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Analysis of the genetic diversity of selected East African sweet potato (*Ipomea batatas* [L.] Lam.) accessions using microsatellite markers

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Sweet potato (*Ipomea batatas* [L.] Lam.) is an economically important crop in East Africa chiefly grown by small holder farmers. Sharing of vines for planting is a very common occurrence among these farmers and eventually varieties are given local names, making it hard to trace the original pedigree. It is therefore important to characterise the sweet potato germplasm for purposes of breeding and germplasm conservation. In this study, 68 sweet potato accessions were evaluated for diversity using 12 microsatellite markers. The genetic relationship of the germplasm was evaluated using the Jaccard's coefficient for dissimilarity analysis, unweighted pair group method with arithmetic means (UPGMA) tree and principal component analysis (PCoA) on DARwin software, while summary statistics was done using PowerMarker and Popgene softwares. The polymorphic information content of the markers ranged from 0.1046 for markers J67b and J67 to 0.3671 for marker J1809a, with a mean value of 0.2723. The total number of alleles amplified was 21. The major allele frequency ranged from 0.5882 for marker JB1809a to 0.9412 for markers J67b and J67c. Cluster analysis divided the accessions into four major clusters. Principle component analysis divided the accession into four groups which were different from those by cluster analysis. This study was able to identify several distinct accessions as well as a few possible duplicate accessions that overlapped on the cluster analysis.

Key words: Sweet potato, cluster analysis, genetic diversity, principal component analysis, Simple Sequence Repeats.

INTRODUCTION

Sweet potato (*Ipomoea batatas*) is a dicotyledonous plant that belongs to the family *Convolvulaceae* (Tortoe, 2010). In many developing countries it is an important food security crop (Korada et al., 2010). In Eastern Africa it is the third most important root crop grown after cassava and Irish potato (FAO 2011). Most sweet potato varieties

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grown in Africa are white, cream or yellow fleshed (Loebenstein and Thottappilly, 2009). Orange fleshed varieties were recently released in many countries and have become popular among the farmers and consumers. Sweet potato is used for human consumption, as a livestock feed, and in industrial processes to make alcohol, starch and other products such as noodles, candy, desserts, and flour (Lebot, 2010, Hazra et al., 2011). Sweet potato brings more income to farmers than any other root crop; both the roots, leaves and tender vines have economic and nutritional values (Antiaobong and Bassey, 2009). About 75% of African sweet potato production is concentrated in East Africa, especially around Lake Victoria where it is a basic subsistence crop (Kapinga et al., 1995; Gibson and Aritua, 2002). In Kenya, sweet potato production is practiced in the western, central and coastal areas of the country. Out of this, over 80% is grown in the Lake Victoria basin (Gruneberg et al., 2004) with Kakamega, Bungoma, Busia, Homa Bay, Rachuonyo and Kisii countries having high acreages of this crop.

Morphological identification (Huaman, 1992) has been widely used to characterise sweet potato accessions (Gichuru et al., 2006; Karuri et al., 2010). Morphological characterisation of parental genotypes for hybridisation schemes is not very efficient due to phenotypic plasticity and environmental effect on morphological traits (Price et al., 2003). According to Naylor et al. (2004), one can use molecular markers as tools to detect the extent and structure of genetic variation; provide insights into the diversity of crop varieties and potential contributions offered by their wild relatives; and to analyze the inheritance of key crop traits (including those that are subject to complex inheritance due to the involvement of numerous genes). Molecular markers concern the DNA molecule itself and, as such, are considered to be objective measures of variation. They are not subject to environmental influences; tests can be carried out at any time during plant development; and, best of all, have the potential of occurring in unlimited numbers, covering the entire genome (de Vicente and Fulton, 2003). Commonly used molecular markers include Restriction Fragment Length Polymorphism (RFLP), Random Amplified Polymorphic DNA (RAPD), Amplified Fragment Length Polymorphism (AFLP), Inter-Simple Sequence Repeats (ISSR) and Simple Sequence Repeats (SSR) (Williams et al., 1990). Such markers are phenotypically neutral, and not influenced by epistatic interactions (Koutita et al., 2005).

Use of microsatellites also called Simple Sequence Repeats (SSR) can be of great help in genetic diversity studies. SSRs are highly variable and evenly distributed throughout the genome (Hajeer et al., 2000). These are short, 2 to 8 nucleotide repeats such as CA or AGC, which are repeated in tandem up to hundreds of times at many independent loci, and are ubiquitous in eukaryote genomes (Lagarcrantz et al., 1993). These markers are easily automated, highly polymorphic, and have good analytical resolution, thus making them a preferred choice of markers (Matsuoka et al., 2002). These polymorphisms are identified by constructing PCR primers for the DNA flanking the microsatellite region (Hajeer et al., 2000; Godwin et al., 2001; Morgante et al., 2001). Since flanking DNA is more likely to be conserved, the microsatellite-derived primers can often be used with many varieties and even other species. Polymorphism is also based on the number of tandem repeat units (Godwin et al., 2001). These repeat motifs are flanked by conserved nucleotide sequences from which forward and reverse primers can be designed to PCR-amplify the DNA section containing the SSR (FAO/IAEA, 2002). SSRs can be exchanged easily between laboratories and multiplex reactions can be run to speed up the assay, where the products have non-overlapping size ranges. It is also possible to amplify SSRs using smaller amounts of DNA. There is a large collection of sweet potato germplasm available in Kenya. The diversity within it is largely unknown since only a few accessions have been characterized in previous studies (Gichuru et al., 2006, Karuri et al., 2010, Yada et al., 2010). If the germplasm is to be utilized in breeding programmes, or if potential duplicates within it has to be identified, then there is a need to undertake further characterization studies. The objective of this study was to characterize selected sweet potato accessions using microsatellite markers.

MATERIALS AND METHODS

Plant material used

A total of 68 sweet potato accessions randomly collected from various sources in East Africa (Kenya and Uganda) were used in this study (Table 1).

DNA extraction

DNA was extracted using a CTAB protocol modified from the Doyle and Doyle (1990) method. The modification involved omission of the ammonium acetate step and a longer DNA precipitation time of 12 h. The quality and quantity of the extracted DNA was checked by running it on a 1% agarose gel and using a nanodrop spectrophotometer. The DNA was then diluted to a working concentration of 30 ng/µl.

Microsatellite markers amplification

Polymerase Chain Reaction (PCR) amplification was done in an Applied Biosystem 2720 Thermo Cycler (Life technologies) using 13 microsatellite primer pairs (Table 2) obtained from Inqaba Biotechnical Industries Ltd. The amplification was performed in a 10µl reaction containing Gotaq Green Master Mix (Thermo scientific), 25 mM $MgCl_2$ (Promega), 10 µM of each primer (Inqaba Biotec), 25 ng DNA working concentration and ddH_20. The pre-amplification conditions were 45 cycles which included (i) initial denaturation at 94°C for 5 min, (ii) denaturation at 94°C for 30 s, (iii) annealing at 51°C for 30 s, (iv) extension at 72°C for 2 min,

Table 1. List of the 68 sweet potato accessions used in the study.

Accession number	Accession name[1]	Origin[2]	Flesh colour[3]
1	52 Nyakisumu	Landrace	Yellow-orange
2	56682-03	Kenya	Cream
3	Kenspot 1	Kenya	Yellow
4	Ejumula x New Kawogo 2	Uganda	Cream
5	Obugi	Landrace	Yellow-orange
6	Amina	Landrace	Orange
7	Ejumula	Uganda	Orange
8	Naspot x New Kawogo 3	Uganda	Yellow-orange
9	Mugande x New kawogo 3	Uganda	Cream
10	Mugande x new Kawogo 4	Uganda	Yellow-orange
11	36 Kalamb Nyerere	Landrace	Cream-yellow
12	Kunyikibuonjo	Landrace	Cream-white
13	Lungabure	Landrace	Cream-white
14	Polo yiengo	Landrace	Yellow
15	Sally boro	Landrace	Orange
16	1-Ujili	Landrace	Yellow
17	Mogesa Gikenja	Landrace	White
18	Naspot x New Kawogo 2	Uganda	Cream
19	Mbita	Landrace	Yellow
20	5-Nyandere	Landrace	Cream-Yellow
21	Odinga	Landrace	Yellow
22	Nangili	Landrace	Yellow-orange
23	Ejumula x New Kawogo 3	Uganda	Yellow
24	Nyarambe	Landrace	Cream
25	SPK 031	Kenya	Orange
26	9-Nduma	Landrace	Purple-cream
27	Nyamuguta	Landrace	Cream-white
28	Wera	Landrace	Yellow
29	Oduogo jodongo	Landrace	White
30	K/KA/2002/12	Kenya	White
31	K/KA/2004/215	Kenya	Yellow
32	Fumbara jikoni	Landrace	Cream
33	K-117	Kenya	White
34	292-H-12	Kenya	Yellow-cream
35	Mwavuli	Landrace	Cream
36	Mugande	Rwanda	White
37	SPK 004	Kenya	Orange
38	29 Kuny kibuonjo	Kenya	Yellow
39	Santo Amaro	Brazil	Cream
40	62 Odhiogo	Landrace	Yellow
41	Naspot x New Kawogo 1	Uganda	Cream
42	Ejumula x New Kawogo 4	Uganda	Yellow-orange
43	Karunde	Landrace	Cream
44	Kibuonjo	Landrace	Cream-white
45	12 Maooko	Landrace	Cream
46	Sinia	Landrace	Yellow
47	Kenspot 2	Kenya	White
48	Kemb 10	Kenya	Yellow
49	Ejumula x New kawogo 1	Uganda	Cream

[1*]All the crosses in this paper are F1 hybrids from a polycross obtained from National Crops Resources Research Institute (NaCCRI), Uganda.[2*]Any accession whose origin we could not determine was assigned as a landrace.

Table 1. Contd.

Accession number	Accession name[1]	Origin[2]	Flesh colour[3]
50	Kenspot 5	Kenya	Orange
51	55 Nganyomba	Landrace	Cream
52	Kenspot 3	Kenya	Orange
53	Nyakagwa	Kenya	Cream
54	24 Kampala	Uganda	Yellow-orange
55	91/2187	Kenya	Yellow
56	Nyawo nyathi odieyo	Landrace	Orange
57	Vita	Landrace	Cream
58	Gachaka	Landrace	Yellow-orange
59	Kenspot 4	Kenya	Orange
60	Naspot 1	Uganda	Yellow
61	Mugande x New kawogo 1	Uganda	Yellow
62	SPK 013	Kenya	White
63	Nyautenge	Landrace	Cream
64	Tainung	Taiwan	Orange
65	Mugande x New kawogo 2	Uganda	Cream
66	Bungoma	Uganda	Cream
67	Alupe-OR	Landrace	Orange
68	Fundukhusia	Landrace	Yellow-orange

and (v) final extension 72°C for 10 min. After amplification, 10ul of each of the amplicons was loaded on a 2% agarose gel (Bioline). Gel electrophoresis was done at a voltage of 80V and a current of 400mA for 1 hour in Tris Borate EDTA buffer. The amplicons were visualised as fluorescent bands under UV light on an Ebox VX5 Transilluminator (Wilber Lourmat). The size of the amplified markers was determined by using O'gene ruler green ready to use 100bp or 1Kb molecular ladder (Thermo Scientific). For each sample, the presence of a band (allele) was recorded as either present or absent.

Statistical analysis

PCR bands (alleles) were scored for all the markers. The data was entered on an excel sheet in a binary form with '0' indicating absence of an allele while '1' its presence. However, for analysis on Popgene the scoring was '2' for presence of an allele and '1' for absence. Any extra amplification on any marker was scored as a separate allele. The data was then analysed using DARwin version 6 software (Perrier and Jacquemoud-Collet, 2006) for Unweighted Pair Group Method with Arithmetic means (UPGMA) tree and Principal Component Analysis (PCoA) while Powermarker version 3 software (Liu and Muse, 2005) was used to compute markers summary statistics. The number of effective alleles was computed using Popgene software (Yeh et al., 1997).

RESULTS

Major allele frequency

The major allele frequency value ranged from 0.5882 to 0.9412 with a mean of 0.7563. Marker JB1809a had the lowest major allele frequency while marker J67b and J67c had the highest major allele frequency (Table 3). These values were quite high with all the values above 0.5. The total number of alleles amplified was 21.

Gene diversity

The gene diversity values ranged from 0.1107 to 0.4844 with a mean value of 0.3384. Markers J67b and J67c had the lowest values while marker JB1809a had the highest value (Table 3).

Polymorphic information content

The PIC values ranged from 0.1046 to 0.3671 with a mean value of 0.2723. Markers J67b and J67c had the lowest values while marker J1809a had the highest value (Table 3).

Effective number of alleles

The number of effective alleles values ranged from 1.0921 to 1.9396 with a mean value of 1.5513. Markers J67b and J67c had the lowest values while marker J1809a had the highest value.

Phylogenetic tree

A UPGMA tree was constructed based on dissimilarity matrix computed using Jaccards coefficient. The

Table 2. List of microsatellite markers and primer pairs used in the study.

Primer	Sequence	Repeat Motif	At (°C)	Reference
IBR03	F GTAGAGTTGAAGAGCGAGCA R CCATAGACCCATTGATGAAG	(GCG)5	53	Benavides (unp.)
IBR12	F GATCGAGGAGAAGCTCCACA R GCCGGCAAATTAAGTCCATC	(CAG)5A	55	Benavides (unp.)
IB242	F GCGGAACGGACGAGAAAA R ATGGCAGAGTGAAAATGGAACA	(CT)3CA(CT)11	54	Buteler et al., 1999
IB275	F GAGTTCCAAAGAGAAGAGTGGAG R AAGCCTACCCGAGAGATAACC	(CT)27	56	Buteler et al., 1999
J175	F ATCTATGAAATCCATCACTCTCG R ACTCAATTGTAAGCCAACCCTC	(AATC)4	54	Solis et al. (unp.)
IB316	F CAAACGCACAACGCTGTC R CGCGTCCCGCTTATTTAAC	(CT)3C(CT)8	55	Buteler et al., 1999
IB324	F TTTGGCATGGGCCTGTATT R GTTCTTCTGCACTGCCTGATTC	*	53	Tseng et al., 2002
IBCIP	F CCCACCCTTCATTCCATTACT R GAACAACAACAAAAGGTAGAGCAG	(ACC)7A	56	Yanez, 2002
IBJ522	F ACCCGCATAGACACTCACCT R TGACCGAAGTGTATCTAGTGG	(CAC)6-7	56	Solis et al. (unp.)
IBS07	F GCTTGCTTGTGGTTCGAT R CAAGTGAAGTGATGGCGTTT	(TGTC)7	53	Benavides (unp.)
J67	F CACCCATTTGATCATCTCAACC R GGCTCTGAGCTTCCATTGTTAG	(GAA)5	56	Solis et al. (unp.)
JB1809	F CTTCTCTTGCTCGCCTGTTC R GATAGTCGGAGGCATCTCCA	(CCT)6(CCG)6	57	Solis et al. (unp.)
IB297	F GCAATTTCACACACAAACACG R CCCTTCTTCCACCACTTTCA	(CT)13	54	Buteler et al., 1999

*At: Annealing temperature.

dissimilarity matrix was computed using 1000 bootstraps. The tree revealed four major clusters (Figure 1). The four clusters had 19, 4, 31 and 14 accessions for clusters I, II, III and IV, respectively.

Principle component analysis

Principal component analysis (PCoA) showed that 68 accessions fell into four major clusters. The first three clusters had 7, 50 and 10 accessions, respectively, while accession 64 formed a cluster of its own (Figure 2).

DISCUSSION

Marker assisted breeding (MAB) is increasingly becoming a crucial part of modern plant breeding in Africa. Genetic diversity using various marker platforms, but more commonly microsatellite markers, is slowly becoming a

Table 3. Table of summary statistics of the 21 alleles amplified in the sweet potato accessions.

Marker	Major allele frequency	Sample size	Allele no.	Availability	ne*	Gene diversity	PIC
IBR03	0.6176	68.0000	2.0000	1.0000	1.8951	0.4723	0.3608
IBR12	0.7794	68.0000	2.0000	1.0000	1.5241	0.3439	0.2847
IB242	0.6471	68.0000	2.0000	1.0000	1.8408	0.4567	0.3524
IB275	0.6765	68.0000	2.0000	1.0000	1.7785	0.4377	0.3419
J175	0.6765	68.0000	2.0000	1.0000	1.7785	0.4377	0.3419
J175b	0.8971	68.0000	2.0000	1.0000	1.1918	0.1847	0.1676
IB297	0.7794	68.0000	2.0000	1.0000	1.4859	0.3439	0.2847
IB316	0.7647	68.0000	2.0000	1.0000	1.5241	0.3599	0.2951
IB324	0.7059	68.0000	2.0000	1.0000	1.7101	0.4152	0.3290
IBCIP	0.6029	68.0000	2.0000	1.0000	1.9187	0.4788	0.3642
IBCIPb	0.8382	68.0000	2.0000	1.0000	1.3349	0.2712	0.2344
IBCIPc	0.7794	68.0000	2.0000	1.0000	1.4859	0.3439	0.2847
IBJ522	0.6029	68.0000	2.0000	1.0000	1.9187	0.4788	0.3642
IBJ522b	0.8971	68.0000	2.0000	1.0000	1.1918	0.1847	0.1676
IBS07	0.7059	68.0000	2.0000	1.0000	1.6741	0.4152	0.3290
J67a	0.6029	68.0000	2.0000	1.0000	1.9187	0.4788	0.3642
J67b	0.9412	68.0000	2.0000	1.0000	1.0921	0.1107	0.1046
J67c	0.9412	68.0000	2.0000	1.0000	1.0921	0.1107	0.1046
JB1809a	0.5882	68.0000	2.0000	1.0000	1.9396	0.4844	0.3671
JB1809b	0.9265	68.0000	2.0000	1.0000	1.1245	0.1362	0.1270
JB1809c	0.9118	68.0000	2.0000	1.0000	1.1577	0.1609	0.1480
Mean	0.7563	68.0000	2.0000	1.0000	1.5513	0.3384	0.2723

ne* = Effective number of alleles.

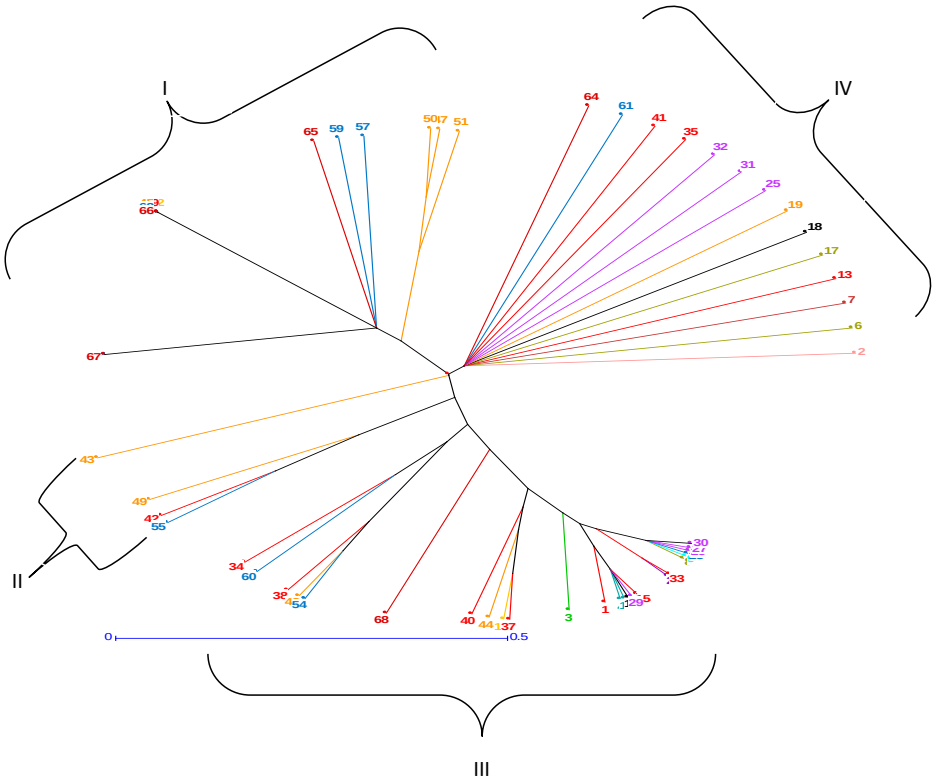

Figure 1. UPGMA tree based on Jaccard's coefficient of dissimilarity.

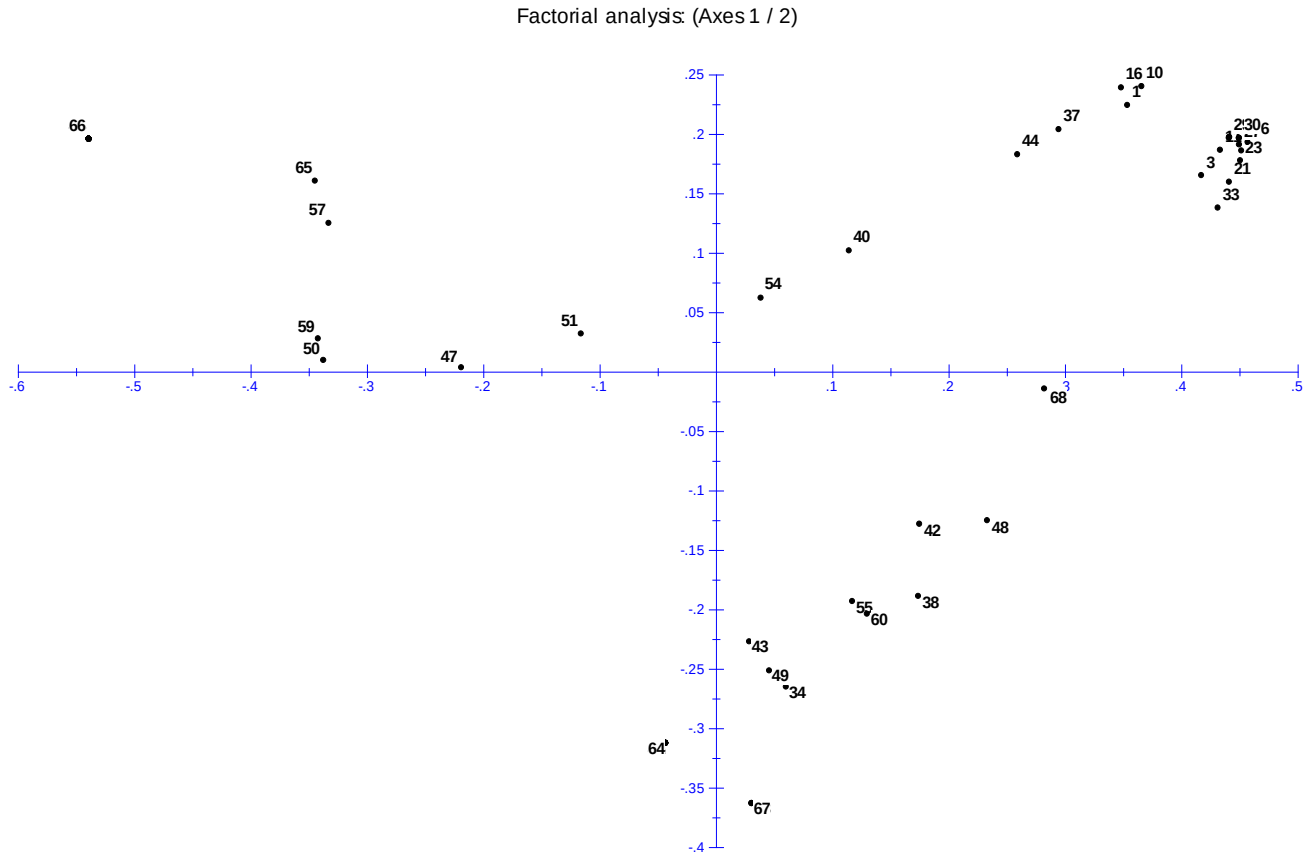

Figure 2. A 1 by 2 factorial analysis (PCoA) diagram of the 68 sweet potato accessions.

common molecular biology tool applied on food crops breeding. The application of MAB increases the efficiency of breeding programs hence reduce the time required to release superior food crop varieties. However, the adoption rate of MAB in breeding programs in most African countries is still very slow due to financial and technical constraints. Genetic diversity studies using molecular phylogenetics form one core application of MAB, most especially on major food crops. Such studies are very important in selecting parents for hybridisation or crossing experiments aimed at improving the food crops varieties. Microsatellite-based genetic diversity studies on East African sweet potato accessions have been done before (Gichuru et al., 2006; Yada et al., 2010; Karuri et al., 2010). In this study, 68 sweet potato accessions were assessed for genetic diversity using 12 primer pairs which amplified a total of 21 alleles.

The UPGMA tree produced four major clusters with cluster three having most accessions overlapping. The tree could only give the general germplasm relatedness and diversity. However, the PCoA gave better resolution in terms of revealing the germplasm relatedness and diversity. From the PCoA the accessions clustered into 4 main axis with accession 64 falling on its own axis. This accession may have clustered alone due to its origin (Taiwan). The PCoA further showed the clustering of the accessions with several accessions overlapping indicating possible duplicates. This is important as identification of duplicates and genetically distinct accessions can help in selecting parents for hybridisation experiments. It is therefore advisable to use both the phylogenetic tree and the PCoA in studying the genetic diversity of germplasm since they complement each other.

The PIC values obtained in this study were quite low since all the values were below 0.5. This might have a direct implication on the discriminatory power of the markers on the accessions used in this study. However, the PIC value of 0.329 for marker IBS07 was higher than the 0.23 obtained by Yada et al. (2010) but lower than the 0.33 obtained by Karuri et al. (2010). The gene diversity values followed the same pattern implying low marker polymorphism. This could be due to the low genetic diversity of sweet potato considering the fact that it is a clonally propagated crop. Another explanation is that farmers in different regions tend to give a particular variety different local name, hence when a breeder collects accessions for hybridization or genetic diversity studies, they might collect the same variety under different names. This means that the breeder might use the same variety as the male as well as the female parent in hybridization leading to inbreeding and low genetic

diversity. It is therefore important to do germplasm characterization before making crosses to determine the genetic diversity of the parental genotypes.

Conclusion

From the results above, SSR markers were successfully used to effectively characterise the selected sweet potato germplasm. The study also revealed that the markers can effectively discriminate the different accessions as seen from the phylogenetic trees and the factorial analysis. Cluster analysis also indicated possible duplicates with several accessions overlapping. It is therefore important to incorporate SSR marker analysis in the selection of genetically distinct germplasm and to identify duplicates in sweet potato germplasm conservation.

Conflict of interests

The author(s) did not declare any conflict of interest.

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Abbreviations: MAS, Marker Assisted Selection; **RFLP,** restriction fragment length polymorphism; **RAPD,** random amplified polymorphic DNA; **AFLP,** amplified fragment length polymorphism; **ISSR,** inter-simple sequence repeats; **SSR,** simple sequence repeats; **PCR,** polymerase chain reaction; **UPGMA,** Unweighted pair group method with Arithmetic means; **PCoA,** principal component analysis.

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