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AFLP analysis of African cassava (*Manihot esculenta* Crantz) germplasm resistant to the cassava mosaic disease (CMD)

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Abstract Amplified fragment length polymophism was assessed in 20 land races and nine elite lines of cassava from Africa, resistant and susceptible to the cassava mosaic disease (CMD). Eleven accessions from a representative core collection from Latin America, previously studied by AFLPs, were included as a reference. AFLP data from all accessions was analyzed by both the unweighted pair group mean average (UPGMA) and multiple cluster analysis (MCA) methods of analysis. Genetic differentiation between clusters and the coefficient of genetic differentiation was also calculated. Results reveal a genetic divergence between African and Latin American accessions, although some overlap was found between them. African land races resistant to CMD, were also found to be genetically differentiated from susceptible land races and from resistant elite lines. AFLP analysis identified a considerable number of duplicates in the African accessions, suggesting a sizeable percentage of redundancy. A unique AFLP fragment, found in a relatively high frequency in African accessions, but absent in the Latin American accessions, was found to be associated with branching pattern by QTL mapping in an F1 progeny derived from African and Latin American parents. The likely source and the utility of the unique AFLP fragment in understanding the processes of genetic divergence in Africa is discussed.

Key words Cassava · AFLP · Genetic distances · QTL

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Introduction

Cassava (*Manihot esculenta* Crantz) is the favored root and tuber crop of the tropics (Nweke 1994; Henry 1995). An important staple, food security, and cash crop, cassava thrives where most other crops fail. The storage roots provide more dietary energy per hectare and working hours than any other staple crop, making it the mainstay of small holders in the tropics with limited access to agricultural inputs (Nweke 1994). The leaves are edible and are a rich source of proteins, vitamin C and other nutrients.

Native to South America, cassava was first introduced from Brazil to West Africa by Portugese traders in the 1500s. An independent introduction was made to East Africa in the 1700 s also by the Portugese. It spread to Central Africa along trade routes from the Congo basin (Jones 1969). Natural cross pollination and selection by farmers is most probably responsible for the large number of morphologically distinct local varieties found today in Africa (Beck 1982). Cassava is a monoecious perennial crop with pistillate flowers maturing before staminate flowers (protogny), making outcrossing the predominant mode of sexual reproduction, and ensuring considerable genetic diversity. Africa accounts for half the world's cassava production (FAO 1991).

The cassava mosaic disease (CMD) a viral disease, transmitted by a white fly (*Bemisia Tabaci*) vector, is the leading constraint of cassava production in Africa, where yield losses can be as high as 100% (Jennings 1976). For example, the Ugandan epidemic of CMD, allegedly caused by a recombinant strain (Harrison et al. 1997), has wiped out cassava production in a large part of the country, with the attendant loss of income for small-scale, resource-poor farmers, and useful cassava germplasm (Otim-Nape et. al. 1997). The cassava mosaic disease (CMD) is caused by a geminivirus, the African cassava mosaic virus (ACMV) consisting of two circular DNA molecules of similar size (2.7 kb) (Bock and Harrison 1985). Four strains of ACMV are reported in the literature: the West Africa-type strain, the Kenya coastal

(C) strain; the Indian strain and the defective strain from Angola (Bock and Harrison 1985; Fauquet and Fargette 1990). Neither the virus, nor its vector (*Bemisia Tabaci*), are known to-date in Latin America.

Combating CMD epidemics has been principally by resistant cassava germplasm. Pioneering work on CMD resistance started in East Africa in 1935 (Storey and Nichols 1938), and gave rise to currently deployed resistance in most of sub-Saharan Africa. Resistance was obtained by interspecific crosses between cassava cultivars, of diverse origins, and accessions of a wild relative, *Manihot glaziovii*. Each cross was followed by three backcrosses to *M. esculenta* to recover desirable root characteristics. Storey's third backcross derivatives have remained the major source of deployed resistance against Eastern and Western strains of ACMV in sub-Saharan Africa. Resistance has been described as being stable and largely additive in nature, with a heritability of about 60% (Hahn and Howland 1972). But Hahn et. al. (1980) later stated that resistance to CMD is recessive. Recently, the International Institute of Tropical Agriculture (IITA) has identified several Nigerian landraces with a high level of resistance to the West African strain of the virus, providing a new source of resistance.

Disease resistance breeding is a considerably slow and cumbersome process, owing to the biological constraints of a heterozygous, vegetatively propagated crop. Considering the time and resources required for developing resistant varieties, it is important to develop varieties carrying as many different genes for resistance as possible. Identifying and pyramiding different disease resistance genes will provide stable resistance against a broad spectrum of the virus, due to the evolution of the virus and/or accidental introduction of infected cassava germplasm. The pyramiding of genes, known only by indistinguishable phenotypes, requires molecular-marker-aided breeding, which itself has to be preceded by gene-tagging studies of resistance genes. The availability of a genetic map of cassava (Fregene et. al. 1997), has made it possible to quickly identify genes responsible for different sources of resistance by marker-assisted genetic analysis of simple pedigreed populations.

As a first step to developing gene-tagging populations, we applied the versatile AFLP technique (Vos et. al. 1995) to a collection of resistant and susceptible African cassava accessions, consisting of land races and improved varieties, to determine possible different sources of resistance to the cassava mosaic disease (CMD). A second objective was to study the genetic structure of the African cassava germplasm collection resistant to CMD. It has been suggested that exisiting genetic variation in African cassava germplasm is structured in response to selection for adaptation to the irreducible uncertainties of biotic and abiotic stresses, declining soil nutrients, agronomic practices, and post-harvest utilization of cassava (Gullberg 1998; Jiggins, personal communication). A small number of Latin American accessions were included; these lines have earlier been analyzed with AFLP markers (Roa et al. 1997). AFLP markers were employed in that study to obtain a quantitative estimate of genetic similarity in a representative sample of the crop's diversity and six wild taxa (Roa et al. 1997).

Materials and Methods

Plant materials

Forty cassava accessions, made up of 20 land races and nine improved, African cassava varieties, resistant or susceptible to the cassava mosaic disease (CMD), and 11 accessions from the cassava core collection at CIAT, Cali, Colombia, were selected for AFLP analysis (Table 1). The South American lines had previously been analyzed by Roa et al. (1997), and served as reference, for scoring AFLP bands. The full AFLP data-set of Roa et al. (1997), analyzed with the same primer combinations was also available for comparison. CMD resistance was available for the African accessions (Table 1). Resistance is measured on a disease damage scale of 1–5, where 1 indicates no symptoms, equivalent to immunity, and 5 indicates severe infection, distorted leaves and stunted growth. Cassava breeders consider anything higher than 3 as susceptible.

DNA extraction and AFLP analysis

Extraction of DNA from all the acccessions was according to Dellaporta (1983) while the AFLP analysis was according to Vos et al. (1995) as modified by Tohme et al. (1996). The AFLP oligonucleotide pre-amplification primers were the corresponding EcoRI core and enzyme sequences plus one selective nucleotide, dATP, (*Eco*RI+A) and the corresponding *Mse*I core and enzyme sequences plus dGTP (*Mse*I+G). Two +3 selective oligonucleotide primer pairs were employed in the second round of selective amplifications: *Eco*RI+ AAC/*Mse*I+GTA and *Eco*RI+ACA/*Mse*I+GTA. The pre-selective and the selective primers described were chosen based on an earlier screening for polymorphism between cultivated cassava and some wild *Manihot* species (Roa et al. 1997). The *Eco*RI +3 selective primers were labelled with [^{32}P] dATP and the radioactive amplified products were size-fractionated on 6% polyacrylamide denaturing gels in 1 × TBE at 40 V/cm 45°C for 2 h. After electrophoresis, gels were dried under vacuum for 1 h at 80°C and exposed to a photographic film for 16 h. All enzymes used were from Pharmacia (Uppsala Sweden) with the exception of *Mse*I (New England Biolabs), and all oligonucleotides from Operon Inc. (Alameda California).

AFLP data analysis

The presence or absence of bands from AFLP analysis using two primer combinations was evaluated in the 40 cassava accessions and a data matrix obtained. The data matrix was converted into a similarity matrix according to the definition of Nei and Li (1979). Mathematically, Sij = 2a/(2a + b + c), where Sij is the similarity between the two individuals i and j; a is the number of bands present in both i and j; b is the number present in i and absent in j; and c is the number of bands present in j and absent in i. The conversion of similarity to genetic distance was by the following equation: Dij = 1 - Sij. The UPGMA (unweighted pair group mean average) method of Seath and Sokal (1973) was employed in analyzing the genetic distance matrix and in constructing a dendogram, using the NTSYS computer program version 1.8 (Rohlf 1993). The genetic differentiation of AFLP clusters was estimated from the coefficient of heterogeneity (Gst), given as Dst/Ht, where Dst is the heterogeneity amongst clusters and Ht is the total genetic heterogeneity. Ht can be obtained from the relationship Ht = Hs + Dst, where Hs is the average heterogeneity within clusters (Nei 1973). To evaluate the effect of AFLP bands with a large effect on clustering, multiple correspondence analysis was performed with the CORRESP command of SAS (SAS Institute 1989).

Table 1 Source of cassava germplasm utilized for AFLP analysis

Accession number	Local name	Source/locality	Resistance to CMD[a]
ARG11		Argentina	nd
BOL3		Bolivia	nd
BRA12		Brazil	nd
BRA110		Brazil	nd
BRA881		Brazil	nd
COL1505		Colombia	nd
COL2061		Colombia	nd
COL2066		Colombia	nd
COL2215		Colombia	nd
CUB74		Cuba	nd
MEX59		Mexico	nd
TMS30572		IITA, Nigeria	R
TMS 4(2)1425		IITA, Nigeria	S
TME1	Antiota	SW Nigeria	MR
TME2	Odungbo	SW Nigeria	S
TME3	2nd Agric	SW Nigeria	HR
TME4	Atu	SW, Nigeria	HR
TME5	Bagi Wawa	Central Nigeria	HR
TME6	Lapai-1	Central Nigeria	HR
TME7	Oko-iyawo	Central Nigeria	HR
TME8	Amala	SW Nigeria	MR
TME9	Olekanga	SW Nigeria	HR
TME10	Orente	SW Nigeria	S
TME11	Igueeba	SW Nigeria	HR
TME12	Tokunbo	SW Nigeria	MR
TME13	MS-20	SW Nigeria	HR
TME14	Abbey-ife	SW Nigeria	HR
TME18	Dankporo	Central Nigeria	MR
TME28	MS-6	SW Nigeria	HR
TME31	Bakince-iri	NW Nigeria	MR
TME38	Adabo	Central Nigeria	MR
TME41	Dan Bussa	Central Nigeria	MR
TME117	Isunikankaniyan	SW Nigeria	S
58308		Moor R.S., Nigeria	MR
TMS60142		IITA, Nigeria	MR
60444		Moor R.S., Nigeria	MR
60447		Moor R.S., Nigeria	S
60506		Moor R.S., Nigeria	S
TMS63397		IITA, Nigeria	MR
TMS90257		IITA, Nigeria	MR

[a] MR: moderately resistant; HR: highly resistant; S: susceptible; nd: not determined

Cloning of a unique AFLP fragment

A unique AFLP fragment found in the African accessions was eluted from the polyacrylamide gel and cloned as follows: the polyacrylamide gel was lined up with the autoradiogram and the position of the AFLP fragment demarcated on the gel by punching through the autoradiogram and gel with a scapel. The region of the gel was then cut out and transferred to an Eppendorf tube; 50 µl of distilled water was added to the tube, left at 4°C, and then boiled for 15 min. Solid contents of the Eppendorf tube were pelleted by centrifugation in a microfuge for 2 min at 12 000 g, and the supernatant was transferred to another tube and precipitated by 2 vol of absolute ethanol with glycogen, at a final concentration of 0.5 µg/ml. The resulting DNA pellet was re-suspended in 20 µl of distilled water. PCR-amplification was performed on 15 µl of the supernantant using the corresponding *Eco*R1+3/*Mse*I+3 primers with the temperature profile for the pre-amplification (*Eco*RI+1/*Mse*I+1) step. Unpurified PCR product (100 ng) was cloned into PGEM-T (Promega) according to the manufacturer's instruction.

Genetic mapping of the unique AFLP fragment

TMS 30572, one of the African accessions employed in this study, is the female parent of a mapping population of 150 F_1 individuals employed in generating a molecular genetic map of cassava (Fregene et al. 1997). The male parent is CM2177–2, a Latin America, analyzed in a previous study (Roa et al. 1997). The AFLP fragment unique to African accessions was present in TMS 30572 and is expected to segregate in a 1:1, presence: absence ratio in the F_1 progeny. The segregating AFLP marker was placed onto the existing linkage groups of the cassava map using the ''group'' command, with a LOD threshold score of 4.0, a recombination fraction of 0.30, and the ''try'' command of Mapmaker 2.0 (Lander et al. 1987) running on a Macintosh 7200 PowerPC.

Association of AFLP markers with trait(s) of agronomic interest

The mapping population has been evaluated at CIAT Headquaters, Palmira, Colombia, for several traits of agronomic interest over the 2 years, 1995 and 1998. The experimental design was a triple partially balanced lattice design with 20 plants per genotype (plot) and three replications. Phenotypic data from trials of the mapping population include plant height, leaf morphology, height of first branching, dry matter yield, percent dry matter, percent post-harvest deterioration, resistance to the cassava bacterial blight (CBB), and earliness. Adjusted means of the phenotypic data for all traits were regressed on the genotype marker classes of the AFLP marker in the F_1 mapping population. Association was declared at a probability level of $P < 0.005$. The above analysis was employed to test if a mapped AFLP marker is associated with a trait of interest, especially if the marker was found in an African accession, resistant to CMD.

Results

AFLP in African and Latin American cassava germplasm

A total of 57 bands were scored for all 40 accessions with the primer pair *Mse*I-GTA/*Eco*RI-AAC, of which 40 bands, or 71%, were polymorphic in at least one of the cassava accessions (Fig 1). Fifty two bands were scored for the second primer pair *Mse*I-GTA/*Eco*RI-ACA; of these 36 bands, or 69%, were polymorphic.. The AFLP analysis was conducted twice; the number of bands produced and the percent polymorphisms were highly reproducible for both pairs of primers. The genetic distance matrices derived from the two primer pairs showed a high level of correlation (r = 0.90), suggesting that a single primer is sufficient for generating distance similarity estimates (Vos et al. 1997). AFLP analysis allowed for discrimination among the accessions, except for ten African cassava land races that fell into three groups of five, three, and two accessions, respectively. Members of the individual groups were indistinguishable, suggesting they are duplicates or else differ at only a few genetic loci. An earlier AFLP analysis of 38 accessions from the CIAT core collection (mostly Latin American accessions with an exception of one African and four Asian accessions) with the same two pairs of primers permitted the unique identification of each individual (Roa et al. 1997).

Genetic relationships amongst African cassava land races and improved varieties as assessed by AFLP analysis

Nei's genetic distance analysis of AFLP bands yielded four major clusters at a 85% truncation level of genetic similarity (Fig. 2). The clusters were mainly along regional lines with the exception of BRA 110 and CUB74, from Brazil and Cuba, respectively, which fell into one of the two clusters of African accessions, and TMS 90257, an improved line from Nigeria, that clustered with varieties from Colombia and Brazil (Fig. 2). The clusters were divided equally between African and Latin American accession. The smaller African cluster has nine accessions made up of four improved lines, TMS 63397, 60142, 4(2)1425 and 30572, from the International Institute for Tropical agriculture (IITA), Ibadan, Nigeria; two improved clones, 60444 and 60446, developed at a Nigerian research station in the 1950s; and a clone, 58308, obtained by selection from open-pollinated seeds of 46106/27, a third backcross derivative from a resistance breeding program in Tanzania, with *M. glaziovii* as the donor parent. African land races in this cluster are susceptible to CMD, while the improved lines have responses ranging from susceptible to resistant. This cluster has a mean genetic similarity of 88%. The second cluster of African accessions was made up, predominantly, of land races and 60506, an improved clone developed in the 1950 s at a Nigerian research station, as well as CUB74 and Bra 110, with a mean genetic similarity of 86%. All land races in this cluster are either resistant or immune to the disease. The mean similarity coefficient within the African accession was 87% compared to the mean genetic similarity of 86% within the Latin American accessions.

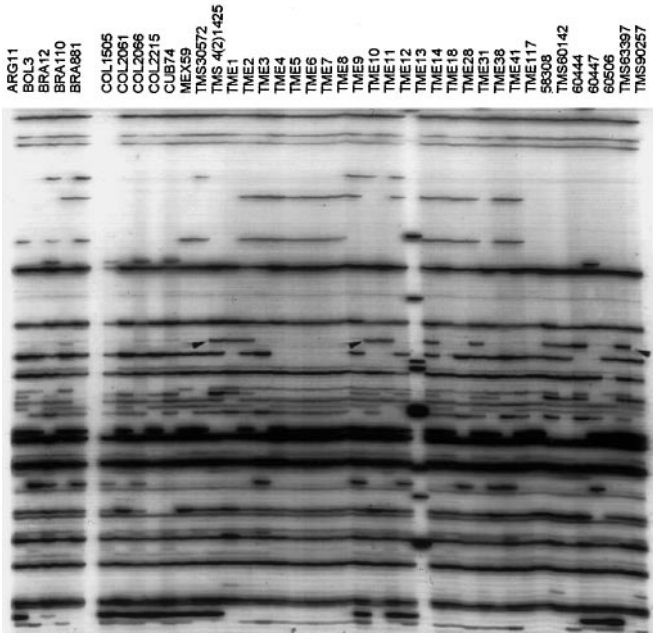

Fig. 1 AFLP phenotypes in cassava accessions from Africa, lanes 12–40, and Latin American, lane 1 to13 detected with primer pair EcoRI+AAC/MseI+GTA. Arrows point to a unique polymorphic AFLP fragment found in some African cassava accessions

Multiple correspondence analysis (MCA) and genetic differentiation of clusters

Ten clusters were obtained by MCA analysis. The first axes of the multiple correspondence analysis reveals a distribution of clusters according to a gradient of resistance to CMD, especially in the African land races (Fig. 3 and Table 1). At one extreme of the gradient is cluster 2, consisting exclusively of African land races, immune or resistant to CMD, and cluster 5, made up of four Latin American accessions, of unknown disease reaction, and one immune African land race. At the middle of the axes lies cluster 1, made up predominantly of African land races, resistant to CMD, with resistance considerably less than the first cluster, on average, and one susceptible elite African accessions. At the other end are clusters 3, 5,7 made up of only susceptible African land races, and elite lines resistant, tolerant or susceptible to CMD. The second axes roughly group the clusters by continent, with the exception of TMS60447, that fell among Latin American accessions, as a single entry cluster. The total genetic heterogeneity (ht) was 0.21, the average genetic heterogeneity within clusters (Hs) was approximately

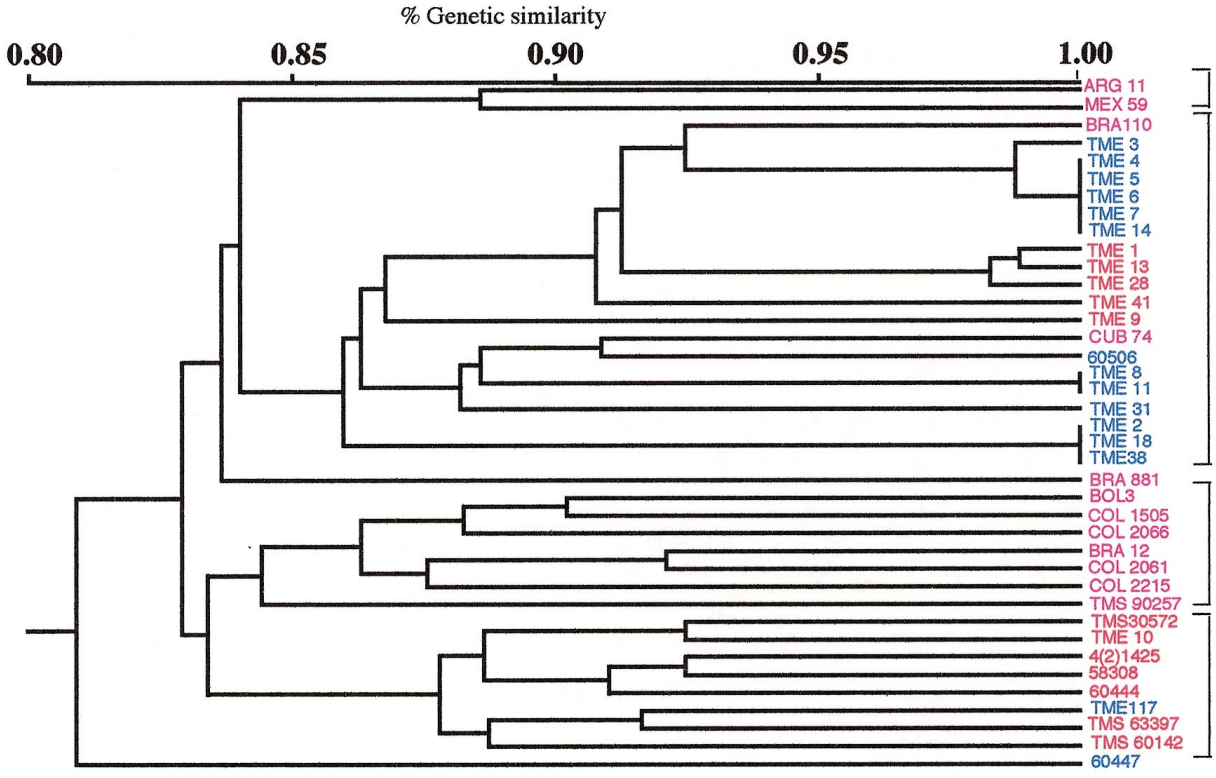

Fig. 2 Dendogram of genetic-distance relationship between some African (*blue and red colour*) and some Latin American (*purple colour*) cassava accession based on AFLP data using UPGMA. The African accessions shown in red have an AFLP fragment absent in Latin American accessions

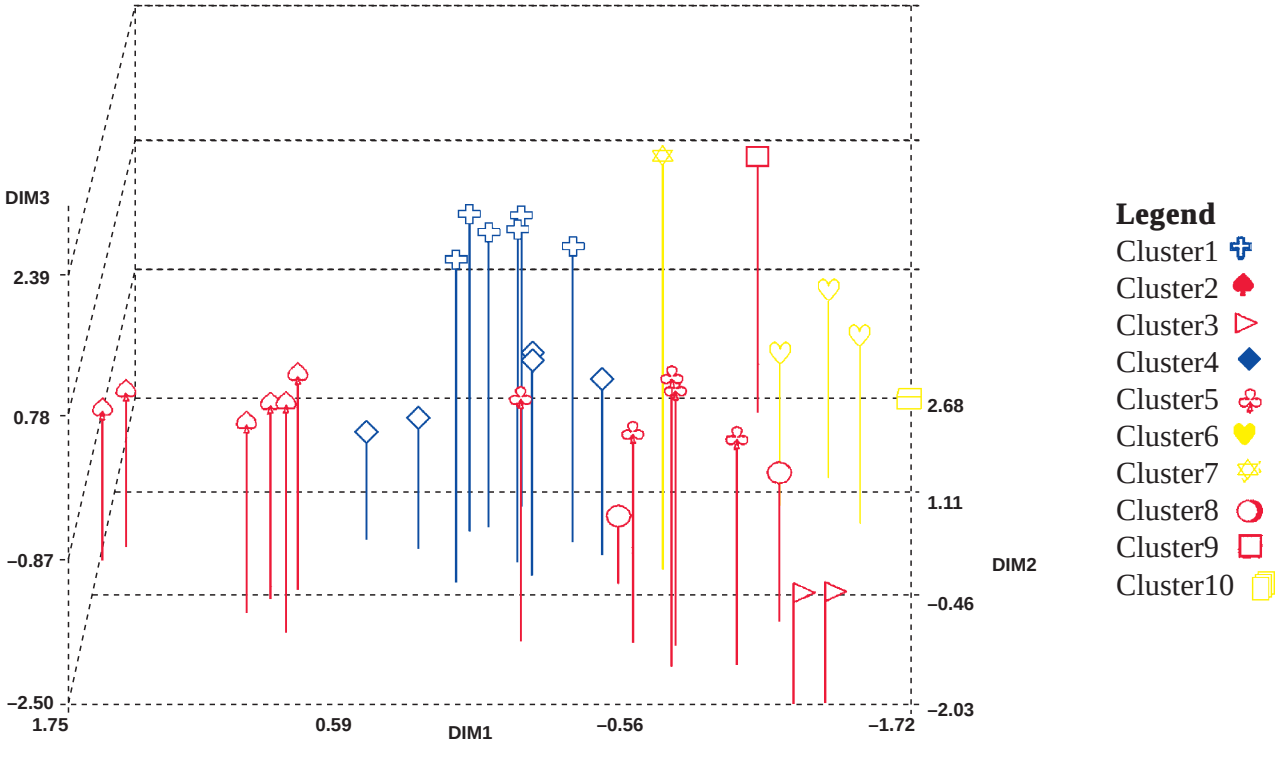

Fig. 3 Multiple correspondence analysis of AFLPs in African Cassava germplasm resistant and susceptible to the cassava mosaic disease (CMD), and a reference set of Latin American accessions. African accessions are shown in red, Latin American in yellow, and mixed clusters in blue

Table 2 Genetic differentiation of clusters derived from multiple cluster analysis showing estimated within-cluster heterogeneity (hs), average heterogeneity (Hs), among-cluster heterogeneity (Dst), total heterogenety (Ht), and coefficient of genetic differentiation (Gst)

Cluster no.	Cassava accessions	hs
1	CUB74, MEX59, TME11, TME18, TME2, TME31, TME38, TME8, TMS60506	0.159
2	TME1, TME13, TME14, TME28, TME3, TME4, TME41, TME5, TME6, TME7	0.074
3	4(2)1425, 58303	0.070
4	BOL3, BRA110, BRA881, COL1505, TME9	0.178
5	TMS35072, TME10, TME117, 60142,TMS63397	0.134
6	BRA12, COL 2061, COL2066	0.150
7	60444, TMS90257	0.175
8	ARG11,	0
9	60447	0
10	COL2215	0

HS	Ht	Dst	Gst
0.120	0.211	0.092	0.434

0.12, while between-cluster heterogeneity (Dst) was 0.09 (Table 2). The genetic differentiation amongst clusters, (Gst), of 0.43, suggests tight clustering.

Genetic mapping of the unique AFLP fragment

A fragment of size 118 bp was found in 11 accessions from Africa but absent from all 11 Latin American accessions representative of the CIAT core collection, and from a core of the collection of 38 clones representative of the Latin American region (Roa et al. 1997). The unique AFLP fragment, found in African accessions, was scored in the cassava mapping population derived from crossing TMS 30572 and CM 2177–2. Segregation data, scored as absence/presence, was in the expected ratio of 1:1 (absence/presence) at a chi-square probability of 95%. The AFLP marker was mapped to the end of chromosome M of the TMS 30572-derived frame-work map of cassava (Fregene et al. 1997), with a LOD > 3.0 (Fig. 4).

Association of a unique AFLP fragment with traits of agronomic interest

The AFLP fragment was found to be associated with the height of first branching in 1995 with a *P* value of 0.003; the association explained 12% of the total phenotypic variance for height at first branching. For the second year, the association was significant at $P = 0.002$, and explained 10% of the total phenotypic variance. The amount of phenotypic variance explained was from the R^2 of the regression. The allelic effect of the fragment was an increase in height of first branching by 20 cm for both years in Palmira. Heritability estimates, calculated from the ANOVA, using SAS (SAS Institute), ranged between 70 and 76% for all environments, suggesting that the trait, height of first branch, is highly heritable (data not shown).

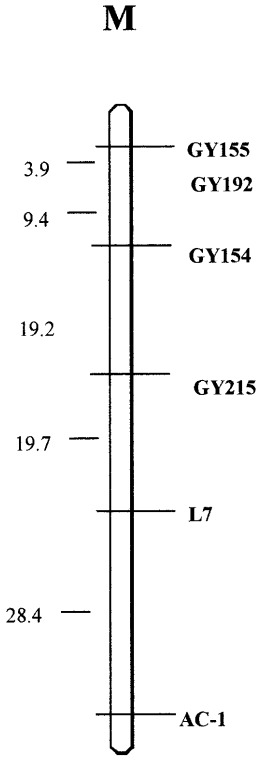

Fig. 4 Linkage group M of the TMS 30572-derived-genetic framework map of cassva. *AC-1* unique AFLP polymorphism which maps to the distal end of the linkage group. Distances at the left are given in centiMorgans. Markers with the prefix *GY* are RFLP markers from a *PST1* library. *L7* is a RAPD marker.

Discussion

The high level of polymorphism found with AFLP markers enabled an estimation of genetic similarities in cassava accessions from Africa that was more informative than other methods used to date, such as isoenzymes, RFLP, and RAPDs (Beeching et al. 1993; Lefevre and Charrier 1993; Marmey et al. 1994). Isozyme, RFLP and RAPD analyses were based on 17, 15, and 20 loci respectively. AFLP analysis was also able to detect possible duplications in 10 of the 20 cassava land races stud-

ied; TME 4, TME 5, TME 6, TME 7 and TME 14 could not be differentiated with 76 polymorphic bands. TME 7 and TME 14 are from the derived rain-forest agroecology of Nigeria, while TME 4, TME 5 and TME 6 are from the southern guinea savannah agroecology of Nigeria. They all have very high levels of resistance to CMD, and a very similar morphology, notably with red petioles. TME 2, from the derived rain-forest agroecology together with TME 18 and TME 38, from the guinea savannah agroecology, both of Nigeria, make up the second group of likely duplicates. They have a moderate level of resistance to CMD. TME 8 and TME 11 constitute the third group, and are from the rain forest agroecology of Nigeria, with high levels of resistance to CMD and moderate resistance to CBB (Dixon, unpublished data). The discovery of a sizeable number of duplicates, 50%, in African cassava land races suggests that a variety can become wide-spread due to its success with farmers, leading to it being given different names and ultimately considered as a different variety by germplasm curators. The speed and purpose of change in cultivar composition has been traced to the active involvement of farmers in continous testing and the adaptation of new plant materials to their agroecology, cropping systems, new market demands and the need to keep useful genetic variation for future needs (Fresco 1986; Nweke 1994).

Multiple cluster analysis revealed a marked genetic differentiation between accessions of Latin American and African origin. Even with a single AFLP primer combination accessions could be separated into regions of origin. This suggests that selection for adaptation to CMD and to several other unique aspects of African agroecologies has changed cassava sufficiently enough from the time of its arrival from the Americas, as to be detected by the sampling of a small number of markers. The pattern of genetic divergence of an introduced species has been shown to be non-random and strongly correlated to the environment (Clegg and Allard 1972). Genetic divergence of African and Latin American cassava germplasm has far-reaching application for cassava breeding, and germplasm collection in the sub-Saharan region of Africa. In addition, genetic differentiation between cassava land races resistant and susceptible to CMD buttress the tentative assumptions of the role which adaptation to adverse environments plays in shaping the present-day genetic constitution of cassava in Africa. The above findings have far-reaching implications for cassava breeding and germplasm collection in the sub-Saharan region of Africa. First, existing genetic variation in African cassava germplasm appears to be structured in response to selection for adaptation to the salient biotic stresses, and the unique African agroecology. Future improvement for response to biotic stress, agroecological niches, other traits of agronomic interest, and different end uses will be far more efficient if parents for breeding populations are carefully chosen based on the existing structure of genetic variation. Secondly, there is a need to sample more systematically the genetic diversity of the crop in Africa, for breeding and conservation purposes. It is important that farmer criteria for choice of cultivar, and other agro-ecological data be included to properly characterise genetic variation in the region. Finally, our study has shown that not too many marker loci are required to obtain an idea of the structure of the genetic diversity of cassava for most important traits.

As would be expected, the African and South American cassava accessions shared most amplified restriction fragments polymorphism. However, one case stands out; one fragment was observed in the African accessions, but not in the South American accessions. This fragment was cloned and mapped onto the existing genetic map of cassava, and association with traits of agronomic interest was sought. The AFLP marker was found to be consistently associated with the height of first branching, with the effect of increasing the height of first branching. The height of first branching in cassava is tightly correlated with flowering, due to the loss of apical dominance and lateral budding that occurs at flowering in cassava. Southern and sequence analysis showed the fragment to be a single-copy sequence hybridizing to two monomorphic fragment in the parents of the cassava mapping population (data not shown), suggesting the fragment to be part of a gene. The relatively high frequency, 38%, of these fragment in accessions from Africa suggests that the clones are related by common descent from a common ancestor possessing the fragment, and/or have been selected for a certain trait in linkage disequilibrum with the fragment. Though pedigree records are not presently available for all accessions with the AFLP fragment, it appears they are somewhat related. TME 1, TME 13 and TME 28 show moderate to high levels of resistance to CMD and have similarity coefficients greater than 97%, implying a similar pedigree. Another member of the group with the AFLP fragment, TME 10, has 95% genetic similarity to improved lines from IITA with the marker. The association of the fragment with an increased height of first branching also suggests the that a second hypothesis of the fragment being in linkage disequilibrum with certain traits of interest to cassava growers and breeder might be true. This opens up the possibility of identifying putative markers for the QTL mapping of poorly understood adaptive traits by screening germplasm pools, adapted to a particular environment, with a sub-set of genome-wide markers. Associations between markers and quantitative traits have been inferred in an oat germplasm pool from the variation and frequencies of RFLP markers (Beer et al. 1997).

Cassava has been cultivated for 10 000 years but not until 400 years ago was it grown outside South and Central America (Bryne 1984). Nevertheless, the genetic similarity in African accesssions was not significantly different from that of Latin American accessions, as observed in this study and comparing with the study of Roa et al. (1997) for a larger and more-representative sample of genetic variability in Latin America. Our results are also in agreement with other studies employing other molecular markers, such as RAPDs and RFLPs (Beeching et al. 1993; Marmey et al. 1994). The incorporation of addi-

tional Latin American cassava germplasm, including a wild relative, *M. glaziovii*, into African germplasm, after the initial few varieties introduced by Portugese traders, has been substantial in the last 30 years.

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