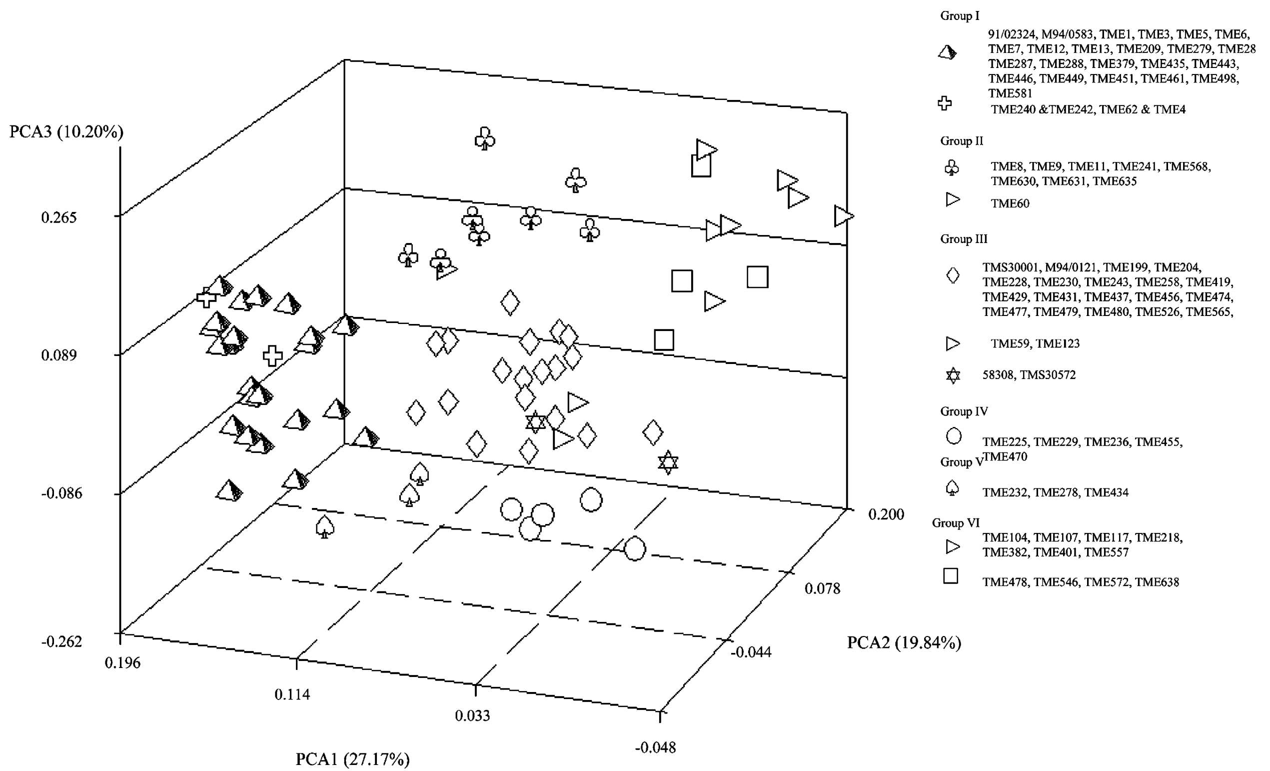

Figure 3. Scatter plot of principal component analysis of CMD resistant cassava accessions and a reference set of CMD susceptible accessions.

Diversity among cassava accessions of different cluster groups

The statistics of genetic diversity among the accessions in the five clusters are given in Table 2. High genetic polymorphism was observed across loci for each cluster group. On average, 95.56% of the loci were polymorphic, the average number of loci detected was 2.68 and the average number of polymorphic loci was 2.73. The highest proportion of heterozygotes was detected in the second and third cluster groups and the average proportion of heterozygotes within accessions was 0.59. Average gene diversity within accessions in cluster groups,

Table 2. Gene diversity analysis among cassava accessions by cluster group.

Cluster Group	P[a]	A[b]	A_P[c]	H_o[d]	He[e]	Hec[f]	Fis[g]
Cluster Group 1	88.9	2.7	2.9	0.498	0.416	0.444	−0.124
Cluster Group 2	94.4	2.6	2.6	0.643	0.437	0.446	−0.437
Cluster Group 3	100	2.8	2.8	0.645	0.483	0.501	−0.289
Cluster Group 4	94.4	2.7	2.7	0.603	0.438	0.460	−0.351
Cluster Group 5	100	2.7	2.7	0.566	0.462	0.519	−0.120
Mean	95.56 ± 4.65	2.68 ± 0.06	2.73 ± 0.09	0.591 ± 0.061	0.447 ± 0.026	0.474 ± 0.034	−0.264 ± 0.142

[a]Percentage of polymorphic loci at the 5% level among accessions
[b]Mean number of alleles per locus within accessions.
[c]Mean number of polymorphic alleles per locus within accessions.
[d]Observed heterozygosity.
[e]Expected heterozygosity under Hardy&Weinberg conditions.
[f]Expected heterozygosity corrected for small sample sizes.
[g]Weighted average of the inbreeding coefficient over all alleles with correction for small sample sizes.

which is an estimate of the probability that any two randomly sampled alleles in a given accession were different, was 0.47. Gene diversity was highest among the accessions in groups three and five. The inbreeding coefficient (Fis) ranged from −0.437 for group two to −0.120 for cluster group five and averaging −0.264 over all five groups.

Genetic diversity and genetic differentiation

Gene diversity at each locus ranged from 0.242 to 0.748 in the population (Table 3). The overall gene diversity (Ht) in the population was 0.51, of which 0.47 was due to the differentiation among accessions within cluster groups (Hs), and this ranged from 0.205 to 0.707 across loci. The difference in gene diversity between the cluster groups (Dst) ranged from 0.006 to 0.167 and accounted for 0.05 of the total gene diversity. Gene differentiation (Gst) averaged 0.096 and ranged from 0.019 to 0.368. Gene differentiation estimated with the *F*-statistic, Fst = 0.121 ± 0.020, further illustrates the low level of inbreeding.

Discussion

The genetic differences between the CMD resistant accessions revealed by their clustering into distinct groups suggests the presence of alternative sources of resistance to CMD other than the *Manihot glaziovii* source of resistance in clone 58308. Based on the hierarchal UPGMA analysis, we identified five groups of CMD resistant accessions, while the PCA, revealed three large CMD resistant groups and three smaller groups with the accessions loosely associated with each other in these groups. The genetic association among some accessions are similar to what has been reported elsewhere. For instance, Fregene et al. (2000) reported the association between 58308 and TMS30572, and identified TME4 and TME6 as possible duplicates, while Raji (2002)

Table 3. Gene diversity analysis among cassava accessions and F-statistic estimated by bootstraps over loci.

Locus	Ht	Hs	Dst	Gst	Fst
SSRY21	0.268	0.251	0.017	0.065	0.022 ± 0.058
SSRY63	0.665	0.584	0.082	0.123	0.226 ± 0.083
SSRY181	0.425	0.404	0.022	0.051	0.125 ± 0.091
SSRY51	0.651	0.547	0.104	0.160	0.165 ± 0.142
SSRY180	0.571	0.500	0.071	0.124	0.208 ± 0.061
SSRY135	0.542	0.455	0.087	0.161	0.165 ± 0.086
SSRY12	0.495	0.442	0.053	0.107	0.153 ± 0.090
SSRY169	0.386	0.369	0.017	0.044	0.039 ± 0.037
SSRY144	0.503	0.478	0.025	0.049	0.178 ± 0.137
SSRY155	0.466	0.460	0.006	0.013	0.025 ± 0.031
SSRY79	0.545	0.501	0.043	0.080	0.080 ± 0.055
SSRY19	0.445	0.415	0.030	0.068	0.119 ± 0.065
SSRY34	0.242	0.205	0.037	0.152	0.200 ± 0.132
SSRY175	0.685	0.672	0.013	0.019	0.016 ± 0.021
SSRY61	0.501	0.492	0.010	0.019	0.030 ± 0.032
SSRY59	0.453	0.287	0.167	0.368	0.500 ± 0.403
SSRY82	0.748	0.707	0.041	0.054	0.045 ± 0.029
SSRY69	0.670	0.622	0.048	0.072	0.126 ± 0.068
Mean	0.514	0.466	0.048	0.096	0.121 ± 0.0204
std	0.138	0.135	0.041	0.083	
95% CI	0.446	0.404	0.032	0.062	
95% CI	0.583	0.533	0.065	0.135	

Ht Diversity in total set cluster groups.
Hs Diversity within cluster groups.
Dst Diversity between cluster groups.
Gst The coefficient of gene differentiation.
Fst Fixation index for each microsatellite locus.
Mean values are given ± standard deviation.

also observed genetic similarities between TME9, TME11 and TME241 based on SSR, AFLP and RAPD markers. Generally, the relationship between accessions in the cluster groups could not be attributed to their origin alone in most cases. However, the genetic stock 58308 and its progeny TMS30572, TME4 and its progeny M94/0583 clustered in the same groups for both plots, while TME1 and its progeny 91/02,324 clustered in group I of the scatter plot, which reflects their similarity in genetic base.

The PCA analysis further provides information about associations of accessions, which are useful to formulate better strategies for breeding. The absence of strong associations among the accessions in the groups implies significant diversity within each cluster group, and the dominant independent role in the cluster groups along each separate principal component implies significant diversity between the groups. It is therefore envisaged that, combining accessions from the different groups as parents in breeding would result in diversifying CMD resistance genes in the breeding population.

The inability of the markers to distinguish between TME240 and TME242 from Togo and TME4 and TME62 from Nigeria based on both the hierarchical and PCA clustering, is an indication that these are duplicates in the collection. Detection of duplicates in a collection is critical to effective management of germplasm. The predominance of accessions from Benin, Nigeria and Togo in the larger cluster groups for both the hierarchical and PCA cluster plots is a reflection of the similarities in cultural practices with respect to cassava cultivation in these neighbouring countries. The collaborative study of cassava in Africa (COSCA) showed that the most preferred characters for the selection of specific cultivars by farmers are early bulking, and high storage root yield (Nweke et al. 1994). A common food product of cassava in West Africa is gari, which requires cassava with these two characters. It is possible that whilst selecting, utilising and distributing landraces with their preferred agronomic and quality traits, farmers have inadvertently added useful CMD resistant accessions to the germplasm available to them. Another important trait preferred by the farmers is the cooking quality of the variety (Annor-Frempong 1992; Otoo et al. 1995). This could explain the associations between the resistant and susceptible accessions; two peculiar cases being the two accessions from Côte d'Ivoire, TME526 and TME557, which clustered together in both plots despite their different CMD resistance status, and TME638 from Ghana and TME546 from Uganda, which clustered with the majority of susceptible accessions in both plots.

Gene diversity (p = 95%, He = 0.45) detected in this study, is higher than the average reported for outcrossing species (p = 64%, He = 0.205) using isozyme markers (Hamrick and Godt 1997). It has been suggested that high levels of polymorphism in microsatellite markers are related to the mechanism of mutations and the high rate at which they occur (Ashley and Dow 1994). High heterozygosity among accessions in the cluster groups was further evident from the negative values of the inbreeding coefficient (Fis. = −0.264), despite the common ancestry between 58308 and TMS30572, between TME1 and 91/02324 and between TME4 and M94/0583.

The estimates of genetic differentiation among the accessions (Gst = 0.10 Fst = 0.12), are lower than the average reported for crop species (Gst = 0.34) and also for the average of 64 outcrossing species (Gst = 0.234, Hamrick and Godt 1997). A recent study on African and South American cassava accessions also revealed similar low levels of genetic differentiation (Gst = 0.11, Fst = 0.09) (Fregene et al. 2003). These results imply that the evolution of CMD resistance accessions in cassava is a result of random genetic drift. Although cassava is generally vegetatively propagated, there is evidence of continuous exchange of genetic material among farmers in Africa. African farmers are also known to use cuttings from spontaneous seedlings for their subsequent planting, which has given rise to a wide range of genetic diversity in the region (Lefevre and Charrier 1993; Nweke et al. 1994).

The implications of this study are of direct relevance to management and control of the spread of CMD, and in breeding for resistance. Recent surveys conducted in Africa between 1998 and 2003 show that severe forms of the disease due to *EACMV-UgV* has graduated from epidemic to pandemic proportions in Uganda and has spread to neighbouring countries such as Sudan, Rwanda, Kenya, Democratic Republic of Congo, Congo Brazzaville and Gabon (Legg and Fauquet 2004). Furthermore, the characteristic severe symptoms

associated with mixed infections of ACMV and EACM identified in some West African countries (Offei et al. 1999; Ogbe et al. 1999), emphasizes the need to intensify efforts in managing the disease. In managing the spread of the disease, cultivating resistant genotypes has been demonstrated to be the most effective means of controlling CMD (Cours-Darne 1968; Otim-Nape et al. 1994; Thresh et al. 1994) as well as being an environmentally sound and sustainable means of managing the spread of the disease. As part of its breeding programme, IITA has distributed seeds of CMD resistant genotypes developed from their breeding population and *in vitro* virus-free clones of their seedlings, to over 30 national programmes in Africa for evaluation and selection under specific agroecologies (Mahungu et al. 1994). Improved CMD resistance cassava accessions TMS30572, TMS60,142 and TMS30337 for example were successfully adopted in Uganda, following the involvement of farmers in multilocational trials (Otim-Nape et al. 1994), and in many other African countries accessions such as TMS30001, TMS30337, TMS30572, TMS4(2)1425, TMS60142 and TMS91934 have been adopted by farmers (Legg and Fauquet 2004). In Southern Nigeria, resistant cassava landraces are usually interplanted with improved resistant types to reduce the rate of spread of the disease (Ogbe et al. 2001). These experiences could be extended to dissemination of these resistant landraces to CMD endemic regions, to control the spread and incidence of the disease, at the same time providing farmers with a source of variable agronomic and consumer qualities.

The large amount of diversity among both the landraces and improved accessions can be utilised to develop durable resistance in the breeding population. Recurrent selection from source populations for target agroecologies and desirable traits including CMD resistance is the breeding strategy used in cassava breeding in Africa. Incorporating the CMD resistant accessions which have other desirable agronomic and consumer quality traits, from the different cluster groups into the breeding programmes as parents, would ensure the diversification of resistance to the disease while creating new genotypes. In addition, by combining different genes that relate to different sources of resistance, epistatic interaction may be identified such that higher levels of resistance can be developed to protect the crop.

In conclusion, this study has shown that despite the damaging effects of the disease on the crop and the exclusive use of resistance from 58308 for several years, there is a significant amount of genetic diversity among the CMD resistant accessions, which could be utilised in breeding to diversify resistance to the disease. The use of spontaneous seedlings as planting material and the frequent exchange of material among farmers may have compensated for any loss of diversity, which may have arisen due to biotic agents in the crop.

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