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Genetic and morphological diversity among sweet potato (*Ipomoea batatas* (L) Lam.) accessions from different geographical areas in Malawi

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An understanding of morphological and genetic diversity of sweet potato landraces is fundamental to any breeding program in a country. Fifty-nine sweet potato accessions from three eco-geographical populations of northern, south eastern and southern Malawi were examined using ten Simple Sequence Repeats (SSR) loci and seven International Board for Plant Genetic Resources (IBPGR) descriptors of sweet potato. The study generated a total of 30 alleles with a mean of 3 alleles per locus and a range of 2 to 5 alleles per locus. The primers were highly polymorphic and discriminatory with Polymorphism Information Content (PIC) mean of 0.55 and a range of 0.29 to 0.75, implying that allelic diversity and molecular relationships revealed by the study are strongly supported. Mean Nei' gene diversity (h=0.30) and Shannon information index (I=0.43) showed moderate genetic diversity of the populations with landraces (h=0.32; I=0.47) exhibiting more genetic diversity than introductions (h=0.25; I=0.38). SSR and morphological markers differently distinguished the accessions as evidenced by poor correspondence of genetic and morphological distance matrices (Mantel' Test, r=0.1095). However, cluster analysis indicated high variability among accessions at morphological (50% dissimilarity) and genetic (64% dissimilarity) level. Therefore, Malawian sweet potato landraces harbour considerably high morphological and genetic diversity warranting use in breeding programs.

Key words: Simple sequence repeats (SSR) loci, morphological diversity, sweet potato accessions, genetic diversity, descriptors, polymorphism.

INTRODUCTION

Sweet potato (*Ipomoea batatas* (L.) Lam) is the seventh most valuable staple crop in the world by fresh weight

and fifth in developing countries after rice, wheat, maize, and cassava (FAO, 2004). In sub-Saharan Africa (SSA) the crop is cultivated on more than 3 million hectares, yielding an estimated ca 13 million tonnes annually (Low and van Jaarswels, 2008).

In Malawi, sweet potato is the second important root crop after cassava and most widely grown in the country. Its production increased by 370% from 1995 to 2006 (FEWS/MoAFS, 1995, 2006) indicating the potential of the crop to alleviate poverty among estimated two million low income small holders farmers who cultivate 0.23 hectare of land on average (Malawi Government, 1999). Sweet potatoes are known to be rich in vitamins (A, C, D and E), highly productive with low demand on labour and inputs as well as tolerant to recalcitrant growing conditions, hence, suitable for marginal lands. These attributes render the crop appealing to low income farmers (Sreekanth et al., 2010) resulting in increasing importance of the crop over other crops in recent years in SSA (Walker et al., 2011).

In general, systematic plant breeding and efficient utilization of agricultural inputs has increased crop productivity in the past century (Warburton et al., 2002). However, increased productivity has often resulted in decreased genetic diversity within gene pools (Fernie et al., 2006) due to many compounding factors including inbreeding. This trend is particularly worrisome among vegetatively propagated crops like sweet potatoes and in particular landraces which have a diverse genetic base but are rarely integrated into the plant breeding programs due to their low production performance. This observation necessitates characterization of sweet potato landraces in Malawi in order to inform rational use and conservation of the present sweet potato genetic resources (Fraleigh, 2006).

Identification and release of sweet potato cultivars in Malawi is mainly based on morphological and agronomical characteristics (Chipungu et al., 1999) making morpho-agronomic characterization the main driver of collection and utilization of sweet potato germplasm in any breeding program in Malawi. While sweet potato morphological descriptors have been variously used (Vimala et al., 2012; Norman et al., 2014; Rahman et al., 2015; Amoatey et al., 2016; Mbithe et al., 2016; Su et al., 2016) and proven useful for preliminary evaluation of accessions due to their considerable discriminatory power, the present trend is to use molecular marker based characterization as a complementary tool to validate morphological characterization findings (Changadeya et al., 2012a; Malviya et al., 2012). Molecular markers have increasingly been employed to investigate sweet potato genetic diversity for germplasm conservation and genetic enhancement (Veasey et al., 2008; Karuri et al., 2010; Moulin et al., 2012; Cruz da Silva et al., 2013; Maquia et al., 2013; Camargo et al., 2013; Ochieng et al., 2015; Naidoo et al., 2016).

Therefore, this study was conducted to assess the level of genetic diversity in Malawian accessions using simple sequence repeats (SSRs) molecular markers and validate the degree of relatedness of the morphologically divergent sweet potato accessions from different geographical sources.

MATERIALS AND METHODS

Accessions collection

A total 268 sweet potato germplasm accessions were collected for morphological characterization from the Northern, South Eastern and Southern (Lower Shire) Regions of Malawi (Figure 1 and Table 1). Prior information on areas of high production and varietal diversity obtained from Karonga, Mzuzu, Blantyre and Shire Valley Agricultural Development Divisions (ADDS) offices facilitated the accession collection (Figure 1).

A total of 59 accessions that showed wide morphological distances within and among geographical populations, namely, the North, South East, the Lower Shire Valley and introductions were sampled for further analysis using SSR markers. Sample leaves for DNA analysis were obtained from the Bvumbwe Agricultural Research Station field where the 268 accessions were planted for morphological characterization. DNA analysis was conducted at University of Malawi, Chancellor College, Department of Biological Sciences, Molecular Biology and Ecology Research Unit (MBERU) DNA Laboratory.

Morphological characterization

Detailed comparisons using morphological descriptors (Table 2) aimed at isolation of potential duplicates among the accessions (Huaman et al., 1999) were carried out at Bvumbwe Agricultural Research Station. Sweet potato vines (25 to 30 cm long) were planted and grown following standard procedures. Characterization of above ground morphology of plants started at 80 to 100 days after planting (Mok and Schmiediche, 1998). Seven IBPGR descriptors for sweet potato (Huaman, 1991; CIP et al., 1991) were used for the discriminatory assessment. The descriptors used had a total of 47 different character states (classes) (Table 2). Morphological indicators on roots were done at harvest (5 months after planting). Data was collected from four randomly sampled plants per accession. These descriptors were qualitatively and quantitatively scored (Huaman, 1991).

Genetic characterization

DNA extraction

Total genomic DNA from freshly harvested leaves was extracted using a modified cetyltrimethylammonium bromide (CTAB) procedure (Doyle and Doyle, 1990; Edwards et al., 1991). Four leaf

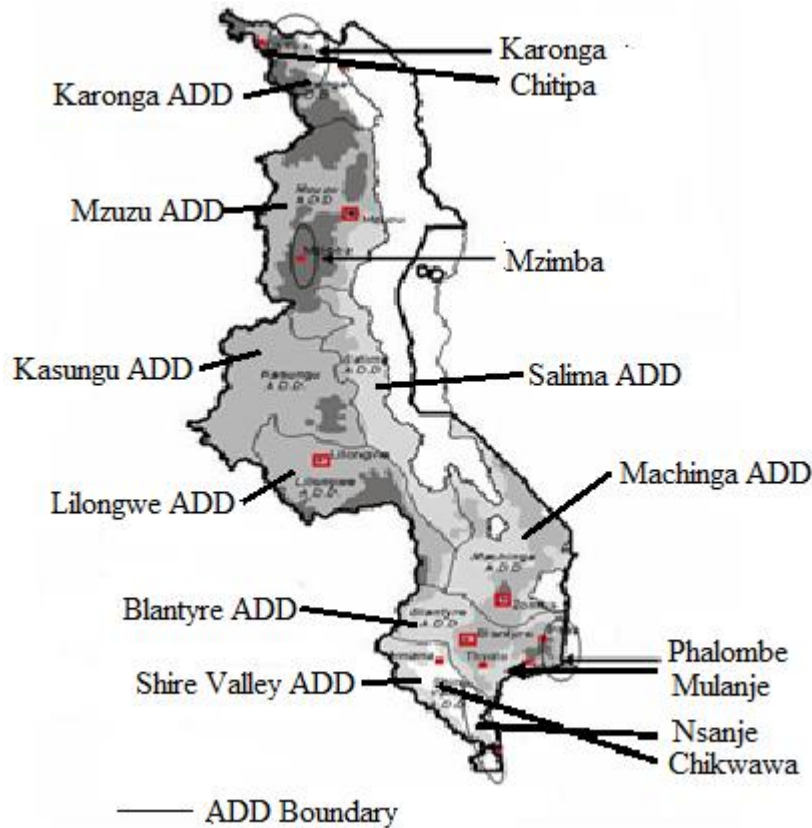*Corresponding author. E-mail: F.chipungu@cgiar.org.

Figure 1. Map of Malawi showing sweet potato collection sites and Agricultural Development Divisions (ADD).

Table 1. Number of sweet potato accessions collected per district and farmer in Malawi.

District	Accessions per district		Number of Farmers per district			Cultivars per farmer	
	Number	%	Interviewed	Donors	%	Mean	Maximum
North							
Chitipa	58	22	20	12	60	4.83	12
Karonga	29	11	20	8	40	3.62	6
Mzimba	46	17	20	13	65	3.54	12
South							
Chikwawa	42	16	20	11	55	3.81	8
Nsanje	77	29	20	12	60	6.41	16
South east							
Phalombe	12	4	20	4	20	3.00	3
Mulanje	4	1	20	2	10	2.00	2
Total	268	100	140	62	44		
Mean	38.26	14.29	20	8.86	44	3.85	8.43
±SD	25.54	-	-	4.34	-	1.40	5.16

SD: Standard deviation.

discs were ground with the aid of carborundum powder in 2 ml microcentrifuge tubes. A total of 500 µl of preheated (60°C) extraction buffer (1.5% CTAB, 100 mM Tris-HCl, 20 mM EDTA, 1.4 mM NaCl, 0.2µl β-mercaptoethanol) was added and the mixture

Table 2. Phenotypic classes of morphological traits used for diversity analysis in sweet potato in seven districts in Malawi.

Character	Abbreviation	Character states	IBPGR/CIP code	No. of classes
Vine inter node length	VL	Very short (<3 cm); short (3-5 cm); intermediate (6-9 cm); Long (10-12 cm); very long (>12 cm)	1, 3, 5, 7, 9	5
Vine inter node diameter	VD	Very thin (< 4 mm); thin (4-6 mm); intermediate (7-9 mm); thick (10-12 mm); very thick (>12)	1, 3, 5, 7, 9	5
Vine tip hairiness	TP	None, sparse, moderate, heavy, very heavy	0, 3, 5, 7, 9	5
Leaf lobe number	LN	1, 3, 5, 7, 9	1, 3, 5, 7, 9	5
Petiole pigmentation	PP	Green; Green with purple near stem; Green with purple near leaf; Green with purple at both ends; Green with purple stripes; Purple with green near leaf; Some petioles purple, others green, totally and mostly purple	1, 2, 3, 4, 5, 6, 7, 8, 9	9
Storage root shape	RS	Round; round elliptic; elliptic; obovate; ovate; oblong; long oblong; long elliptic; long irregular or curved.	1, 2, 3, 4, 5, 6, 7, 8, 9	9
Predominant flesh colour	FC	White; Cream; Dark cream; Pale yellow; Dark yellow; Pale orange; Intermediate orange; Dark orange; Strongly pigmented with anthocyanins	1, 2, 3, 4, 5, 6, 7, 8, 9	9
Total classes	-	-	-	47

incubated at 60°C (water bath) for 60 min. An equal volume (500 µl) of chloroform: isoamyl-alcohol (24:1, v/v) was added and the homogenate mixed on shaker for 20 min. The mixture was centrifuged at 15000 rpm for 15 min in a Tomy high speed microcentrifuge. Thereafter, 450 µl of supernatant was transferred into 2.0 ml microfuge tubes, 100 µl of 20% SDS added, mixed and incubated at 65°C for 10 min in a water bath. Potassium acetate (500 µl; 5 M) was added and mixture incubated at 4°C for 20 min and centrifuged at 15 000 rpm for 10 min. The DNA in the supernatant was precipitated in 700 µl cold isopropanol at -20°C for 1 to 2 h. After centrifugation at 15000 g for 15 min, the alcohol was decanted, and the DNA pellets were rinsed with 70% cold ethanol and centrifuged again for 5 min. The DNA pellets were air dried for 15 min before suspension in 50 µl Tris-EDTA buffer (pH 8.0). The DNA extracts were further purified by repeated phenol-chloroform and chloroform: isoamylalcohol procedures in order to remove PCR inhibitors before resuspension in 50 µl TE after air drying.

DNA amplification and visualisation

The Polymerase Chain Reaction (PCR) using ten SSR primers (Table 3) was carried out in a mini-cycler model PTC-150 (MJ Research Inc, Watertown, USA). PCR final volume for each tube was 13.11 µl, comprising 2 µl of 25 ng/µl genomic DNA, 5.7 µl double distilled water, 1 µl of 10 mM dNTP mix, 1.25 µl of 10X PCR buffer, 1.6 µl of 25 mM Magnesium Chloride (MgCl₂), 0.75 µl of 15 pmol of both forward and reverse primers and 0.06 µl of 5 u/µl *Taq* DNA polymerase stored in buffer A (Promega, 2000), was used.

PCR steps included the following: initial denaturing at 94°C for 2 min, then 30 amplification cycles of denaturing at 94°C for 30 s, annealing at an optimal temperature for a specific primer pair for 15 s and extension at 72°C for 30 s. The final extension was at 72°C for 20 min followed by a soaking temperature of 4°C. The amplified products of PCR were resolved using 6% polyacrylamide gel electrophoresis in BIORAD Sequi-Gen® GT Nucleic Acid Electrophoresis Cell where pGem DNA marker (Promega, 2000) and φ X174 DNA/*Hinf* 1 (Promega, 2000) were used as band size standard markers.

Data analysis

Statistical analysis for loci variability

In order to investigate the genetic variation among sweet potato accessions in the study, the 59 accessions were assigned to five population groups, namely, North (1), South East (2), Shire Valley(3), Landraces (a combination of North, South East and Shire valley) (4) and introductions (5). Allelic variation was estimated by the total number of alleles amplified per loci and population. Polymorphism Information Content (PIC), a measure of variability of each locus was calculated as described by Saal and Wracke (1999):

$$PIC = 1 - \sum_{i=1}^{n} p_i^2$$

where p_i is the frequency of the ith allele out of the total number of alleles at a SSR locus and n is the total number of different alleles for that locus.

Analysis of genetic variation

Owing to difficulty in estimation of exact number of copies of individual alleles among polyploids like sweet potatoes, allelic data is usually analyzed at binary data matrix and SSRs are considered as dominant markers (Lian et al., 2003). Therefore, each allelic band was considered as a binary character and was scored as 1 (present) or 0 (absent) for each sample, hence, generating a data matrix usable in POPGENE freeware version 1.31 (Yeh et al., 1999). Two measures of genetic diversity; Nei's genetic

Table 3. Microsatellite (SSR) primers used in the study.

Primer	Primer sequences (5´-3´)	Primer source	Observed allele size range	Expected allele size range	Ta[1] (°C)
IB-S09	F GCTGCTCAATCCCTCTCCTT R GGAACTCGATACAGCGTGGT	Benavides (2002-2203)	46-52	*	60
IB-S10	F CTACGATCTCTCGGTGACG R CAGCTTCTCCACTCCCTAC	Benavides (2002-2203)	350-396	*	60
IB-R13	F GTACCGAGCCAGACAGGATG R CCTTTGGGATTGGAACACAC	Karuri et al. (2010)	222-226	*	60
IB-R16	F GACTTCCTTGGTGTAGTTGC R AGGGTTAAGCGGGAGACT	Karuri et al. (2010)	218-240	131-237	60
IB-R19	F GGCTAGTGGAGAAGGTCAA R AGAAGTAGAACTCCGTCACC	Karuri et al. (2010)	218-222	190-208	60
IB-CIP-5R	F CCTCAACGAATTTGACCTC R GATGACGGTGTGTCTGAAG	Yanez (2002)	120-128	*	65
IB-242	F GCGGAACGGACGAGAAAA R ATGGCAGAGTGAAAATGGAACA	Buteler et al. (1999)	124-132	95-135	58
IB-286	F AGCCACTCCAACAGCACATA R GGTTTCCCAATCAGCAATTC	Buteler et al. (1999)	100-106	90-122	57
IB-297	F GCAATTTCACACACAAACACG R CCCTTCTTCCACCACTTTCA	Buteler et al. (1999)	126-138	130-200	58
IB-324	F TTTGGCATGGGCCTGTATT R GTTCTTCTGCACTGCCTGATTC	Tseng et al. (2002)	126-134	*	56

Ta[1]=Optimal annealing temperature, *Required information the fields not available.

diversity (h) (Nei, 1973a, b) and Shannon's information index (I) (Lewontin, 1974) were computed in POPGENE.

Pearson's correlation coefficient was calculated to estimate the degree of association among indices. The significance of the coefficients was calculated at $P<0.05$ using the t- statistics (Sokal and Rholf, 1969).

Cluster analysis comparison using SSR and morphological markers

The data on morphological traits and SSR of the 59 accessions were transformed into binary data matrixes. The presence of a SSR allele at a particular locus and a character state in a particular class for morphological traits was recorded as 1 and 0 for present and absent, respectively. Based on the presence/absence, dissimilarity coefficients were generated using the SIMINT module (NTSYS pc 2.11c software (Rholf, 2001)). The default parameter DIST (average genetic distance) was used to generate the binary data matrix. Dendrograms were generated from the sequential, agglomerative, hierarchical, and nested (SAHN) clustering method using the

Unweighted Pair Group Method and Arithmetic Average (UPGMA) (Sneath and Sokal, 1973; Rholf, 2001) using NTSYS pc 2.11. Correlations between similarity matrices from morphological and SSR coefficients were calculated by Pearson's product-moment. The significance of the correlation was tested by Mantel's test (Mantel, 1967) using the NTSYS program (MXCOMP option).

RESULTS AND DISCUSSION

Variation of SSR markers

Number of alleles and size range

The total number and size range of alleles at each locus among the five populations are presented in Table 4. The total number of alleles scored varied among the ten loci and five populations. The highest number scored with reference to all populations was at locus IB-297 (5

Table 4. Number of alleles (A) and size ranges (SR) in base pairs (bp) in 59 sweet potato accessions at ten SSR loci.

Population	Locus IB-R16		Locus IB-324		Locus IB-297		Locus IB-242		Locus IB-286	
	A	SR	A	SR	A	SR	A	SR	A	SR
Shire Valley	2	218-240	3	126-134	5	126-138	4	124-132	4	100-106
South East	2	218	3	126-134	5	126-138	4	124-132	4	100-106
North	2	218-240	3	126-134	5	126-138	4	124-132	4	100-106
*Landraces	2	218-240	3	126-134	5	126-138	4	124-132	4	100-106
Introductions	2	218-240	3	126-134	5	126-138	4	124-132	4	100-106
All populations	2	218-240	3	126-134	5	126-138	4	124-132	4	100-106

Population	Locus IB-R19		Locus IB-R13		Locus IB CIP-5R		Locus IB-SO9		Locus IB-S10	
	A	SR	A	SR	A	SR	A	SR	A	SR
Shire Valley	2	218-222	2	222-226	3	120-128	3	46-52	2	350-396
South East	2	218-222	2	222-226	3	120-128	3	46-52	2	350-396
North	2	218-222	2	222-226	3	120-128	3	46-52	2	350-396
*Landraces	2	218-222	2	222-226	3	120-128	3	46-52	3	350-396
Introductions	2	218-222	2	222-226	3	120-128	3	46-52	2	350-396
All populations	2	218-222	2	222-226	3	120-128	3	46-52	2	350-396

*Landraces = combination of South East, North and Shire valley populations.

alleles) and the least at loci IB-R16 (2 alleles), IB-R19 (2 alleles), IB-R 13(2 alleles) and IB-S10 (2 alleles). A total of 30 allele sizes with a range of two to five alleles and a mean of three alleles per locus were observed in the study. Gichuru et al. (2006) also generated two to five alleles in 57 sweet potato landraces from Kenya, Uganda and Tanzania using four SSR primers. Another study on sweet potato by Kiarie et al. (2016) which used ten SSR markers revealed a total number of alleles of 18 with an average of 3 alleles per locus. Low total numbers of alleles (23) were also recorded among Kenyan sweet potato in a study by Karuri et al. (2010) which employed six SSR markers. The average number of alleles per locus

in their study was 3.67. Such findings from Kenya, which is a secondary centre of sweet potato diversity implies the Malawian accession are equally genetically diverse given that Karuri et al. (2010) genotypes revealed high levels of observed heterozygosity ranging from 0.21 to 1.0. High genotypes diversity among Kenyan sweet potato has been previously observed by other researchers (Gichuru et al., 2006; Njuguna, 2005; Karuri et al., 2009). A study by Roullier et al. (2013c) of 369 landraces in Papua New Guinea, another secondary centre of sweet potato diversity, revealed 16 alleles at six SSR loci with a mean of 6.7 alleles per locus. A higher number of total alleles was however reported by Zhang et al.

(2000) who reported 70 SSR variants from six loci in 113 accessions from three geographic origins, averaging 11.67 variants per loci. The high number of variants generated in this study could be attributed to the large number of accessions and the wide geographical sampling range (Zhang et al., 2000). Random mutations that occur over time as a result of asexual propagation of sweet potato via vines can explain the allelic diversity observed in the present study (Villordon and LaBonte, 1995; Zohary, 2004; Purugganan and Fuller, 2009; Roullier et al., 2011; Roullier et al., 2013b). Such mutations are also the cause of allelic diversity among banana cultivars which are also vegetatively propagated (Changadeya et al.,

Table 5. Polymorphism information content (PIC), Nei's (h) gene diversity measure and Shannon information index (I) at six SSR loci for all sweet potato accessions.

Population	N	Primer PIC values										Mean PIC	± SE	h	I
		IBR-16	IB-324	IB-297	IB-242	IB-286	IB-R19	IB-R13	IB-CIPR5	IB-S09	IB-S10				
Shire Valley	19	0.42	0.66	0.75	0.71	0.46	0.45	0.46	0.63	0.66	0.46	0.57	0.02	0.37	0.49
South East Region	7	0.00	0.60	0.75	0.67	0.67	0.42	0.44	0.62	0.63	0.46	0.53	0.04	0.25	0.36
Northern Region	24	0.38	0.66	0.77	0.69	0.39	0.48	0.46	0.64	0.66	0.47	0.56	0.03	0.32	0.47
Introductions	9	0.30	0.57	0.73	0.69	0.42	0.46	0.47	0.59	0.63	0.38	0.52	0.01	0.25	0.38
Landraces	50	0.37	0.66	0.77	0.70	0.46	0.46	0.46	0.64	0.66	0.47	0.57	0.04	0.32	0.47
Mean	59	0.29	0.63	0.75	0.69	0.48	0.45	0.46	0.62	0.65	0.45	0.55	-	0.30	0.43
± SE	-	0.01	0.04	0.05	0.03	0.06	0.04	0.03	0.02	0.02	0.03	0.04	-	0.05	0.06

SE: Standard error.

2012b). Ultimately, genetic diversity of the studied materials is the most important factor limiting average number of alleles identified per SSR locus during screening. However, factors such as number of SSR loci and repeat types and methodologies employed for detection of polymorphic markers influence allelic differences (Legesse et al., 2007). This study used Polyacrylamide Gel Electrophoresis (PAGE) which is considered second best to Automated Sequencer Capillary Electrophoresis (ASCE) in terms of efficiency of resolving allelic variations at a finer scale than Metaphor® Agarose Gel Electrophoresis (MAGE) (Sanchez-Perez et al., 2006).

Polymorphism Information Content (PIC) of the six SSR loci

A summary of PIC, Nei's gene diversity (h) and Shannon information index (I) is presented in Table 5.

Mean PIC for the primers ranged from 0.29 (IB-R16) to 0.75 (IB-297) with mean value of 55. On average the primers revealed the highest polymorphism in Shire valley and landraces populations (PIC, 0.57) and the lowest in introduction population (PIC, 0.52). The primers mean PIC of 0.55 implies that the loci used in the study were highly polymorphic and discriminatory since any PIC value > 0.5 indicates highly polymorphic locus (Botstein et al., 1980). Therefore, the allelic diversity and molecular relationships in this study are strongly supported. The mean PIC value reported in this study is higher than 0.46, 0.28, 0.39, 0.47, 0.27, 0.42, and 0.36 reported for sorghum (Geleta et al., 2006), cucumber (Danin-Poleg et al., 2001), potato (Ashkenazi et al., 2001), sweet potato (Karuri et al., 2010), sweet potato (Ochieng et al., 2015), sweet potato (Naidoo et al., 2016), sweet potato (Kiarie et al., 2016), respectively. Hao et al. (2006) recommended that any objective evaluation of genetic diversity among germplasm collections needs to consider, both, the number of alleles per locus and their respective PIC values in combination. The PIC values per locus in the

current study showed a significant and positive correlation with the number of alleles per locus (r = 0.81, P < 0.05). The results are consistent with those of Yu et al. (2003) and Jain et al. (2004) in rice (r = 0.62, 0.72, respectively) and by Vaz Patto et al. (2004) in maize (r = 0.85). The findings, therefore, suggest that in general the sweet potato accessions harbour high genetic divergence and the highest are exhibited by landraces and Shire valley accessions and the lowest are in introductions. This observation also indicates that local allelic diversity in landraces can be relied upon in breeding programs other than imported diversity in introductions.

Genetic diversity among geographical populations

Genetic diversity among the populations as measured by Nei's gene diversity (mean h=0.30) measure and Shannon information index (mean I=0.43) showed that the populations were moderately diverse (Table 5). The two indices

were positively and significantly correlated (r=0.84) and the differences among populations for h and I indices were significant at p< 0.05. The indices confirmed the findings from individual population PIC indicating that Shire valley (h=0.37; I=0.49) and Landraces (h=0.32; I=0.47) accessions were the most genetically diverse and introductions (h=0.25; I=0.38) and south east region (h=0.25; I=0.36) accessions were the least diverse (Table 5). Ochieng et al. (2015) in their study of 68 sweet potato accessions and 12 SSR loci reported similarly moderate mean gene diversity (h=0.34). Kiarie et al. (2016) using ten SSR loci on 18 sweet potato accessions recorded moderate gene diversity of 0.41. Crus da Silva et al. (2013) detected moderate mean gene diversity (h=0.27) using RAPD molecular markers on Northeastern Brazilian sweet potato. Similar moderate to low gene diversity values have been registered in other crops such in mulberry population (h= 0.20) (Zhao et al., 2006) and *Medicago citrina* populations (h= 0.15) (Juan et al., 2004). However, some sweet potato studies in some parts of the world, have documented very high gene diversity (h); Mesoamerica (h=0.71), Venezuela-Colombia (h=0.70) and Peru-Ecuador (h= 0.52). Such findings are an indication of the richness of the Latin American gene pool as a centre of sweet potato diversity (Zhang et al., 2000).

Comparison between morphological and SSR data

UPGMA-based cluster analyses on binary data of seven morphological traits and 59 sweet potato accessions are shown in Figure 3. The morphological clustering grouped the accessions into three main clusters A, B and C consisting of a singleton accession in clusters B, 27 in cluster A and 31 accessions in cluster C. The clusters A and C comprised of accessions from all sources under study namely North, South East, Shire Valley and introductions while the singleton cluster contains accession Tchubatchuba from the Northern population. The clusters A and C were further sub grouped to establish any possibilities of the accessions to cluster according to sources of origin.

The composition of sub clusters I, II, III and IV of main cluster A contained accessions from all sources of origins while sub cluster V contained accessions from the Northern population and included Yoyera which was also sampled in the Shire Valley. While sub cluster I of main cluster C contained accessions from all sources of origins, sub cluster II contained accessions from the North including Tsambalimodzi which was also sampled from the Shire valley and an introduction A45, which originates from the Republic of South Africa. All the accessions in sub cluster III of C originated from the Shire valley.

In SSR analysis, a dendrogram for landraces (north, south east and Shire valley) (50) excluding nine introductions was generated. SSR clustering grouped the

50 landrace accessions into two main groups A and B composed of 16 and 34 accessions, respectively (Figure 3). Groups A and B generated sub clusters I to II and I to IV, respectively. The accessions in group A and its sub clusters I and II did not show the tendency to cluster according to the three eco-geographical sources. However, sub clusters I and III of main cluster B grouped accessions according to eco-geographical origins. Sub cluster I contained accessions from the North while sub cluster III contained accessions from the Shire valley.

Generally, morphological clustering of sweet potato was different from SSR clustering in the present study as different clusters contained different accessions. This implies that the two methods distinguished the genotypes in the accessions differently. This was further evidenced by the Mantel (1967) matrix correspondence test that demonstrated that there was low correspondence between the distance matrices generated from SSR and morphological traits (r = 0.1095). Low association between SSR and morphological data has been reported in different crops indicating independent nature of morphological and genetic variation since SSR loci are part of non-coding DNA which is not expressed and therefore not subjected to the same forces of selection which shape phenotypic characters (Kjaer et al., 2004; Vieira et al., 2007).

High variability was detected at both morphological (50% dissimilarity) and genetic (64% dissimilarity) level as expressed in the clustering patterns. However, both cluster (Figures 2 and 3), accessions exhibited some degree of clustering according to eco-geographical associations, suggesting a genetic distinction. This observation is contrary to what Gichuru et al. (2006) showed where morphological clustering was irrespective of geographical origin but SSR analysis tended to cluster Tanzanian landraces together from the Kenyan and Ugandan accessions. The tendency of sweet potato to cluster according to geographical source was also reported using other molecular methods such as random amplified polymorphic DNA, RAPD (Gichuki et al., 2003), Amplified Fragment Length Polymorphism (AFLP) (Zhang et al., 1998) and Selective Amplification of Microsatellite Polymorphic loci (SAMPL) (Tseng et al., 2002). The pattern of some accessions in this study to cluster irrespective of eco-geographical origin implies some similarity among them which could be due to gene flow which is facilitated by long term tradition of sharing vines among farmers as well as recent increased efforts by NGOs to distribute massively sweet potato vines especially during years of drought. Other studies have documented human mediated sweet potato gene flow since prehistorical era (Roullier et al., 2013a).

Conclusions

Morphological and SSR markers displayed considerably high genetic diversity of the sweet potato accessions as
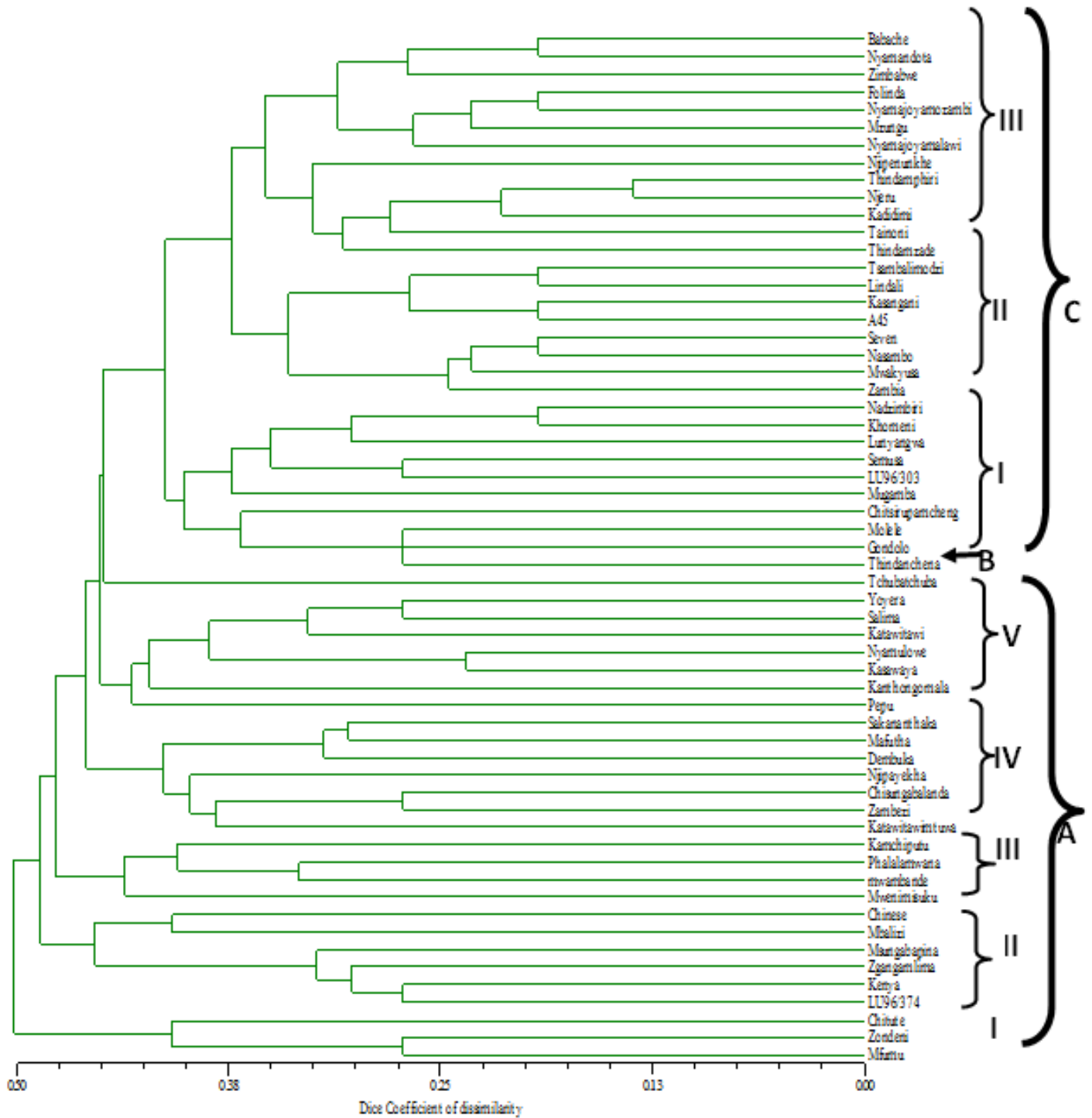
Figure 2. Cluster analysis of 59 sweet potato accessions on seven IBPGR morphological traits.

substantiated by diversity measures used in the study, therefore the landraces can be used in breeding programs. Each method of characterization distinguished the genotypes in the accessions differently thus can be used effectively in any sweet potato characterization program regardless of low correlation between morphological and SSR markers.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.
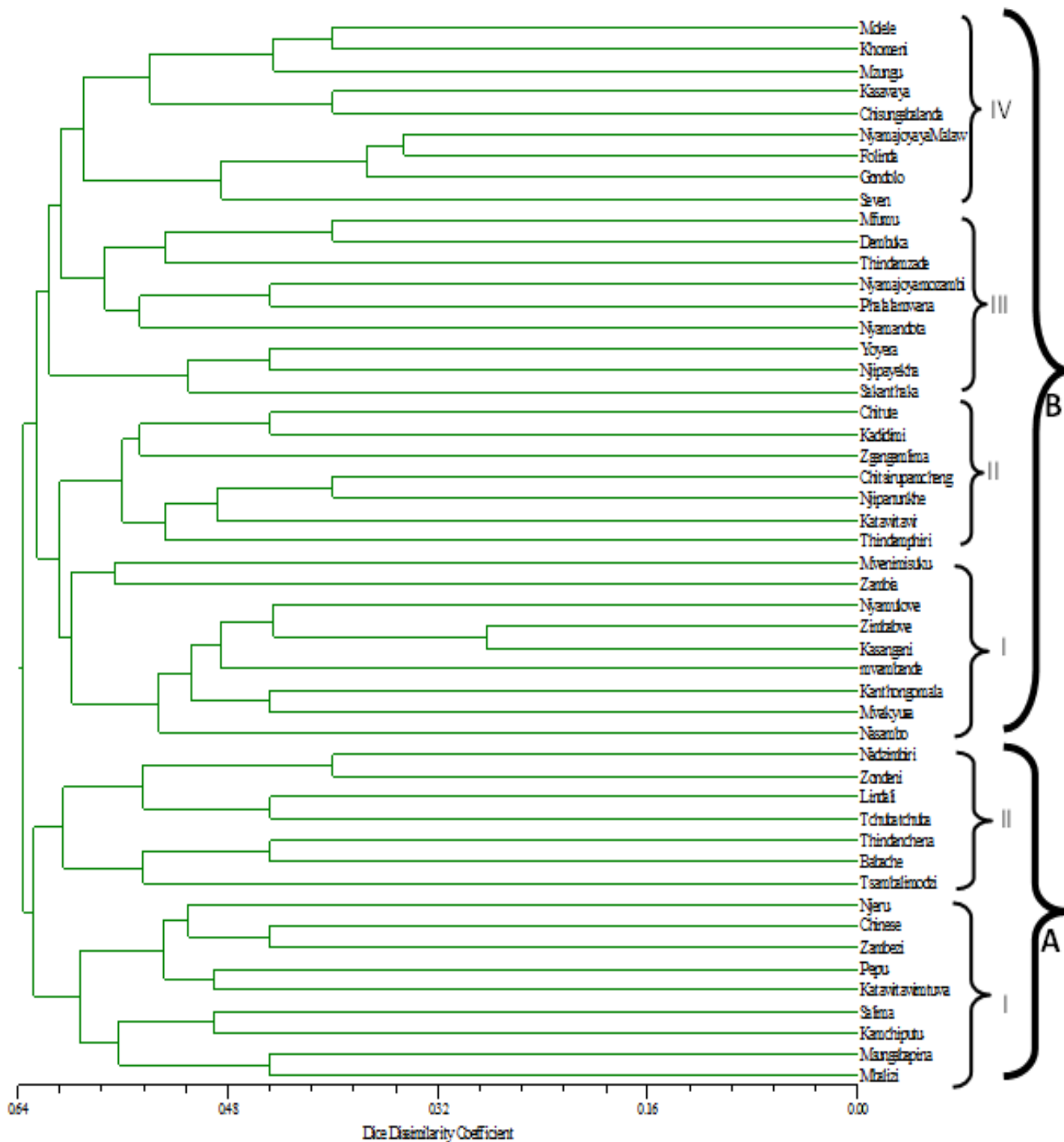
Figure 3. Cluster analysis of 50 landrace sweet potato accessions using ten SSR loci.

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