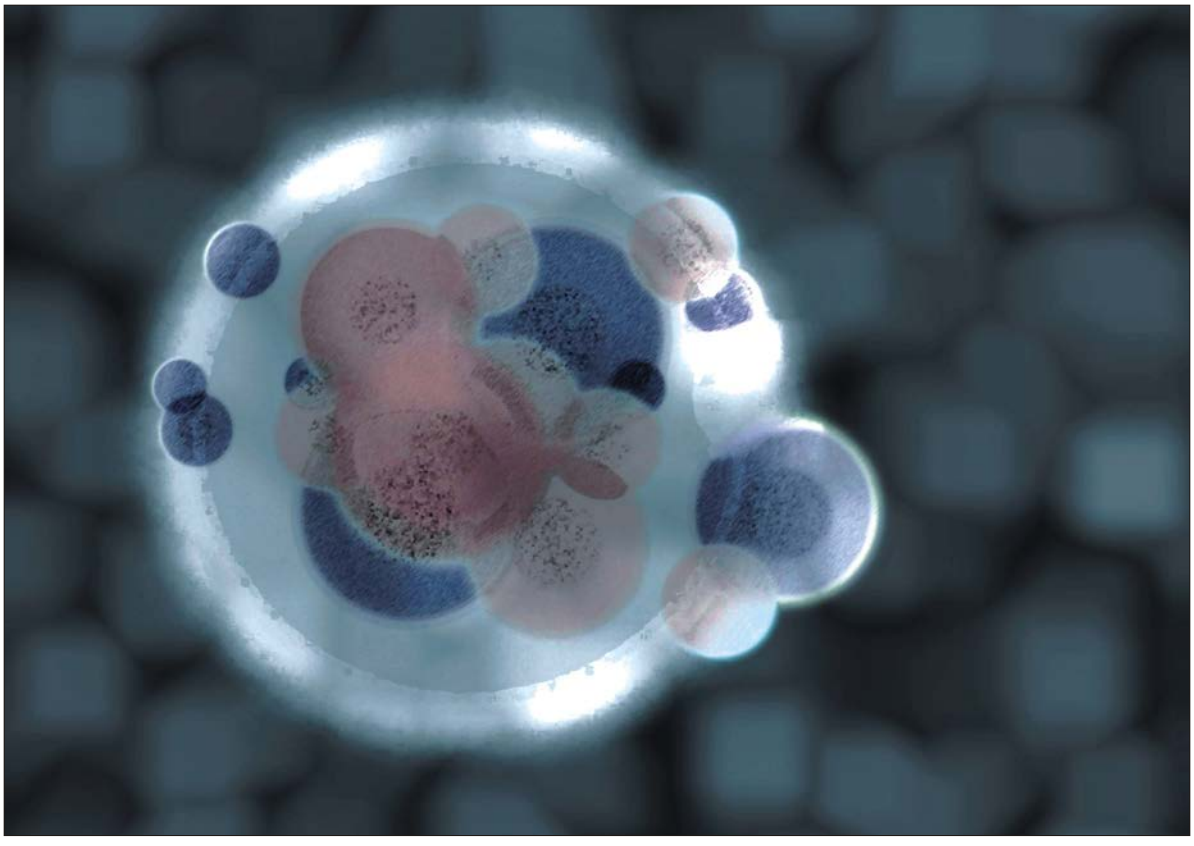

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Ethnobotany, Morphology and Genotyping of Cassava Germplasm from Malawi

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Abstract: The objectives of this study were to collect and characterise Malawian cassava germplasm using ethnobotany, morphological and Amplified Fragment Length Polymorphism (AFLP) markers. Exploration of accessions with the help of indigenous knowledge was done. Ninety three accessions collected from farmers fields and commercial programs were planted and morphologically characterised at Chitedze Agricultural Research Station (Malawi). A subsample of 28 accessions was used for DNA fingerprinting. Preferences of farmers for traits in cassava varieties were diverse according to use and areas. Ethnobotany revealed wide genetic diversity in the germplasm, as did morphological characterisation, but morphological characterisation failed to uniquely differentiate all analysed accessions. AFLP markers showed narrow genetic diversity but managed to distinguish all accessions. Hence, there is need to use all three techniques at different levels to identify genetic diversity.

Key words: AFLP, ethnobotany, *Manihot esculenta* Crantz, genetic diversity, morphology

INTRODUCTION

Cassava (*Manihot esculenta* Crantz) is a woody shrub with edible tuberous roots, which grows in tropical and subtropical areas of the world. The starchy tuberous roots of cassava have become the most important source of dietary energy in half of Sub-Saharan Africa (Scott *et al*., 2000; FAO, 2001). It provides more dietary energy per hectare than any other staple crop (Fregene *et al*., 2000) and grows in marginal areas (Aina *et al*., 2007). More than 30% of the Malawi population, especially those living along the lake shore, depend on cassava as their staple food crop, while in the rest of the country, cassava is grown for food security, as a snack and cash crop (Moyo *et al*., 1998). Cassava is increasingly becoming an important industrial crop (Benesi *et al*., 2004; Nthonyiwa *et al*., 2005). Leaves of cassava are used as a vegetable in Malawi and Africa as a whole (Moyo *et al*., 1998; Fregene *et al*., 2000). Cassava tuberous roots are an excellent source of carbohydrates but contain little protein.

Commercialisation of cassava and adoption of improved varieties increases production and income but encourages genetic erosion (Mkumbira, 2002; Benesi *et al*., 2004). The main biotic stresses, such as cassava mosaic disease and cassava bacterial blight and the outbreak of cassava brown streak disease reported in the 1990's threatens genetic diversity (Perez *et al*., 2005; Cuambe *et al*., 2007). This calls for the collection, characterisation and conservation of local germplasm for use in crop improvement programmes in Malawi, the region and at global level. An insight into the magnitude of variability present in a crop species is of utmost importance, as it allows effective selection (Beeching *et al*., 1993; Jarvis and Hodgkin, 2000).

The objectives of this study were to explore cassava germplasm in Malawi with the aid of indigenous knowledge and to assess the diversity of Malawian cassava accessions using ethnobotany, morphological and AFLP markers in order to assist the breeding efforts.

MATERIALS AND METHODS

A collection of Malawian cassava germplasm with the aid of indigenous knowledge was conducted with detailed passport data, based on farmers' knowledge, preferences and special attributes for particular varieties. A total of 78 accessions were collected from Baka, Mkondezi, Chitala, Chitedze and Makoka Agricultural Research Stations. Sixty three accessions represented a Malawian working collection while 15 were local landraces (from farmers fields). The working collection comprised of

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eight locally recommended accessions, 23 introductions from the International Institute of Tropical Agriculture (IITA) in tissue culture form and 32 local accessions. Of the 78 accessions, 72 (92%) sprouted clean. Another 87 accessions were collected from farmers' fields in January 2003 of which 76 (88%) sprouted clean at Chitedze Agricultural Research Station. It is normally difficult to get clean [free from Cassava Mosaic Disease (CMD) symptoms] planting material from farmers fields, especially along the lake shore where it is a high pressure area for CMD. The high rate of sprouting of clean accessions was due to careful selection of planting material at collection points by an experienced exploration team and the help of farmers themselves. Chitedze is a low pressure area for CMD and cassava brown streak disease, hence no single accession was lost or re-infected by viruses in the field gene bank by December 2005.

Eighteen cassava accessions of the 2001/02 and 75 accessions of the 2003 collections were planted at Chitedze Agricultural Research Station in January 2003. Morphological characterisation of above and below ground parts was conducted using a modified cassava descriptor of Nweke *et al.* (1994). The 12 morphological characters scored were: shoot colour, shoot pubescence, leaf lamina colour, leaf lobe shape, petiole colour, tender shoot colour, mature stem colour, branching habit, root outer skin colour, root inner skin colour, root pulp colour and taste. Morphological data for 93 analysed accessions was converted into a binary matrix. Traits that had only two categories of description were scored normally in the binary matrix. The number of leaf lobes were scored as (0) for 5 and (1) for 7. Root constriction is classified as absent (0) or present (1). Root texture was scored as smooth (1) and rough (0). All the traits concerning colour, shape and position were coded by considering the whole range of diversity of that trait and scored against that particular class. For example, the colour of root surface ranged from 1=(white/cream), 2=(light brown) to 3=(dark brown). If a genotype's root skin colour was white, it was scored as one against CRS1=(white/cream) and 0 for CRS2=(light brown) and CRS3=(dark brown). If a genotypes' root skin colour was dark brown, it was scored as one against CRS3=(dark brown) and 0 for CRS1=(white/cream) and CRS2=(light brown). Morphological characterisation was completed by March of 2004.

DNA was extracted from a subsample of 28 cassava accessions selected from 93 morphologically characterised accessions. These 28 accessions represented different morphological and agronomical traits preferred by farmers and cropping systems. They represented all major clusters of the dendrogram generated based on morphological characterisation. The method of Edwards *et al.* (1991) was used for DNA extraction. AFLP analysis was performed according to Vos *et al.* (1995) as modified by Herselman (2003) and Benesi (2005) using the AFLP Plant Mapping Kit for regular genomes (Promega, Madison, USA). *Eco*RI primers were either FAM (E-ACA and E-ACT) or NED (E-AAC and E-ACC) labelled. Six commercially available AFLP primer pairs (E-ACA/M-CAA, E-AAC/M-CAA, E-ACA/M-CAT, E-AAC/M-CAT, E-ACT/M-CTT and E-ACC/M-CTT) were used. PCR products were resolved on an ABI Prism 310 Automated Capillary Sequencer. DNA fingerprint analysis was done on a Macintosh (iMac) computer using GeneScan 3.1 (Perkin-Elmer Corporation, 1997). Fragments were scored into a binary matrix as present (1) or absent (0).

Similarity coefficients for morphological and AFLP data were calculated using Dice similarity coefficients (Dice, 1945; Nei and Li, 1979) using NTSYSpc version 2.11c computer package (Rohlf, 2000). Dendrograms were constructed using the unweighted pair-group method using arithmetic averages (UPGMA) clustering and the SAHN programme parameters of NTSYSpc (Rohlf, 2000). The goodness of fit of clustering to data matrices was calculated using the COPH and MXCOMP programmes. Comparison of morphological and AFLP analysis was done by correlating Dice similarity confidents for each method using (Agrobase, 2000). This part of the project was completed at the end of 2004.

RESULTS

The field collection data revealed that preferences of cassava varieties by farmers in terms of taste varied according to the intended use and modes of utilisation of cassava in that particular area (Table 1). In the North where farmers rely on cassava as a staple crop, they preferred very bitter cassava varieties. In the centre, farmers generally preferred sweet cassava varieties, but preferences varied according to area and intended use of cassava. Along the lake shore, vast areas of very bitter cassava varieties were grown as a staple crop. In the upland and the rest of the central region, vast areas of sweet cassava varieties were grown for the fresh market, as a snack or for food security. In the South, farmers preferred very sweet cassava varieties (Table 1) intended for the fresh market, as a snack or processed into *makaka* (scraped or peeled cassava tuberous roots, cut and split into chips and directly sun dried) for food security or sale. Since submerged fermentation is not practiced, bitter varieties cannot be used to make *makaka*. The few farmers who grow bitter cassava in the south, practice heap fermentation for one week before drying.

Table 1: Taste and maturity periods as preferred by farmers at points of collections

Collection areas	Collected Acc.		Farmers' preferences of sweet and bitter cassava varieties												Farmers' preferences of maturity period of cassava varieties											
			Very sweet			Sweet			Bitter			Very bitter			Early			Medium			Late			Very late		
	No.	(%)	No.	[%]	(%)	No.	[%]	(%)	No.	[%]	(%)	No.	[%]	(%)	No.	[%]	(%)	No.	[%]	(%)	No.	[%]	(%)	No.	[%]	(%)
North	44	46	10	23	10	10	23	10	9	21	9	15	34	16	23	52	24	18	41	19	2	5	2	1	3	1
Centre	25	26	10	40	10	1	4	1	7	28	7	7	28	7	8	32	8	14	56	15	2	8	2	1	4	1
South	27	28	17	63	18	7	26	7	1	4	1	2	7	2	5	19	5	20	74	21	0	0	0	2	7	2
Total	96	100	37	-	38	18	-	18	17	-	18	24	-	25	36	-	37	52	-	54	4	-	4	4	-	4

Acc: Accessions; No.: No. of collected accessions; [%]: Percentage collected accessions within region; (%): Percentage collected accessions across the country

All farmers across the country concurred on early to medium maturing (Table 1), good ground storage, high yields, pest and disease resistance, tuberous roots with no fibres, leaves suitable as a good vegetable and varieties with high multiplication ratio in terms of production of planting materials as important and positive characteristics. Since the crop is vegetatively propagated, appropriate varieties need to have many, long, healthy and quality stems to avert shortage of planting materials and withstand dry spells soon after planting (IITA, 1990).

Cluster analysis for morphological characterisation revealed three major clusters (I, II and III), with the third cluster (III) containing only two accessions. Cluster I consisted of most of the genotypes (Fig. 1). Sub-cluster A contained 41 local cultivars from all three regions of the country, three introductions from IITA and one local clone (CH92/082). Accessions did not cluster according to geographic distribution. The most distant accessions in this cluster were Kanıphunobii and TMS4(2)1425 with a Dice Similarity Coefficient (GS) of 0.434. Accessions in this cluster were characterised by green shoot tips, silvery green mature stems and white root outer skin colour.

Sub-cluster B contained 30 local cultivars from all three regions of the country, one introduction from IITA (Maunjili) and one local variety (Sauti) (Fig. 1). Accessions in this cluster were characterised by hairless unexpanded apical leaves.

Cluster II contained 14 accessions and were subdivided into two sub-clusters (C and D). Nine of them were local cultivars from the central and northern regions of Malawi. Mkondezi (MK91/478), Yizaso (CH92/112) and MK95/054 were local while LCN8010 and Silira (TMS60142A) were introductions from IITA (Fig. 1). The diversity of accessions belonging to this cluster was wide and GS values ranged from 0.333 to 1.000. The unique trait for cluster II was light brown to orange mature stems.

Cluster III contained Thipula and Unknown2 with a GS of 0.609. These accessions were characterised by green and hairless unexpanded apical leaves, lanceolate shaped central leaf lobes, purple petioles and green shoot tips. Green unexpanded apical leaves, purple petioles and non-branching growth habit made them unique from the rest of the accessions. Their uniqueness was also captured in the ethnobotany since farmers indicated that tuberous roots of these accessions were not fibrous and good for *Chiphuwa* (fermented and roasted cassava).

Morphological markers failed to uniquely distinguish all 93 accessions. This might be due to the limited number of morphological markers - 12 morphological traits were used which generated 41 markers. Morphological characterisation resulted in the following accessions clustering together: Balaka1 and Balaka2 and Mbundumali3; Manyokola5, Mbundumali1 and Mbundumali2; Mwaya1 and Mwaya2; Chithekere1 and Chithekere2; Six months1, Six months2, Kabuthu, Nakalasi, Manyokola1 and Manyokola2 in sub-cluster A with narrow genetic diversity. This indicated that most of these accessions were closely related. The other morphologically similar accessions were Matuvi and Depwete and Chilikhano1 and Chilikhano2 in subcluster B and Gomani1 and Gomani2 in cluster II (Fig. 1).

Although, some morphologically similar accessions were identified, morphological markers revealed wide genetic diversity for Malawian cassava germplasm. The germplasm covered a wide range of GS values from 0.083 to 1.000.

In the next step, dendrograms were generated using morphological and AFLP data for the subset of 28 accessions selected for AFLP analyses to enable the comparison of these two methods (Fig. 2, 3). Morphological characterisation of the subset of 28 accessions revealed two clusters, I and II. Most of the accessions belonged to cluster I which further formed two major sub-clusters A and B (Fig. 2) and two singletons (Fyoka and Thipula).

The dendrogram for AFLP analysis of the subset of 28 accessions revealed two clusters I and II (Fig. 3). Only Fyoka belonged to cluster II, while the rest were in cluster I. Sub-cluster a (within cluster I) contained 10 local accessions from all three regions of Malawi and Yizaso Accessions in this cluster were characterised by lanceolate shaped central leaf lobes, silvery green mature stems and white root outer skin colour.

Sub-cluster b (within cluster I) contained Masangwi, Mgwalangwa and Matuvi from the southern and the other three accessions from the Northern regions of Malawi

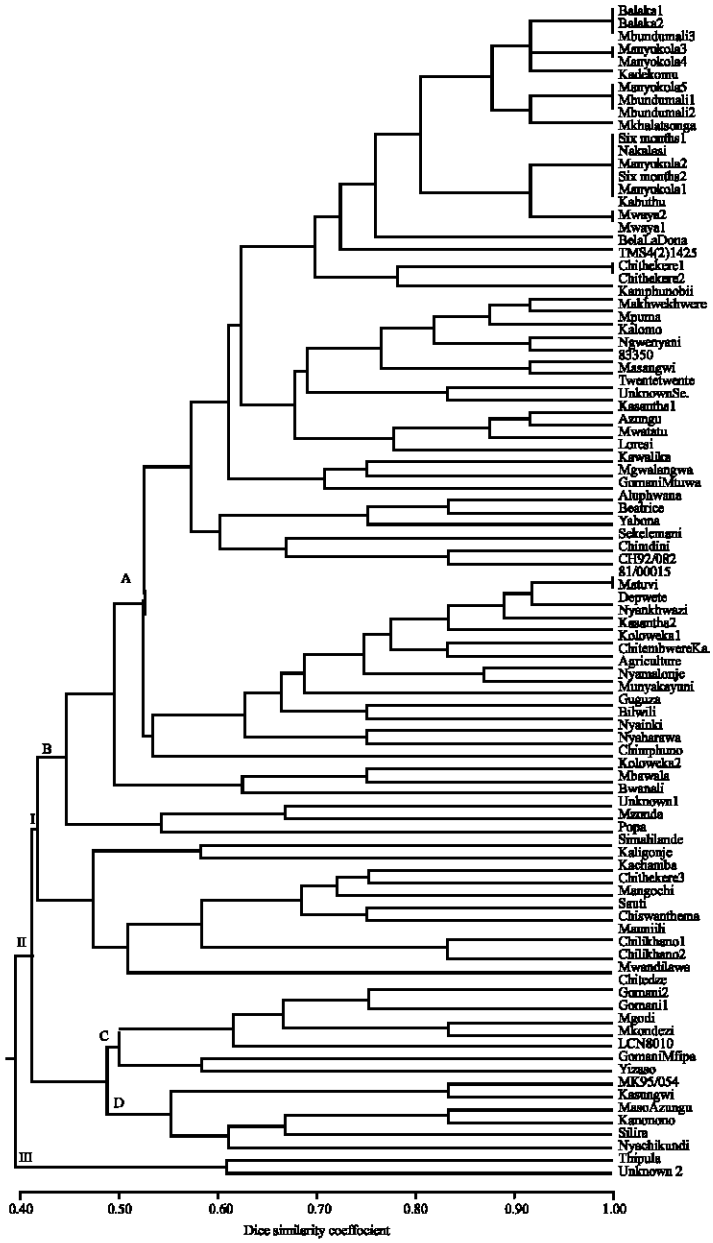

Fig. 1: Dendrogram for morphological characterisation of 93 cassava accessions

(Fig. 3). The GS range of 0.860-0.889 revealed narrow genetic diversity within this cluster. Accessions in this cluster were characterised by silvery green mature stems and white root outer and inner skin colours.

Sub-cluster ii contained two local accessions, both from the northern region of Malawi (Fig. 3). These accessions were characterised by glabrous unexpanded apical leaves and silvery green mature stems. Sub-cluster B had eight accessions, of which accessions Sauti (CH92/077), MK95/054 and CH92/082 were local and the remaining five were introductions from IITA. This cluster contained only local and introduced clones. There could exist a close genetic relationship between the introduced and local clones since seeds for the local clones were obtained from IITA and could have been generated from the same gene pool. The closest accessions in this cluster were TMS4(2)1425 and 81/00015 with a GS of 0.885. Accessions in this cluster were characterised by white root inner skin colour. Fyoka was separated from the rest of the characterised accessions which was an indication of uniqueness in terms of genetic constitution.

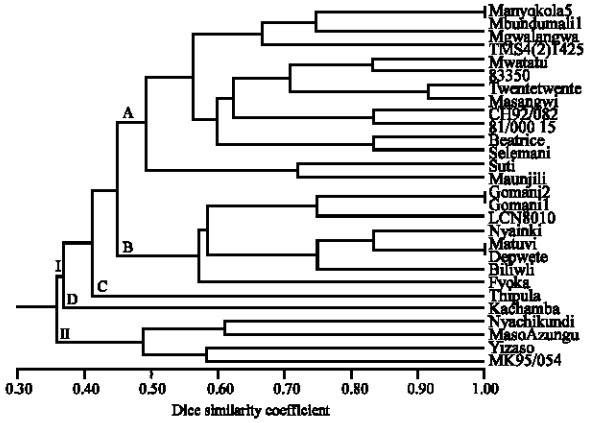

Fig. 2: Dendrogram for morphological characterisation of a subset of 28 accessions using NTSYS computer package, Dice similarity and UPGMA clustering

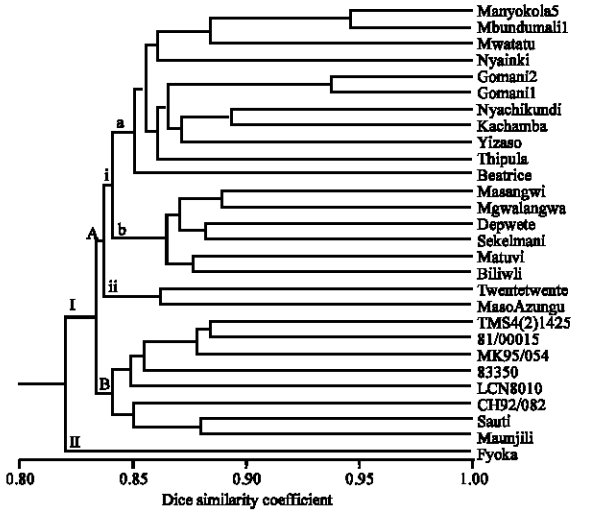

Fig. 3: Dendrogram for characterisation of a subset of 28 cassava accessions using six AFLP primer pairs with the aid of NTSYS computer package, Dice similarity coefficient and UPGMA clustering

Morphological and AFLP dendrograms were similar for clusters and genetic distances. Gomani1 and Gomani2 and Mbundumali1 and Manyokola5 clustered together in both dendrograms and were the most similar accessions based both on morphological and AFLP markers. However, Matuvi and Depwete that were 100% similar based on morphological data (Fig. 2) clustered separately based on AFLP data (Fig. 3) but still within sub-cluster b. Matuvi, Depwete and Biliwili, as well as Maunjili and Sauti clustered together in both dendrograms. These accessions are morphologically and genetically similar. Therefore, AFLP analysis confirmed morphological characterisation.

Accessions in sub-cluster B of the AFLP dendrogram grouped together in sub-cluster A of the morphological dendrogram, except for LCN8010 and MK95/054. Most of the accessions in sub-cluster B and cluster II of the morphological dendrogram (Fig. 2) clustered together in sub-cluster A of the AFLP dendrogram, except for LCN8010, MK95/054 and Fyoka.

Some differences were observed between morphological and AFLP dendrograms for some clusters and genetic distances. Fyoka clustered separately from other accessions in the AFLP dendrogram, but within sub-cluster B of the morphological dendrogram. Accessions in cluster II of the morphological dendrogram clustered separately from other accessions but randomly within the AFLP dendrogram.

Correlations between genetic distance matrices based on AFLP, morphological and a combination of AFLP and morphological traits were significant (data not shown).

DISCUSSION

Results of farmer preference for either bitter or sweet varieties agreed with previous studies of Cardoso *et al.* (1998) in Mozambique and Chiwona-Karltun *et al.* (1998, 2000) in Malawi. This is because farmers regarded bitterness and toxicity of tuberous roots as a protector of cassava against theft, spoilage by animals and unplanned harvesting by family members in order to guarantee food security. Farmers are aware that tuberous roots from bitter cultivars need to be processed before consumption, while roots from sweet cultivars could be eaten fresh or directly cooked (Chiwona-Karltun *et al.*, 1998). Farmers have reliable detection methods for distinguishing toxic and safe cassava and have processing methods for treating toxic cassava to become safe (Chiwona-Karltun *et al.*, 2000; Mkumbira *et al.*, 2001).

Colour traits, shape of central lobes and branching habit typified many accessions, hence were important salient characters used by farmers to identify varieties. Elias *et al.* (2001) reported that colour variables played a crucial role in differentiating cassava varieties. Morphologically close varieties have a greater possibility of being confused. The confusion can happen either when one farmer acquires cuttings from another farmer or even in the farmer's own field as he or she selects plants to be planted in the next field (Elias *et al.*, 2001). This could be one of the reasons for the high number of morphologically similar accessions observed in this study.

Morphological characterisation showed a high range of Genetic Similarity (GS) values of 0.087-1.000 compared to AFLP with a GS range of 0.778-0.946. Similar results were obtained when DNA techniques were used by

Gepts (1991) on common beans, Miller and Tanksley (1990) on tomato and Keim *et al.* (1990) on soybean. This could be due to the fact that morphological characters are controlled by a few major genes and may be caused by changes in a few loci (Halward *et al.*, 1992) that might be subjected to intense selection pressure. As a result, morphological variation is likely to increase during domestication while molecular markers which are not subjected to direct selection, often decrease (Gepts, 1991). However, these results disagreed with the findings of cassava genetic diversity studies of Roa *et al.* (1997) and Wong *et al.* (1999) who found a wide GS range in cassava using AFLP analysis.

No relationship was found between genetic structure and taste (sweet and bitter cassava varieties). The lack of relationship agreed with findings of Narváez-Trujillo *et al.* (2001) which confirmed the polygenetic control of cassava root taste, hence being influenced by both genotype and environment.

Cassava cultivars collected in the northern region were more diverse compared to the central and southern regions. Most of the accessions collected in the central and southern regions were closely related as revealed by morphological characterisation, while those collected along the lake shore in the central and northern regions were genetically diverse. Chiwona-Karltun *et al.* (2000) and Mkumbira (2002) observed that in the north, even between adjacent villages, varieties differed greatly, while in the southern and upland of the central region, except for the northern part of the lakeshore, varieties grown across the region were more uniform.

AFLP analysis showed a narrow range of GS values of 0.778-0.946 between all tested genotypes (Fig. 3). This was in agreement with a study by Fregene *et al.* (2000) who reported a GS range of 0.83-1.00 despite the fact that they characterised accessions which comprised landraces from Nigeria, improved clones from IITA, Africa and accessions from a core collection of CIAT, South America. However, most accessions clustered according to geographic origin and they concluded that AFLP was efficient and the germplasm was diverse although several accessions were found to be duplicates. Results of the current study (Fig. 3) clustered accessions according to origin. Introductions were clustered separately from Malawian land races except for Yizaso which was clustered together with local landraces in subcluster a. No duplicates were found even when using single primer pairs. The clustering together of both tissue culture derived genotypes and clones from open pollinated seeds introduced from IITA, indicated that they might have originated from the same gene pool. Malawian cassava landraces formed different clusters, suggesting existence of genetic diversity within the local germplasm.

These results showed that a high degree of relationship existed between AFLP and morphological diversity analyses methods. Nemera (2003) reported a significant correlation between AFLP and morphological genetic diversity analyses on sorghum. The correspondence between morphological and AFLP analyses found in the current study might be due to the fact that mainly salient traits were used as recommended by Berthaud (1997) and Elias *et al.* (2001) since they are less affected by the environment and developmental stage of the plant. AFLP uses the same principle of revealing salient fragments, which typify individuals or populations. Salient traits should be compared with AFLP analysis (Elias *et al.*, 2001), while polygenic traits should be compared with Quantitative Trait Loci (QTL). Traditionally, genetic diversity estimates and segregation of genes and hybrids in crop species were based on differences in morphological characters and quantitative traits (Schut and Stam, 1997) which have been accurately done. On the other hand, DNA markers are perceived as reliable, since it is not influenced by environmental factors and give rise to a high number of polymorphic loci (Karp *et al.*, 1997). However, DNA markers require specialised knowledge, laboratory equipment and chemical supplies making them more expensive than morphological descriptors. Indigenous ethnobotany is mostly neglected, leading to little or no contribution to the formal breeding schemes, which has resulted in low adoption of improved varieties.

Farmers reported diverse characters which assisted them in the identification and selection of preferred cultivars and for economic gains. These characters ranged from sweet/bitter, maturity period, food quality, processing amenability and suitability in intercropping with various crops and cropping systems. Cassava accessions were diverse in each of these characters. The entire range of cultivars were available in the farmers fields. For example, cultivars ranged from very sweet to very bitter, from early maturing to late maturing, from susceptible to resistant to cassava diseases and pests prevalent in Malawi, low and high yielding cultivars and unstable and stable. Ethnobotany recorded high levels of phenotypic variation of cultivars in farmers fields. This correlated with high levels of genetic diversity detected using morphological analysis (GS of 0.083-1.000), in contrast with low levels of diversity detected using AFLP analysis (GS of 0.778-0.946). This could be due to the fact that farmers do selection based on morphological characters which are used in morphological characterisation.

To conclude, the involvement of experienced farmers and scientists in the exploration significantly contributed in gathering of indigenous knowledge, reduction in

numbers of accessions which were to be sampled while covering the diverse germplasm and ensured collection of clean cuttings for conservation, evaluation and use in cassava improvement programmes. Strict implementation of crop hygiene and setting up of a gene bank in a low pressure area for pests and diseases eased management of the germplasm and reduced the risk of losing accessions through secondary infections.

Characterisation of cassava accessions at DNA level can help to identify genetically representative, non-redundant sets of germplasm for cassava breeding and conservation purposes. AFLP markers were more powerful than morphology in distinguishing accessions. DNA-based markers clearly suggested that conventional methods have been effective in selecting unique collections and that diversity assessed by molecular markers may efficiently represent the genetic diversity in morphological traits. Morphological and AFLP characterisations confirmed the diversity of Malawian cassava germplasm reported by farmers in the field.

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