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Evaluating Diversity among Kenyan Sweet Potato Genotypes Using Morphological and SSR Markers

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ABSTRACT

Genetic diversity of 89 sweet potato genotypes was evaluated using morphological and molecular markers. Eighteen aerial and sixteen storage root characters were used in the morphological characterization. Analysis of variance showed that all the characters evaluated were significantly different ($P<0.01$) between the genotypes. The dendrogram obtained using phenotypic characters separated the genotypes into two major clusters with a Euclidean distance ranging from 0.0 to 6.98. Twenty three unique alleles, ranging from 3 to 6 per locus were detected using six simple sequence repeats (SSR) markers. Cluster analysis showed a Jaccard co-efficient ranging from 0.5 to 1.0 indicating high genetic diversity. Comparison between morphological and molecular data using the mantel test revealed a low correlation ($r = -0.05$) between the two data sets. Despite the poor correlation both techniques showed a high degree of variation among the genotypes suggesting great genetic diversity in Kenyan sweet potato genotypes that can be utilized in breeding programs. © 2010 Friends Science Publishers

Key Words: Genetic diversity; SSR markers; Morphological characters; Sweet potato; Cluster analysis

INTRODUCTION

Morphological characterization has been used for various purposes including identification of duplicates, studies of genetic diversity patterns and correlation with characteristics of agronomic importance (CIAT, 1993). Sweet potato cultivars are generally distinguished on the basis of morphological traits and have a wide variability of botanical characteristics. Morphological and agronomic characters coupled with reaction to pests, diseases and other stresses have been used to characterize sweet potato. Phenotypic characterization in sweet potato is done by assessing variations in the vine, leaf, flower and storage root characteristics (Huaman, 1991) and it has been traditionally used for identification of sweet potato cultivars. Morphological characterization is an important first step in the assessment of sweet potato diversity, but has certain limitations due to morphological plasticity and parallel evolution (Prakash & He, 1996). Therefore, genetic differences exhibited as presence/absence of polymorphisms that exist between accessions can be combined with phenotypic analyses to augment germplasm characterization. Simple sequence repeats (SSR) are considered to be the most efficient markers for genetic diversity studies in many plants (Rakoczy-Trojanowska & Bolibok, 2004) including sweet potato (Zhang *et al*., 2000). This is because of their high levels of allelic variation and their co-dominant character, which means that they deliver more information per unit assay than any other marker system (Rakoczy-Trojanowska & Bolibok, 2004). Successful conservation of any given gene pool is largely dependent on understanding the diversity and its distribution in a given region (Zhang *et al*., 1999). Studying the diversity of important crops enables identification of land marks for *in situ* germplasm conservation, the creation of core genotypes for genetic analysis and the extension of knowledge, useful for breeding programs. Kenyan sweet potato germplasm has been characterized using molecular markers. Njuguna (2005) used ISSR markers to fingerprint 22 popular sweet potato varieties from Kenya, but none of the ISSR primers was able to discriminate the varieties. Gichuru *et al*. (2004) analyzed the diversity among sweet potato cultivars from distinct agro ecologies in Kenya, Uganda and Tanzania using morphological and SSR markers. However, few morphological and SSR markers were used in the study. Kenya being a secondary centre of sweet potato diversity has a wide array of genotypes and it's important to carry out a detailed evaluation of the high genetic diversity. An accurate assessment of the levels of genetic diversity in sweet potato is invaluable for various purposes including identification of diverse parental combinations to develop segregating progenies with maximum genetic variability for further selection (Barrette & Kidwell, 1998). The objective of the current study was to characterize Kenyan sweet potato genotypes using morphological and molecular markers and to compare the efficiency of these two methods in evaluating genetic diversity in sweet potato.

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MATERIALS AND METHODS

Plant material: Sweet potato genotypes (89) were collected in 2007 as vine cuttings from five provinces in Kenya namely; Kakamega, Vihiga, Bungoma and Busia districts in Western province; Homabay, Migori, Kisii and Rachuonyo districts in Nyanza province; Thika and Kirinyaga districts in Central province; Embu, Makueni and Machakos districts in Eastern province and Kwale, Malindi and Kilifi districts in Coast province (Table I). The plants were propagated in a greenhouse at the Kenya Agricultural Research Institute's National Agricultural Research Laboratories (KARI-NARL).

Evaluation of morphological characters: For each genotype, six cuttings were planted in the field in single rows on ridges spaced 1 m apart and 0.3 m within a row. The experiment was conducted at the KARI-NARL farm, which lies at 01º15'S and 36º46'E with an altitude of 1650 m. The site is located in the semihumid climatic zone with a total bimodal rainfall of over 970 mm per annum. The soils are humic nitosols. Morphological characterization of the above and below ground parts was conducted using CIP, AVRDC, IBPGR, (1991) guide at 3 and 5 months after planting, respectively. A total of 34 characters (18 aerial & 16 storage root characters) were evaluated for each genotype (Table II). Eighteen aerial characters were recorded for the vines (Twining, plant type, ground cover, vine internode length & diameter vine color & vine tip pubescence) and leaves (General outline of the leaf, leaf lobes type, leaf lobe number, shape of central leaf lobe, mature leaf size, leaf & petiole color). The storage root characters that were recorded included, storage root shape, root surface defects, root skin and flesh color, root formation, root cracking, latex production and oxidation in roots and quality characteristics of boiled storage root.

DNA extraction and SSR analysis: DNA was extracted from fresh leaves of each genotype using a modified cetyl trimethyl ammonium bromide (CTAB) extraction method (CIP, 2000). PCR amplification was done using six labelled SSR primers (Table III). Each PCR reaction contained 10 pmol μl^{-1} of each primer, 2.5 mM $MgCl_2$, 10 mM dNTPs, 0.1 U Amplitaq Gold Polymerase (Applied Biosystems) and 5X PCR buffer (Applied Biosystems). Amplification was carried out using the Gene-Amp PCR system 9700 (Applied Biosystems) in following thermocycling conditions: 1 cycle of 94°C for 2 min, followed by 15 cycles of 94°C for 1 min, 60°C for 2 min, 72°C for 1.5 min, 94°C for 1 min, 50°C for 2 min and 72°C for 1.5 min and a final extension step of 10 min at 72°C to reduce the probability of false scoring of stutter bands as alleles. The PCR products of each sample were separated on a 2% agarose gel. DNA was quantified by spectrophotometry and visual comparison with lambda DNA on ethidium bromide stained gels. The PCR products were screened by capillary electrophoresis on the ABI 3730 genetic analyzer (Applied Biosystems). The GeneMapper ver. 3.7 software (Applied Biosystems) was used to size peak patterns using the internal Genescan-500 LIZ size standard and Genotyper 3730 (Applied Biosystems) for allele calling.

Data analysis: Cluster analysis was done on standardized morphological data based on the Euclidian distance co-efficient and un-weighted pair group method with arithmetic means (UPGMA) using NTSYS-pc version 2.11T (Sokal & Michener, 1958). Analysis of variance of the morphological characters was done using Genstat statistical package, 8th edition. Sequential, agglomerative, hierarchical and nested clustering parameters (SAHN) programme in Numerical Taxonomy and Multivariate Analysis System (NTSYS) was used to generate dendrograms. Similarity matrix values for SSR data were generated using similarity for qualitative data (SIMQUAL) function of NTSYS based on the Jaccard co-efficient. The dendrogram was generated using UPGMA and employing SAHN program of NYTSYS (Rohlf, 2000). Comparison between morphological and SSR data was performed by calculating the correlation between the two data sets using the mantel test with 250 permutations in the matrix comparison (MxCOMP) program of NTSYS.

RESULTS

Morphological analysis: The genotypes exhibited high morphological diversity in aerial and storage root characters. An analysis of variance showed that all the characters evaluated were significantly different ($P<0.01$) between the genotypes. The dendrogram obtained using phenotypic characters (Fig. 1) separated the genotypes into two major clusters (1 & 2) with an Euclidean distance ranging from 0.0 to 6.98. Cluster 1 contained 64 genotypes and consisted of 4 sub-clusters. The genotypes ALPFS Mbita and KAK/04/2007 did not fall into any sub-group. Cluster 2 contained 25 genotypes and formed two sub-clusters with K9 (1V) being an outlier. The general outline of the leaf and shape of the central leaf lobe separated the genotypes into the two major clusters (1 & 2). Cluster 1 had genotypes that had lobed leaves with a semi-elliptic central leaf lobe, while cluster 2 contained genotypes that had a triangular leaf outline with a toothed central lobe. Storage root characters and geographic locations had no influence in the grouping of genotypes. K16 (1V) and K16 (2V) were the only genotypes that showed morphological similarities. Genotypes that shared a common name clustered together, but showed differences in a single phenotypic character. For instance, Marooko (1), Marooko (2) and Marooko (3) were grouped together in sub-cluster 2A, but they had different predominant vine colour.

SSR analysis: A total of 23 alleles were detected and the number of alleles per locus ranged from 3 for IB-CIP-13, IB-R12 and IB-S07 to 6 for IB-R19 with an average of 3.67 alleles per locus. The polymorphic information content (PIC) of the markers varied from 0.33 to 0.81 with an average of 0.47. Marker IB-R19 revealed the highest PIC of 0.81, while marker IB-S07 had the lowest PIC of 0.33. Observed heterozygosity ranged from 0.21 to 1.0 with a

Table I: Sweet potato genotypes collected from five Kenyan provinces

Genotype	Origin (Province)	Genotype	Origin (Province)
TVT/07/2007	Coast	TVT/09/2007	Coast
TVT/03/2007	Coast	Kanini kaseo	Coast
OP-LNA-006-08	Coast	MLD/05/2007	Coast
Karoti (2)	Coast	TVT/02/2007	Coast
TVT/12/2007	Coast	KARI Mtwapa OP-T21	Coast
MLD/01/2007	Coast	Kikanda (2)	Eastern
Kiazi cha nduma	Coast	Yellow 1	Central
WFTC/02/2007	Coast	KWL/04/2007	Coast
Ys Kemb 10	Western	Farmer 5 Bungoma	Western
ALPFS Were	Western	KAK/07/2007	Nyanza
YS Dada Mowar	Western	YS/05/2005	Western
Marooko (3)	Western	ALPFS 2002/141	Western
SYA/01/2007	Western	Sadak	Nyanza
YS Masaba	Western	TS/01/2007	Western
ALPFS Mbita	Western	YS Sample 2	Western
Big G	Western	YS Nyanguyegwo	Western
MKN/06/2007	Eastern	KKFS Salyboro	Western
MCK/21/2007	Eastern	K16(2V)	Central
KRG/01/2007	Central	Kemb 10 (1)	Nyanza
S2 Msichana Nairobi	Western	S13 Nyatonge (2)	Nyanza
S4 Kuny kibuojo	Western	S5 Nyatonge (1)	Nyanza
S11 (Nyatonge (3)	Western	Polista	Central
K9 (1V)	Western	K9 (2V)	Central
Tainung	Western	S6 Mwavuli	Nyanza
SPK 004 (1)	Western	K16 (1V)	Central
S6 Ondiek chilo	Nyanza	Kemb 23	Central
S1 Amina (2)	Nyanza	K15	Central
Muibai	Central	Bungoma	Nyanza
Kamau (1)	Central	Amina (1)	Western
Naspot	Nyanza	MCK/17/2007	Eastern
MKN/08/2007	Eastern	Mwei umwe (1)	Eastern
MKN/07/2007	Eastern	MKN/04/2007	Eastern
BGM/02/2007	Nyanza	Kikuyu cha kikamba	Eastern
Katumani (2)	Eastern	Mwei umwe (5)	Eastern
KBZ/01/2007	Eastern	WFTC/03/2007	Coast
Kikuyu (3)	Western	Katumani (5)	Eastern
Katumani (7)	Western	Kiluu	Eastern
Ilukwasi	Eastern	MKN/02/2007	Eastern
Kikanda (1)	Eastern	Kikamba (2)	Eastern
SPK 004 (Katumani)	Western	KAK/04/2007	Nyanza
MCK/23/2007	Eastern	Nyakuwili	Nyanza
YS Sopalla	Western	Marooko (1)	Nyanza
KKFS Mwavuli	Nyanza	KKFS NK-L-22	Central
MKN/13/2007	Eastern	Marooko (2)	Nyanza
BSA/02/2007	Westen		

Table II: Vegetative and storage roots characteristics used for evaluation of sweet potato genotypes

Plant Organ	Character Scored
Vine	Twining, plant type, ground cover, vine internode length and diameter, Predominant and secondary vine color, vine tip pubescence
Leaf	General outline of the leaf, leaf lobes type, leaf lobe number, shape of central leaf lobe, mature leaf size, abaxial leaf vein pigmentation, Mature and immature leaf color, petiole length and pigmentation
Storage root	Root shape, root surface defects, root skin color (Predominant & secondary skin color, intensity of predominant skin color), root flesh color (predominant & secondary flesh color, distribution of secondary flesh color), root formation, root cracking, latex production and oxidation in roots, quality characteristics of boiled storage root (consistency, undesirable color, texture & sweetness of boiled storage root).

Source: CIP, AVRDC, IBPGR (1991)

Table III: Sweet potato microsatellite primers used in the study

Marker name	Primer forward 5'-3'	Primer reverse 5'-3'	Repeat motif	Tm (°C)	Expected product size
IB-R03	GTAGAGTTGA AGAGCGAGCA	CCATAGACCC ATTGATGAAG	(GCG)5	73	243-258
1B-S07	GCTTGCTTGTG GTTCGAT	CAAGTGAAGT GATGGCGTTT	(TGTC)7	69	162-178
IB-R12	GATCGAGGAG AAGCTCCACA	GCCGGCAAAT TAAGTCCATC	(CAG)5A	71	303-342
IB-R16	GACTTCCTTGG TGTAGTTGC	AGGGTTAAGC GGGAGACT	(GATA)4	76	201-213
1B-R19	GGCTAGTGGA GAAGGTCAA	AGAAGTAGAA CTCCGTCACC	(CAG)5b	76	190-208
IB-CIP13	CGTGCTTGAGG TCTGAGTAGAA	TTCCCTAGAA GCTGCGTGAT	ACC)3+(CCG)2+(TGC)3+(GTC)2	68	196-373

Table IV: Characteristics of amplified fragments in 89 sweet potato genotypes using 6 SSR markers

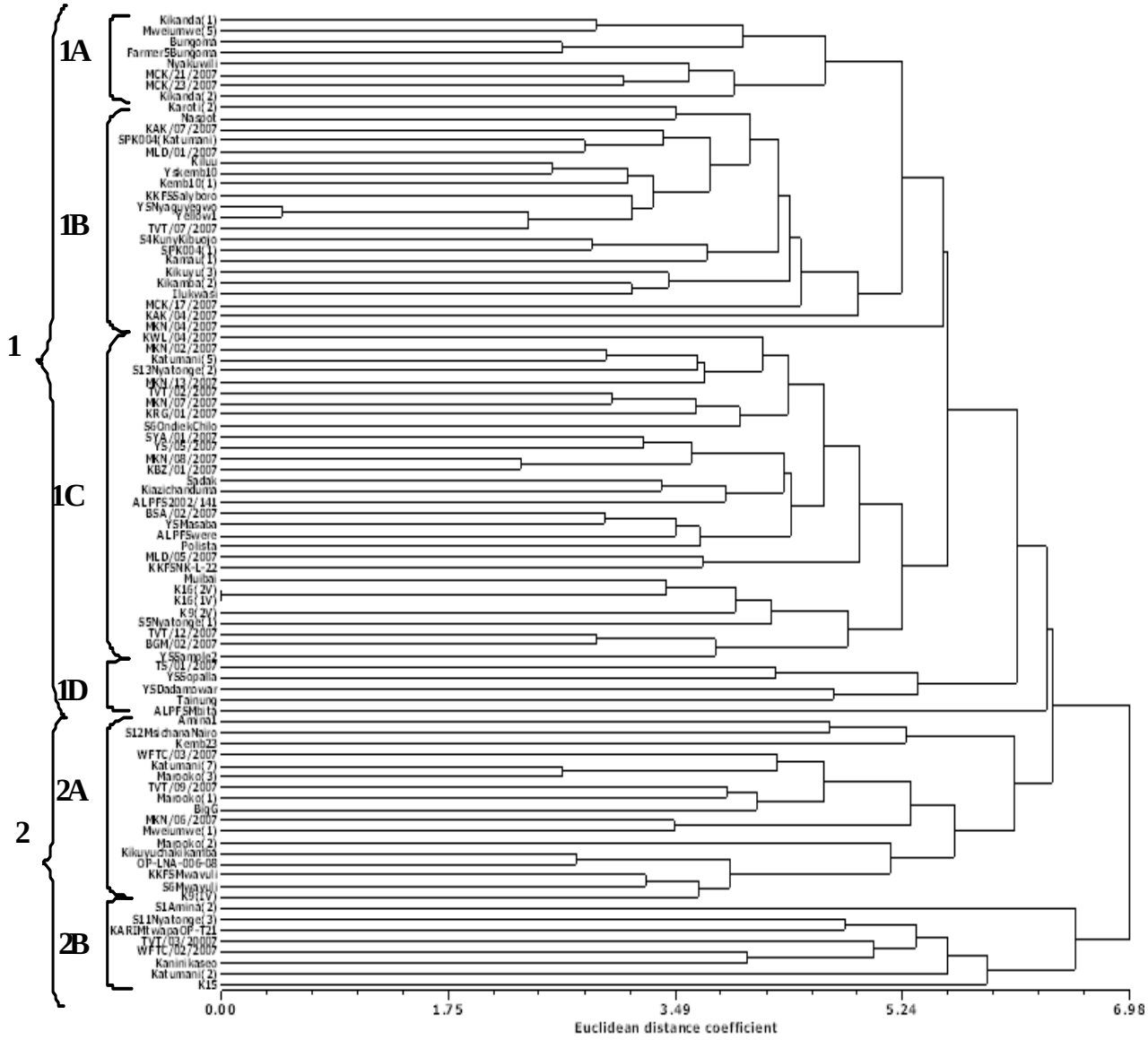
Marker name	Total no of alleles	Allele size range	Abundant Allele (%)	Rare Allele (s) (<=5%)	[a]PIC values	[b]Observed heterozygosity
IB-R16	4	202-214	41.55	None	0.69	0.99
IB-R19	6	190-208	26.62	None	0.81	0.87
IBCIP-13	3	206-374	56.08	206	0.53	0.87
IB-R12	3	318-339	41.18	None	0.65	0.56
IB-SO7	3	175-191	87.91	191	0.33	0.21
IB-R03	4	243-258	29.08	None	0.74	1.00
Mean	**3.83**		**47.07**		**0.47**	**0.75**

[a] PIC=1-Σ(pi^2) (where Pi is the frequency of the i^{th} allele detected) and [b] Frequency at which heterozygous individuals occur in a population at a given locus

mean of 0.75 across the six SSR loci. The highest observed heterozygosity was in marker IB-R03 with a value of 1.0, while the lowest was 0.21 in marker IB-S07 (Table IV). All the 89 genotypes had the same alleles of 206, 374 and 175 at loci IB-R16, IBCIP-13 and IB-S07, respectively. The highest (17) number of alleles across the six loci was observed in genotypes YS Masaba, Amina (1) and MCK/17/2007, whereas the lowest was 10, which was observed in genotypes TVT/02/2007, ALPFS were, Kikanda (1), Kikamba (2) and SPK 004 (Katumani). Dendrogram (Fig. 2) based on UPGMA analysis grouped the 89 genotypes into two major groups A and B and the Jaccard's co-efficient ranged from 0.5 to 1, with an average of 0.75 accounting for 50% variation among the 89 genotypes. Cluster A contained 71 genotypes, while B contained 17 genotypes. The genotypes did not form specific groups according to geographic regions and genotypes that shared a common name did not show genetic similarities {Marooko (1), Marooko (2) & Marooko (3)}.

Comparison between morphological and SSR data: The mantel test showed quite low correlations between morphological and molecular dendrograms (r = -0.05). Both the morphological and genetic analyses allowed separation of the sweet potato genotypes into different clusters and two main groups were formed for each method used. Despite the low correlation between morphological and SSR matrices there were similar grouping of genotypes in the respective dendrograms (sub-cluster A in Fig. 1 & 2). However, there

Fig. 1: UPGMA dendrogram (based on Euclidean distance coefficient) of 89 genotypes generated by morphological characters

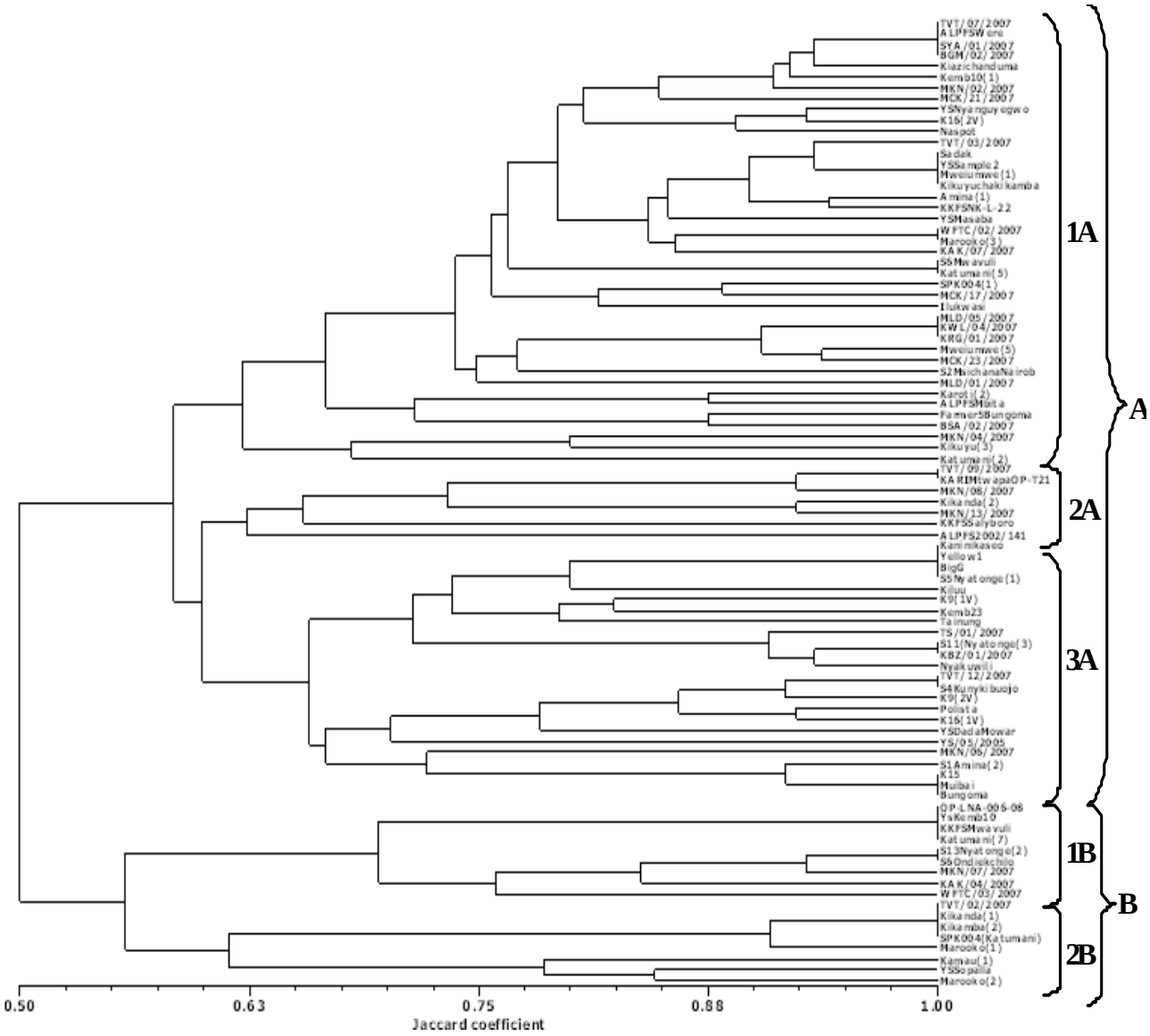


were some discrepancies between the two dendrograms. For instance, the genotypes K16 (1V) and K16 (2V), which were morphologically similar in sub-cluster 1C (Fig. 1) were grouped in separate sub-clusters (sub-cluster 1A & 3A) in SSR analysis (Fig. 2). Similarily, genotypes that appeared similar in SSR analysis had different morphological characters and were clustered in different groups. An example of these genotypes include TVT/07/2007, ALPFS were, SYA/01/2007 and BGM/02/2007, which were grouped together in sub-cluster A (Fig. 2), but they exhibited different morphological characters as indicated by their grouping into sub-cluster 1B and 1C (Fig. 1).

DISCUSSION

A comprehensive analysis of the extent and distribution of the genetic variation in sweet potato is essential for sound genetic conservation strategies. Conservation and sustainable use of genetic resources is essential to meet the demand for future food security. Successful conservation of any given gene pool is largely dependent on understanding the diversity and its distribution in a given region (Zhang *et al*., 1999). In this study, high level of genetic diversity was exhibited in sweet potato. Morphological characters were highly variable among the genotypes studied. This high variability in Kenyan sweet potato genotypes has been previously reported (Gichuru *et al*., 2004; Njuguna, 2005; Karuri *et al*., 2009) and it is a result of natural mutations for traits like root and skin color, leaf and vine characteristics. Accumulation of random mutations results from asexual propagation of sweet potato via stem cuttings and adventitious buds arising from storage

Fig. 2: UPGMA dedrogram (based on Jaccard's similarity coefficient) of 89 sweet potato genotypes generated using SSR markers



roots (Villordon & LaBonte, 1996). The general outline of the leaf and shape of the central leaf lobe allowed separation of the 89 genotypes into two major clusters. These characters are not affected by the environment (Huaman, 1992) and they have been reported to be a major expression of the crop's diversity (Gichuru *et al*., 2004; Tairo *et al*., 2008; Karuri *et al*., 2009). Genotypes that shared a common name {Marooko (1) (2) & (3)} were similar except for the predominant vine color, which is a character influenced by the environment and may not be easily distinguishable by local farmers. The high ploidy level in sweet potato may also be responsible for the variability in qualitative traits due to the increased mutation rates associated with polyploidy (Mogie, 1992).

The six SSR markers were able to discriminate between the different genotypes. Studies have shown that SSR loci give good discrimination between closely related individuals in some cases even, when only a few loci are employed (Powell *et al*., 1996). High levels of genetic diversity are present in Kenyan genotypes as revealed by the high number of alleles observed. This may be due to the mating system of the crop and natural cross-pollination (Ozias-Akins & Jarret, 1994) and the fact that Kenya is a secondary center of sweet potato diversity. A measure of the amount of heterozygosity can be used as a general indicator of the amount of genetic variability in a population. The genotypes used in this study showed high levels of observed heterozygosity ranging from 0.21 to 1.0. Zhang *et al*. (1999) attributes the high levels of heterozygosity in sweet potato to its outcrossing and hexaploid nature. High genetic

variation (50%) was also depicted in the dendrogram, which showed the great genetic variation maintained by local farmers in different regions. The effects of morphological plasticity, which is the capacity of organisms with the same genotype to vary in morphological characters according to varying conditions and parallel evolution, where two organisms are phenotypically similar, but genetically different (Austin, 1997) were evident in some genotypes such as Marooko (1) (2) and (3) (Fig. 1 & 2), which clustered differently in morphological and SSR analyses. There were no defined groups according to geographic regions probably due to the exchange of sweet potato genotypes between farmers across provincial borders.

The mantel test for association among the matrices derived from SSR and morphological data indicated a poor matrix correlation, showing that these methods discriminated very differently among the genotypes. Low correlation between morphological and molecular markers has been reported in many crops (Koehler-Santos *et al*., 2003; Ferriol *et al.,* 2004; Bushehri *et al*., 2005) and these authors suggest that it could be as a result of the independent nature of morphological and molecular variations. The low correlation could also be due to the fact that a large portion of variation detected by molecular markers is non-adaptive and is therefore not subject to either natural or artificial selection as compared with phenotypic characters, which in addition to selection pressure are influenced by the environment (Vieira *et al*., 2007).

CONCLUSION

Morphological and molecular markers revealed high genetic diversity in Kenyan sweet potato genotypes. SSR markers exhibited remarkable discriminatory power and are therefore suitable for genetic diversity analysis in sweet potato. Despite the poor correlation between morphological and molecular markers, both techniques can be used effectively in sweet potato characterization. The genotypes used in this study can be used in breeding programs aimed at improving the crop for various traits of economic importance.

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