MOLECULAR CHARACTERIZATION AND GENETIC DIVERSITY OF WILD AND CULTIVATED YAMS (*DIOSCOREA* SPP) IN MALAWI

Richard M. Luhanga*

Wisdom J. Changadeya

Aggrey J. D. Ambali**

Abstract

Yam is an important food crop in the tropical countries especially in the 'yam belt' of West Africa. In Malawi, the crop is grown by small-holder farmers without much government support to enhance its productivity. An investigation was carried out on 80 wild yam accessions, and 55 cultivated yam accessions from 18 districts across the country using nine microsatellite DNA markers to determine the genetic differences within and between wild and cultivated yam accessions. Microsatellite analysis distinguished wild from cultivated accessions and established unique genetic profiles that identified three predominant species; *D. dumetorum*, *D. schimperiana* and *D. alata*. All cultivated *D. alata* accessions were classified into four 'genetic groups', which matched with the four morphotypes established from the same accessions using morphological traits analysis. The data from several diversity statistics suggested a generally high level of genetic variation among the accessions most particularly the wild ones. A total of 39 alleles were generated with a range of 1-10 alleles per locus and a mean of 2.56 in cultivated *D. alata* and 3.56 alleles per locus in wild species. However, a high level of genetic similarity (70%) which tallied with one observed using morphological analysis (70%) was evident among

* Department of Biological Sciences, University of Malawi, Chancellor College, P.O. Box 280, Zomba, Malawi

** NEPAD African Biosciences Initiative, Policy Alignment and Programme Development Directorate, NEPAD Agency, c/o CSIR Building 10F, Meiring Naude Road, Brummeria, Pretoria, Republic of South Africa

cultivated accessions. The four morphological and genetic groups should be conserved since they hold evolutionary heritage and serve as a measure of diversity of cultivated yams in Malawi and the diversity of wild yams provides a valuable genetic resource base for exploitation of useful traits for the crop' improvement

Keywords: Cultivated yams, wild yams, genetic characterisation, Microsatellites, loci

Introduction

The word yam is derived from English transcription of the African term 'nyami' which refers to the edible root of the *Dioscorea genus* (Schulthei and Wilson, 1998). According to Onwueme (1978) and Degras (1982), the term yam is applied to entire *Dioscorea* genus. The genus is represented on all continents at varied latitudes and altitudes and is of the tropical and subtropical origin. Yams are believed to have originated and brought into cultivation in three independent areas of the tropics: West Africa (for *Dioscorea rotundata (*Poir) Miege , D.*cayenensis* Lam, D. *dumetorum (*Kunth) Pax, South East Asia (for D. *alata* L and *D. esculenta* (Lour) Burkill), and tropical America. The probable centre of origin of African food yams is along Niger River valleys in Nigeria, which has been observed as their centre of genetic diversity (Hahn *et al*., 1995). Yams are popular in Africa, South East Asia, West Indies and parts of Central and South America. There is also a relatively high abundance of yams in tropical Asia (Milne-Redh, 1975).

Yam is a multi-species root crop (Coursey, 1967; Onwuema, 1978; Degras, 1993). A total of thirteen species occur in Malawi according to National Herbarium and Botanical Gardens (NHBG) records (Mwanyambo and Kananji, 2002) and are distributed countrywide (Gondwe *et al*., 2003). Some of the crop's wild relatives (wild yams) are used as foods in times of famine. The crop has great potential to improve food security if fully exploited. However, yams are grown at small scale by farmers with little government support despite the crop' economic potential though the country faces challenges on how to feed the ever-increasing population from a narrow food base dominated by maize. Maize is the principal crop and main staple food whose production depends on heavily subsidized farm inputs by government (Malawi Government, 1999). In contrast, yams require minimal or no fertilizer inputs (Hahn *et al*., 1995), therefore,

have high potential to improve food security in the country following proper crop exploration, characterization and evaluation.

Despite yams' advantages and prospects, no research has so far been carried out to determine the genetic profile of yams in Malawi. Such data is required for rational design of breeding programmes, conservation of the germplasm and management of yam genetic resources. This study therefore endeavoured to characterize and assess the extent of genetic diversity in wild and cultivated yam germplasm sampled across the country using microsatellite markers with a view to contributing knowledge for designing of effective management and conservation programmes for sustainable utilization of yam genetic resources at the farm level.

Materials and Methods

Plant material

Tubers of 135 yam accessions (80 wild and 55 cultivated) collected from 18 districts across the country were planted and maintained at Bvumbwe Agricultural Research Station (BARS), in Thyolo district, Malawi (Figure 1, Tables 1, 2 and 3). The germplasm collections were made in 1998, 2000, 2002 for cultivated yams and 2003 for wild yams. The cultivated yam accessions were provided by the farmers in form of tubers. The wild yams were sampled from their natural habitats and germplasm materials collected were bulbils or tubers or both. A wild yam distribution data sheet sourced from NHBGM (2002) was used as a guide to identify sampling sites for wild yams. The accessions had been characterized morphologically according to the International Plant Genetic Resources Institute (IPGRI, 1997) descriptors for yam and classified into morphotypes (Msowoya, 2005). DNA analysis was done at the Molecular Biology and Ecology Research Unit (MBERU) of the Department of Biological Sciences, Chancellor College, University of Malawi, Zomba.

Table 1 Summary of species and number of accessions used in the study

Species	Number of accessions
Cultivated yams	
D. alata	55
Wild yams	

D. dumetorum	41
D. schimperana	23
D. odoratissima	4
D. cochleari-apiculata	1
D. quartiniana	5
D. sansibarensis	2
D. bulbifera	3
D.hylophila	1

DNA extraction

Total genomic DNA was extracted from young leaves using a modified CTAB procedure as outlined in Gawal and Jarret (1991). Two to three leaf discs were punched into 2.0ml microfuge tube and ground with the aid of Carborundum using a glass micropestle mounted on Industro Power Tools grinder. CTAB DNA extraction buffer (400µl; preheated at 60°C) was added, and the mixture incubated in an Advantec water bath at 60°C for 30 min with mild shaking. Thereafter, 400µl of chloroform:isoamylalcohol (24:1) was added to the homogenate followed by centrifugation at 15000 rpm for 10 min. The supernatant (350µl) was precipitated in 210µl cold Isopropanol in 1.5 ml microfuge tube placed under -20°C. The DNA pellet was separated from the suspension by centrifugation at10000 rpm for 5min and decanting the supernatant. The pellet was rinsed in 500µl 70% ethanol and centrifuged again at 10000 rpm for 1 min. The ethanol was decanted and the DNA air-dried for 15min and rehydrated in 50µl low TE buffer and stored at -20°C.

Table 2 List of cultivated yam accessions (*D. alata*) analyzed in this study

Accession Number	Cultivar	Origin	Accession Number	Cultivar	Origin
BAR1	Cultivar 4	Thyolo	MSY22	Cultivar 4	Nkhota kota
BAR2	Cultivar 2	Mulanje	MSY23	Cultivar 4	Nkhota kota
BAR3	Cultivar 1	Thyolo	MSY25	Cultivar 4	Nkhota kota
BAR4	Cultivar 4	Zomba	MSY26	Cultivar 4	Nkhota

					kota
BAR5	Cultivar 3	Mulanje	MSY28	Cultivar 4	Nkhota kota
BAR6	Cultivar 3	Thyolo	MSY32	Cultivar 4	Nkhota kota
MUN2	Cultivar 2	Mangochi	MSY33	Cultivar 4	Nkhota kota
MUN4	Cultivar 4	Mangochi	MSY34	Cultivar 4	Salima
MUN12	Cultivar 4	Karonga	MSY36	Cultivar 4	Salima
MUN14	Cultivar 2	Chitipa	MSY37	Cultivar 4	Salima
MUN17	Cultivar 4	Nkhata Bay	MSY38	Cultivar 4	Salima
MUN18	Cultivar 4	Nkhata Bay	MSY39	Cultivar 4	Dedza
MUN22	Cultivar 4	Kasungu	MSY41	Cultivar 4	Dedza
MUN23	Cultivar 3	Kasungu	MSY42	Cultivar 4	Dedza
MSY2	Cultivar 4	Nkhata Bay	MSY46	Cultivar 4	Ntcheu
MSY4	Cultivar 4	Nkhata Bay	MSY48	Cultivar 4	Ntcheu
MSY5	Cultivar 4	Nkhata Bay	MSY50	Cultivar 4	Ntcheu
MSY7	Cultivar 3	Nkhata Bay	MSY52	Cultivar 4	Ntcheu
MSY8	Cultivar 4	Nkhata Bay	MSY53	Cultivar 4	Ntcheu
MSY10	Cultivar 3	Nkhata Bay	MSY54	Cultivar 4	Chikwawa
MSY11	Cultivar 4	Nkhata Bay	MSY55	Cultivar 4	Chikwawa
MSY12	Cultivar 4	Nkhata Bay	MSY56	Cultivar 4	Chikwawa
MSY13	Cultivar 4	Nkhata Bay	MSY57	Cultivar 4	Chikwawa
MSY14	Cultivar 4	Nkhata Bay	MSY58	Cultivar 4	Chikwawa
MSY15	Cultivar 4	Nkhata Bay	MSY59	Cultivar 4	Nsanje
MSY16	Cultivar 4	Nkhata Bay	MSY60	Cultivar 4	Nsanje

MSY17	Cultivar 4	Nkhotakota
MSY18	Cultivar 4	Nkhotakota
MSY21	Cultivar 4	Nkhotakota

Table 3 List of wild yam accessions analyzed in this study

Accession Number	Species	Origin	Accession Number	Species	Origin
MH5	**D.dumetorum**	Mangochi	MH3	**D.schimperiana**	Mangochi
MH13	*D.dumetorum*	Mangochi	MH4	*D.schimperiana*	Mangochi
MH14	*D.dumetorum*	Mangochi	MH10	*D.schimperiana*	Mangochi
MH16	*D.dumetorum*	Mangochi	MH7	*D.schimperiana*	Mangochi
MH19	*D.dumetorum*	Mangochi	MH8	*D.schimperiana*	Mangochi
MH22	*D.dumetorum*	Mangochi	MH17	*D.schimperiana*	Mangochi
MH23	*D.dumetorum*	Mangochi	NCU39	*D.schimperiana*	Ntcheu
MH24	*D.dumetorum*	Mangochi	DDZ55	*D.schimperiana*	Dedza
MH25	*D.dumetorum*	Mangochi	DDZ57	*D.schimperiana*	Dedza
MH32	*D.dumetorum*	Mangochi	DDZ58	*D.schimperiana*	Dedza
MH27	*D.dumetorum*	Mangochi	MCU63	**D.schimperiana**	Mchinji
MH29	*D.dumetorum*	Mangochi	MCH68	*D.schimperiana*	Mchinji
NCU30	*D.dumetorum*	Ntcheu	MCH71	*D.schimperiana*	Mchinji
NCU31	*D.dumetorum*	Ntcheu	MCH75	*D.schimperiana*	Mchinji
NCU33	*D.dumetorum*	Ntcheu	KAS78	*D.schimperiana*	Kasungu
NCU35	*D.dumetorum*	Ntcheu	NTC85	*D.schimperiana*	Ntchisi
NCU36	*D.dumetorum*	Ntcheu	DOW88	*D.schimperiana*	Dowa
NCU37	*D.dumetorum*	Ntcheu	DOW89	*D.schimperiana*	Dowa
NCU43	*D.dumetorum*	Ntcheu	DOW90	*D.schimperiana*	Dowa
NCU44	*D.dumetorum*	Ntcheu	DOW93	**D.schimperiana**	Dowa
NCU45	*D.dumetorum*	Ntcheu	DOW94	*D.schimperiana*	Dowa
NCU46	*D.dumetorum*	Ntcheu	DOW99	*D.schimperiana*	Dowa
NCU47	*D.dumetorum*	Ntcheu	LLW101	*D.schimperiana*	Lilongwe
NCU48	*D.dumetorum*	Ntcheu	NCU34	*D.quartiniana*	Ntcheu
MCH65	**D.dumetorum**	Mchinji	NCU40	*D.quartiniana*	Ntcheu
MCH69	*D.dumetorum*	Mchinji	NCU41	*D.quartiniana*	Ntcheu
NTC107	*D.dumetorum*	Ntchisi	NCU42	*D.quartiniana*	Ntcheu
LLW60	*D.dumetorum*	Lilongwe	DDZ61	*D.quartiniana*	Dedza

MCH62	*D.dumetorum*	Mchinji	MH1	*D.odoratissima*	Mangochi
MCH64	*D.dumetorum*	Mchinji	MH8	*D.odoratissima*	Mangochi
MCH70	*D.dumetorum*	Mchinji	MCH66	*D.odoratissima*	Mchinji
MCH72	*D.dumetorum*	Mchinji	MCH76	*D.odoratissima*	Mchinji
MCH73	*D.dumetorum*	Mchinji	ZOM104	**D.bulbifera**	Zomba
MCH77	*D.dumetorum*	Mchinji	ZOM105	*D.bulbifera*	Zomba
NTC111	*D.dumetorum*	Ntchisi	ZOM103	*D.bulbifera*	Zomba
NTC112	*D.dumetorum*	Ntchisi	DOW97	*D.hylophila*	Dowa
NTC113	*D.dumetorum*	Ntchisi	MH28	*D.cochleari-ap*	Mangochi
NTC114	*D.dumetorum*	Ntchisi	MH60	*D. cochleari-ap*	Lilongwe
NTC115	*D.dumetorum*	Ntchisi	MH20	*D.sansibarensis*	Mangochi
NTC116	*D.dumetorum*	Ntchisi	MH21	**D.sansibarensis**	Mangochi
NTC106	*D. dumetorum*	Ntchisi			

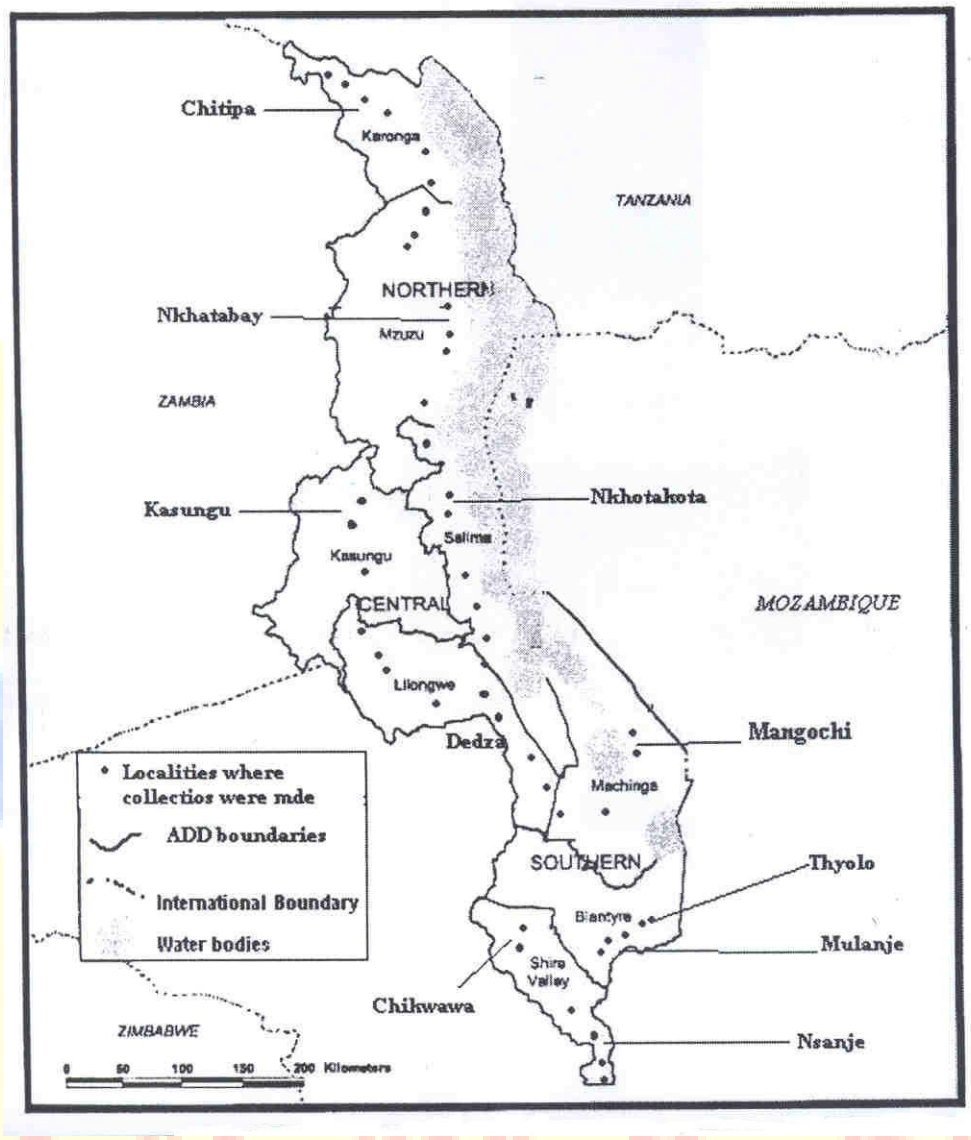

Figure 1: Map of Malawi showing districts from which yam germplasm was collected.

DNA amplification and PCR products detection

Microsatellite amplification reactions were done using nine microsatellite DNA markers which were polymorphic except YM1 (Table 4). The template DNA (2µl) was placed in 0.2ml PCR tube to which 10.5µl of the PCR master mix was added making a final reaction volume of 12.5µl. The PCR master mix cocktail consisted of 5.7µl PCR grade water (ddH_2O), 1µl of 10mM

dNTP mix, 1.25 µl of 10 x PCR buffer, 1.6µl of 25mM magnesium chloride ($MgCl_2$), 0.2µM (0.75 µl) each of both forward and reverse primers, 0.06µl of 5U *Taq polymerase*. The amplification were carried out in a minicycler model PTC-150 (MJ Research Inc, Watertown, USA) with the following PCR conditions: one cycle at 95°C for 5 minutes, followed by 10 amplification cycles, each consisting of a 30s denaturing step at 94°C, 15s annealing step at primer specific temperature, and a 30s extension step at 72°C. This was followed by another 25 cycles, each consisting of denaturing step at 89°C for 30s, annealing step at primer specific temperature for 15s and extension step at 72°C for 15s. The final extension was at 65°C for 20min followed by a soaking temperature of 4°C.

Amplified microsatellite bands were visualized using silver sequence staining procedure on 6% polyacrylamide gels (26 ml of ultra pure water, 7.5 ml of 40% acrylamide:bis (19:1) solution, 5.0 ml of 10 X TBE, 14µl of TEMED (N,N,N',N'-tetramethylethylenediamine), and 357µl of 10% Ammonium persulphate) which were poured in BIORAD Sequi-Gen® GT nucleic acid electrophoresis cell. Two band size standard markers {pGem DNA marker and X174 DNA *Hinf* 1(Promega, USA)} were used to score the bands over a light box.

Table 4 Primer sequences used in this study and their annealing temperatures

Primer	5'-nucleotide sequence	T (°C)
YM-1	TTGTCAGCGAAATAAGCAGAGA-F CAACAGACGCAGCCCAACT-R	57
YM-2	ACTCGACAACTCAATGAAACAAAA-F CGCTGGGGGTGGCTTAT-R	56
YM-5	AATGAAGAAACGGGTGAGGAAGT-F CAGCCCAGTAGTTAGCCCATCT-R	57
YM-13	TTCCCTAATTGTTCCTCTTGTTG-F GTCCTCGTTTTCCCTCTGTGT-R	57
YM-15	TACGGCCTCACTCCAAACACTA-F AAAATGGCCACGTCTAATCCTA-R	55
YM-19	CCACCCTCTACCTCAAGT-F GAGGCTTCTCCCACTAAGT-R	57

YM-26	AATTCGTGACATCGGTTTCTCC-F ACTCCCTGCCCACTCTGCT-R	57
YM-28	GGAGTGCGGGGAGAGGAG-F CGGCGGGAGCTATTGGTGTGT-R	57
YM-30	GGTCCTCTTCTATCCCAACAA-F CACGTATTAACTCCATCTATCCAA-R	57

Data analysis

A number of genetic diversity measures were estimated for each collection of wild and cultivated yam accessions using basic descriptive statistics and POPGENE version 3.2a (Yeh *et al*., 1999) computer software package. Allelic variation was estimated by the number and size range of alleles observed at each locus, the total number of alleles generated, mean number of observed alleles per locus, the number of polymorphic alleles, the frequency of polymorphic alleles, mean number of polymorphic alleles per locus and number of private alleles. Genotype variation was estimated by the number of genotypes assayed per locus, the total number of genotypes observed, the mean number of genotypes assayed per locus and the proportion of heterozygous genotypes within each accession group.

The presence of an amplification product (allele) at a particular locus was recorded as 1 and absence as 0 for all accessions. Based on presence/absence data, Nei genetic distance matrix was compiled by NTSYS-pc 2.11c software (Rolhf, 2001) using Nei's Unbiased genetic distances, to estimate all pair-wise differences in the alleles for all accessions. Based on the distance matrix, multidimensional scaling (MDS) was done to estimate genetic relationship among accessions.

Results and Discussion

Allelic variation

A total of 39 alleles were recorded at nine microsatellite loci among all accessions. Thirty-two and 23 alleles were scored from wild and cultivated yams respectively. The number of alleles were locus-dependant, with a range of one at locus YM-1 to 10 at locus YM-19 and a mean of 4.3 alleles per locus (Table 5). The mean number of alleles per locus in this study (4.3), for both cultivated and wild yams, was lower than what Mignouna et al. (2002) found (9.7) among 45 accession of *D. rotundata* (cultivated yams) indicating lower genetic diversity among both wild

and cultivated yams in Malawi compared to cultivated yams of Cameroun where yams are grown at larger scale. Among wild yams, mean number of alleles varied between species from 2.1 (*D. schimperiana*) to 3.1 (*D. dumetorum*) (Table 6). The mean number of alleles among cultivated yams (*D. alata*) was 2.56 (Table 5). Within-species number of alleles of cultivated yams represented 59% of the total number of alleles illustrating a considerable distinction between cultivated and wild yams with wild yams showing a greater allelic diversity (82%). This observation is further evidenced by wide differences in allelic size ranges between cultivated (64-294 bp) and wild (70-308 bp) yams (Table 6).

Table 5 Number of alleles and size range estimated at 9 SSR loci in wild and cultivated yam groups

SSR Locus	Wild (n = 80)		Cultivated (n = 55)		Combined collection (n = 135)
	A	SR	A	SR	A
YM-1	1	126	1	126	1
YM-2	6	136-206	2	156-206	6
YM-5	2	144-198	2	144-198	2
YM-13	2	94-114	2	94-220	3
YM-15	2	70-86	2	86-116	3
YM-19	9	114-226	4	64-128	10
YM-26	5	138-230	5	120-248	8
YM-28	2	152-172	2	152-172	2
YM-30	3	290-308	3	290-294	4
Total	32		23		39
Mean	3.56±0.82		2.56±0.41		4.33±1.01

Table 6 Number of alleles and size range estimated at 9 SSR loci in dominant yam species

SSR Locus	*D.dumetorum* (n = 41)		*D. schimperiana* (n = 23)		*D.alata* (n = 55)	
	A	SR	A	SR	A	SR
YM-1	1	126	1	126	1	126
YM-2	3	136-206	3	140-164	2	156-206
YM-5	2	144-198	2	144-198	2	144-198
YM-13	2	94-114	2	94-114	2	94-220
YM-15	2	70-86	2	70-86	2	86-116
YM-19	9	114-226	3	114-226	4	64-128
YM-26	5	138-230	2	120-176	5	120-248
YM-28	2	152-172	2	152-172	2	152-172
YM-30	3	290-308	2	290-292	3	290-294
Total	28		19		23	
Mean	3.11±0.52		2.11±0.12		2.56±0.4	
% of total	72		49		59	

Eight of the 9 SSR loci analyzed in this study exhibited allelic variation. The total number of polymorphic alleles detected within the collection was 32, representing 82% of the total allele number. The study revealed variation in the number of polymorphic alleles in wild (25) and

cultivated (12) yams. The frequency of polymorphic alleles between species was estimated at 54% in *D. dumetorum*, 27% in *D. schimperiana* and 31% in *D. alata* with mean polymorphism of 2.63, 1.25 and 1.50 alleles per locus respectively (Table 7). The results indicate that *D. dumetorum*, *D. schimperiana* (wild) accessions were more genetically variable than *D. alata* (cultivated) accessions considering their sample size (Table 6). The low genetic polymorphism observed among the cultivated accessions is in harmony with morphological findings which showed high levels of similarity (70%) among cultivated *D. alata* collection (Msowoya-Mkwaila et al., 2013).

Table 7 Number of polymorphic alleles, frequency of polymorphic alleles and mean polymorphism in selected species of yam

Comparison	*D. dumetorum* (Wild)	*D.schimperiana* (Wild)	*D. alata* (Cultivated)
Number of loci assayed	9	9	9
Number of polymorphic loci	8	8	8
Total alleles	31	20	23
Number of polymorphic alleles	21	10	12
Frequency of polymorphic alleles	54%	27%	31%
Mean polymorphism	2.63	1.25	1.50

Private alleles

Private alleles are unique to a particular population, species or group of individuals. They hold maximum phylogenetic legacy of an evolutionary lineage or any biological entity and can therefore differentiate between populations, varieties or species. A total of 15 and 8 private alleles were recorded in wild and cultivated yam respectively, thereby delineating these two groups (Table 8). At species level, private alleles were scored in *D. alata*, *D. schimperiana* and *D. dumetorum* (Data not shown). At cultivar level within cultivated yam (D. alata), private alleles were scored in Cultivar 1 (Table 2) which contained accession BAR3 (64, 74, 120 bp); Cultivar 2 (Table 2) composed of accessions BAR2, MUN2 and MUN14 (248, 294 bp) and Cultivar 3 (Table 3) with accessions BAR5, BAR6, MUN23, MSY7 and MSY10 (136 bp) (Table

9b). Out of 23 alleles that were documented in *D. alata*, 16 alleles were shared by all accessions, representing a percentage similarity of 70%. However, on the basis of the private alleles and accessions from which they were amplified, four morphotypes were distinguished in *D. alata* which were similar to those identified by cluster analysis using morphological traits by Msowoya-Mkwaila et al. (2013). Cultivar 1 (accession BAR3) with three private alleles 64, 74 (locus YM-19) and 120 (locus YM-26) was morphologically characterized by a simple sagittate leaf, extensive stem branching, purplish tuber flesh, branched tuber and high anthocyanin level. Cultivar 2 (BAR2, MUN2 and MUN14) was identified by private alleles 248 (locus YM-26) and 294 (locus YM-30). This cultivar was defined by numerous roots on tuber surface, short and unbranched tuber, smooth tuber flesh texture and low anthocyanin level. Private allele 136 (locus YM-26) was scored in cultivar 3 which comprised accessions BAR 5, BAR6, MUN23, MSY7 and MSY 10. It was distinguished by long cylindrical tubers and little tuber branching. The rest of accessions belonged to Cultivar 4, which had simple broad leaves, highly branched tubers and very grainy tuber flesh. This cultivar revealed allele 138 bp at locus YM-26. The four morphotypes confirmed by both morphological and molecular characterization are the known genotypes of cultivated yams currently known in Malawi.

Table 8. List of private alleles (bp) in wild and cultivated yam collections

Locus	Wild yam collection	Cultivated collection
YM-2	136, 140, 164	-
YM-13	114	220
YM-15	70	116
YM-19	116, 118, 156, 176, 222, 226	64, 74
YM-26	174, 226, 230	120, 136, 248
YM-30	308	294
Total	15	8

Genotypes

The number of genotypes varied from 18 in cultivated yam to 25 in wild yam. There was considerable variation present in the genotypes observed among wild and cultivated accessions as indicated by high levels of heterozygous genotypes (0.78 in *D. dumetorum* compared to 0.71

in *D. alata*) (Table 9a). Genotypic variation was also observed among cultivars of *D. alata* at loci YM-19, YM-26, YM-28 and YM-30 that resulted in four distinct cultivars (Table 9b). as revealed by morphological analysis (Msowoya-Mkwaila et al. (2013).

Table 9a Genotype number, mean number of genotypes per locus and observed heterozygosity in wild and cultivated yams and selected species

	Genotype number	Mean genotypes per locus	Heterozygosity
Wild yams	25	2.78	81
Cultivated yams	18	1.89	71
D. dumetorum	23	2.56	78
D. alata	18	1.89	71

Table 9b Genotypes that distinguish cultivated *D. alata* accessions at loci YM-19, 26, 28 and 30

Locus	Cultivar 1	Cultivar 2	Cultivar 3	Cultivar 4
YM-19	**64/74**	114/128	114/128	114/128
YM-26	**120**/176	176/**248**	**136**/176	138/176
YM-28	172/172	172/172	152/172	152/172
YM-30	290/292	290/**294**	290/290	290/290

Note: alleles shown in **bold** are unique (private) to that particular cultivar

Genetic relationship among the cultivars

Multidimensional scaling plot (Figure 2) of Nei's unbiased genetic distances among the accessions divided the yams into two distinct groups of wild (A) and cultivated (B) yams probably due to allelic differences existing between the two yam groups as revealed by group specific private alleles (Table 8). The groups conformed to Principal Component Analysis results based on morphology in which cultivated yams were clearly distinguished from wild yams (Msowoya-Mkwaila et al. 2013)

In Figure 2, Group A included accessions of wild species, i.e. *D. dumetorum, D. schimperiana, D. quartiniana, D. cochlearei-apiculata, D. bulbifera, D. odoratissima, D. sansibarensis* and *D. hylophila*. It was divided into two groups A1 and A2. The A1 group included mainly *D.*

dumetorum accessions while A2 was dominated by *D. schimperiana* accessions. A1 plants were vigorous with vines circumnutating to the left. The leaves, petioles and stems were covered with hairs and spines. The leaves were compound palmate with three leaflets borne on long petioles. The tuber flesh was cream yellow in colour. Accessions of other wild species (*D. odoratissima, D. cochleari-apiculata, D. hylophila, D. sansibarensis* and *D. bulbifera*) were in the group because of shared traits such as cream-white tuber flesh and composite leaves (*D. quartiniana*). However their smaller sample sizes compared to *D. dumetorum* (Table 1) meant that little variation among them was captured to delineate them from the rest of *D. dumetorum* accessions in cluster A1. Morphologically, accessions of *D. schimperiana* were vigorous plants with vines twining in anti-clockwise direction. The stems and leaves were covered with silvery gray indumentum. The leaves were large, dull green, ribbed and cordate shaped. The tuber flesh was reddish orange and was fibrous at the proximal end but grainy at the distal end.

Group B consisted of accessions of cultivated yam (*D. alata*), separated into subgroups B1 and B2. The B1 group comprised most of the accessions of cultivated yam, which belonged to cultivars 2, 3 and 4 (Table 2). The B2 group contained accession **BAR** 3 of cultivar 1. B1 subgroup consisted of accessions that had simple cordate shaped leaves. The stems had few branches, and the tuber flesh was white in colour. The anthocyanin level ranged from low to medium. Accession **BAR** 3 was characterized by a simple sagittate shaped leaf, extensive stem branching, purplish tuber flesh, branched tuber and high anthocyanin level. The genetic classification corresponded with cluster analysis results of the same cultivated yams based on morphological traits reported by Msowoya-Mkwaila et al. (2013) which showed BAR3 of cultivar 1 (cluster 1) separated from the rest of the cultivars at 70% similarity.

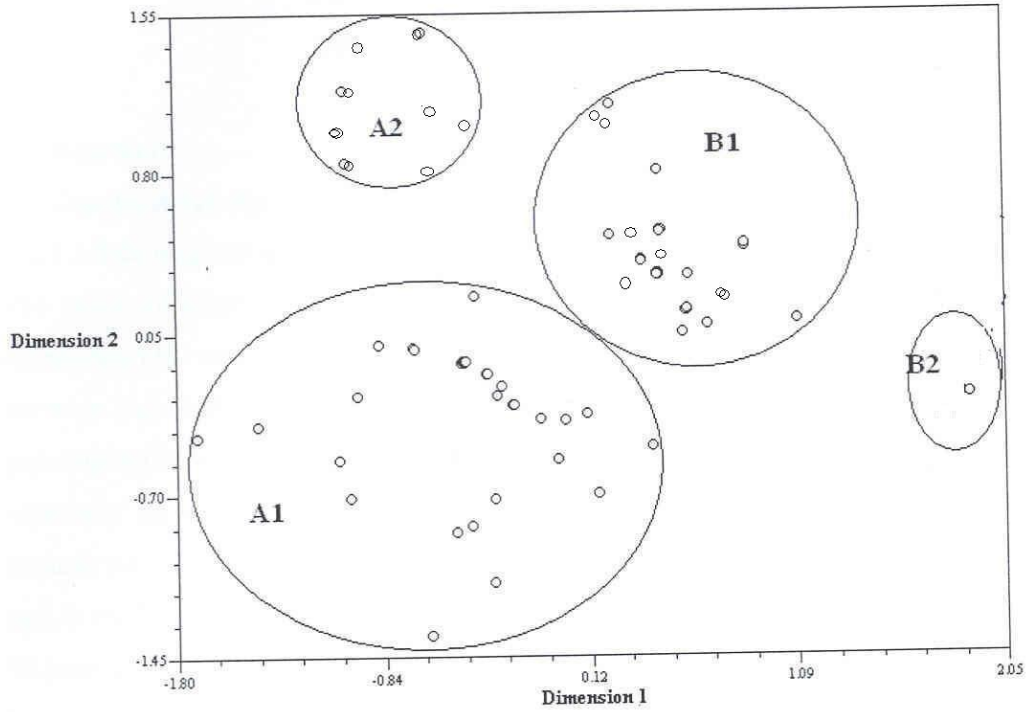

Figure 2 Multidimensional scaling plot of the genetic distance values for individual yam accessions showing distance between and within wild and cultivated yams

Key **A= Wild**

B= Cultivated

A1= Accessions of *D. dumetorum* plus other wild species

A2= Accessions of *D. schimperiana*

B1= Accessions of *D. alata*, cultivars 2, 3 and 4

B2= Accessions of *D. alata*, cultivar 1

Conclusions

This study illustrated the potential of molecular markers for the analysis of genetic resource collections, in addition to morphological descriptors.

The genetic analyses showed that wild and cultivated yams are genetically different and in general, there is considerable genetic diversity among the yam collection base on private alleles which separate wild from cultivated yams. Wild yams are richer in both species and genetic

diversity compared to cultivated yams. The diversity of wild yams provides a valuable genetic resource base for exploitation of useful traits for crop improvement.

Cultivated yams registered a high level of similarity both morphologically and genetically. Nevertheless four genotypes were identifiable at molecular level as genetic units of *D. alata*. Such morphologically and genetically distinct collections should be maintained and conserved as they hold evolutionary heritage and serve as a measure of the extent of genetic diversity of cultivated yams in Malawi.

Acknowledgement

The study was funded by the Rockerfeller Foundation (USA). The authors are also indebted to Mr. E.S. Kathumba of National Herbarium and Botanical Gardens of Malawi and Mr. S. Chitekwere of Bvumbwe Agricultural Research Station for technical assistance offered to the researchers.

References

Coursey DG (1967). Yams. Longmans. London. pp 230

Degras L, Martin FW (1982). Tropical Yams and Their Potential. Part 6: Minor Cultivated *Dioscorea spp*. Science and Education Administration, USDA, Washngton, DC.

Degras L (1993). The Yam: A Tropical Root Crop. The Macmillan Press, London.

Gawal NJ, Jarret RL (1991). Cytoplasmic genetic diversity in banana and plantain. Euphytica 52: 19-23.

Gondwe WT, Ambali AJD, Chitekwere SF, Luhanga R, Msowoya W (2003). Yam (*Dioscorea species*) Germplasm Evaluation for Yield and Quality. Department of Agricultural Research Services. In-House Scientific Conference. September 1-2, 2003, Chitedze Agricultural Research Station, Lilongwe.

Hahn SK, Osiru DSO, Akoroda MO, Otoo JA (1995). Production of yams: Present role and future prospects. International Institute of Tropical Agriculture, Research Guide, 5-30.

IPGRI/IITA (1997). Descriptors for Yam (*Dioscorea* spp.). International Institute of Tropical Agriculture, Ibadan, Nigeria/ International Plant Genetic Resources Institute, Rome, Italy

Malawi Government (1999). Malawi Agricultural and Natural Resources Research Master Plan, National Research Council of Malawi, Lilongwe.

Mignouna, HD (2002). *DNA fingerprinting techniques in white yam*. Genetic Resources and Crop Evolution 47**:** 269-276.

Milne-Redh E (1975). Flora of Tropical East Africa, Polhill pp 1-25.

Msowoya WE (2004). Morphological Characterization of Cultivated and Wild Yams (*Dioscorea spp*) in Malawi. Masters Thesis. University of Malawi.

Msowoya-Mkwaila WE, Changadeya WJ, Ambali AJD (2013). Morphological Characterization of Cultivated and Wild Yam (*Dioscorea* spp) in Malawi. IJPSS *(in press*)

Mwanyambo LM, Kananji SI (2002). Ethnobotanical Inventory of an edible wild tuber, mpama, (*Dioscorea odoratissima* Pax), Malawi National Herbarium and Botanical Garderns, Zomba. unpublished.

National Herbarium and Botanical Gardens of Malawi (2002). Distribution of *Dioscorea* species in Malawi. Zomba. Unpublished.

Onwuema IC (1978). The tropical tuber crops. Yams, Cassava, Sweet potaoes, Cocoyams. John Wiley and Sons, NewYork. 3-100.

Promega Life Science Catalog (2000). 4.8-4.9.

Promega Silver Sequence™ DNA Sequencing System Technical Manual (2000). Madison, USA.

Rholf JR (2001). NTSYSpc Version 2.11c Numerical Taxonomy and Multivariate Analysis System, Exeter Software, New York.

Schultheis RJ, Wilson G (1998). What is the difference between a sweet and a yam. North Carolina Cooperative Extension Service: http://www.ces.ncsu.edu/depts/horthil-23-9.html.

Yeh FC, Yang R, Boyle T (1999). Microsoft window-based freeware for population genetic analysis: Quick user guide. POPGENE version 1.31. University of Alberta/International Forestry Research.