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ORIGINAL RESEARCH ARTICLE

Molecular genotyping of sweet potato (*Ipomoea batatas* L. Lam) accessions using microsatellites

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ABSTRACT

The experiment was conducted to ascertain the level of genetic diversity in sweet potato accessions using microsatellites. Thirty sweet potato accessions obtained from the International Potato Center (CIP), Kumasi, Ghana, Mozambique, and local germplasm of the National Root Crops Research Institute (NRCRI), Umudike, Abia State, Nigeria, as well as sweet potato vines from local farmers' fields in Jos, Plateau State, and Bauchi State, Nigeria, were analyzed for genetic diversity using five microsatellite markers. The results showed that the polymorphic SSR loci revealed diverse relationship among the sweet potato cultivars, which was grouped into four major clusters by unweighted pair group method analysis (UPGMA) method. Cluster analysis showed a Jaccard co-efficient ranging from 0.0 to 3.0 indicating high genetic diversity. The primers detected a total of 18 alleles and the number of alleles per locus was 4 for IBR-19, IBR-286, IBR-297 and 3 for IBR-16 and IBR-242 with an average of 3.67 alleles per locus. The polymorphic information content (PIC) of the markers varied from 0.35 to 0.72 with an average of 0.497. Marker IBR-19 revealed the highest PIC of 0.72, while marker IBR-297 had the lowest PIC of 0.35. Observed heterozygosity ranged from 0.32 to 0.89 with a mean of 0.675 across the five SSR loci. The results from the Analysis of molecular variance (AMOVA) which was used to quantify the diversity level and genetic relationship among the thirty sweet potato accessions indicated that a high diversity was mostly distributed within the populations for sweet potato accessions (75.12%) and (15.67%) among the populations.

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INTRODUCTION

Sweet potato (*Ipomoea batatas* [L.] Lam) is a major crop grown in 119 countries for food, feed, and industrial raw materials (FAOSTAT, 2013). It is a hexaploid crop that is typically propagated clonally through stem cuttings, but true seed production is easily accomplished through open pollination (Martin and Jones, 1986). Sweet potato, (*Ipomoea batatas* (L.) Lam, belongs to the family of *Convolvulaceae* and is the sixth most important food crop worldwide, following rice, wheat, potatoes, maize, and cassava (International Potato Centefr, 2018). This important root crop plays a critical role in food security, especially in developing countries. The origin of cultivated sweet potato is unclear (Rajapakse *et al*., 2004; Roullier *et al*., 2013$_b$). The most recent proposed origin of sweet potato is of autopolyploid origin with *I. trifida* as the sole relative (Munoz-Rodriguez *et al*., 2018). Another hypothesis proposed that *I. batatas* is an allo-autohexaploid ($2n = 6x = 90$), with a $B_1B_1B_2B_2B_2B_2$ genome composition resulting from an initial crossing between a tetraploid ancestor and a diploid progenitor followed by a whole genome duplication event (Magoon *et al*., 1970; Yang *et al*., 2016). Assessment of genetic diversity at the molecular level is

more meaningful than at the phenotypic level as the later involves data on morphological traits, which are environmental dependent. Different molecular marker systems have been successfully employed to assess the genomic stability of regenerated plants regardless of the presence or absence of obvious phenotypic alterations earlier. Molecular markers have been proved to be valuable tools in the characterization and evaluation of genetic diversity within and between species and populations.

Simple sequence repeat (SSR) microsatellites are molecular markers that facilitate rapid differentiation between related individuals because they are abundant and evenly distributed throughout the genome. In addition, due to their high polymorphism rate, co-dominant inheritance and multi-allelic nature, SSR markers are highly reproducibility and require small amounts of DNA (Oliveira *et al*., 2006; Oliveira and Silva, 2008). Several studies with sweet potato culture using SSR markers have observed high genetic diversity among accessions, including Veasey *et al*. (2008) and Koussao *et al*. (2014). 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). Despite being a major producer of sweet potato in Africa, there is a scarcity of information on the genetic diversity of sweet potato germplasm collections in Nigeria. As a result, there is a need to determine the level of genetic diversity of sweet potato accessions in Nigeria in order to ensure continuous germplasm improvement. The study's objective was to assess the level of genetic diversity in sweet potato accessions using microsatellites.

MATERIALS AND METHODS

Plant material

A total of thirty sweet potato accessions were collected from the International Potato Center (CIP), Kumasi, Ghana, Mozambique, and National Root Crops Research Institute (NRCRI), Umudike, Umuahia, Abia State as well local farmers' fields from different locations in Jos, Plateau State and Bauchi State to analyze the level of genetic variability among them. Collections were made at widely separated locations to include all possible diversities but carefully to avoid collection of identical or very closely related clones (Table 1).

Sample collection

The thirty sweetpotato accessions were characterized using five simple sequence repeats (SSR) markers. The primers were obtained from the Africa's Genomic Company, Inqaba, Biotec West Africa Ltd. located in South Africa. The apex of the young sweetpotato leaves were collected using a hand glove, labeled in an envelope and kept at -20^{o}C in an ice pack.

Table 1. Passport data of the sweetpotato accessions used in the study.

S. No.	Accession Code	Local Name	Donor/Source	Country/ Region/ Town
1.	A	FARAAPC	CIP	GHANA
2.	B	44216 PC	CIP	GHANA
3.	C	SANTOMPONA	CIP	GHANA
4.	D	LOCAL X 105193-4	CIP	MOZAMBIQUE
5.	E	LOCAL X 105141-8	CIP	MOZAMBIQUE
6.	F	MUSG 0621-07X105097-12	CIP	MOZAMBIQUE
7.	G	MUSG 0621-07X105193-4	CIP	MOZAMBIQUE
8.	H	MUSG 0621-07 X 105199-29	CIP	MOZAMBIQUE
9.	I	MUSG 0621-07X105141-8	CIP	MOZAMBIQUE
10.	J	MUSG 0621-07X105053-3	CIP	MOZAMBIQUE
11.	K	UMUSPO3 OP	UMUDIKE	NIGERIA
12.	L	CENTEMMAL OP	CIP	GHANA
13.	M	TIS 07/0087	UMUDIKE	NIGERIA
14.	N1	LAMBU	CHA AH	JOS
15.	N2	JANKAROT	K-VOM	JOS
16.	N3	FARIN GANYE	KURU	JOS
17.	N4	JAN IRI	COGON	JOS
18.	N5	DUHU DUHU	MADARA	JOS
19.	N6	BOLOMBOLO	YELWA	BAUCHI
20.	N7	FARI	COGON	JOS
21.	N8	JAN BAWO	GEL	JOS
22.	N9	KANKULE	KURU	JOS
23.	N10	CHIKA KWANDO	TORO	BAUCHI
24.	N11	ZAKI	MADARA	JOS
25.	N12	ZAUNA INUWA	HAIPANG	JOS
26.	N13	YAR TORO	YELWA	BAUCHI
27.	N14	KURU	K-VOM	JOS
28.	N15	DANKALI	FADAMADA	BAUCHI
29.	N16	YELLOW	TORO	BAUCHI
30.	N17	YAR TILDE	TORO	BAUCHI

Table 2. Sweetpotato microsatellite (SSR) primers used in the study.

S. No.	Primer	Primer Sequences (5'- 3') Forward(F) and Reverse(R) Primer sequences	Primer source	Expected size (bp)	Tm (°C)
1	IB-R16	F GACTTCCTTGGTGTAGTTGC R AGGGTTAAGCGGGAGACT	Karuni *et al.* (2010)	131-237	60
2	IB-242	F: GCGGAACGGACGAGAAAA R:ATGGCAGAGTGAAAATGGAACA	Buteller *et al.* (1999)	95-135	60
3	IB-286	F:AGCCACTCCAACAGCACATA R:GGTTTCCCAATCAGCAATTC	Buteller *et al.* (1999)	90-122	57
4	IB-R19	F:GGCTAGTGGAGAAGGTCAA R:AGAAGTAGAACTCCGTCAC	Karuni *et al.* (2010)	109-208	58
5	IB-297	F: GCAATTTCACACACAAACACG R: CCCTTCTTCCACCACTTTCA	Buteller *et al.* (1999)	130-200	58

*bp- base pairs; Tm- annealing temperature.

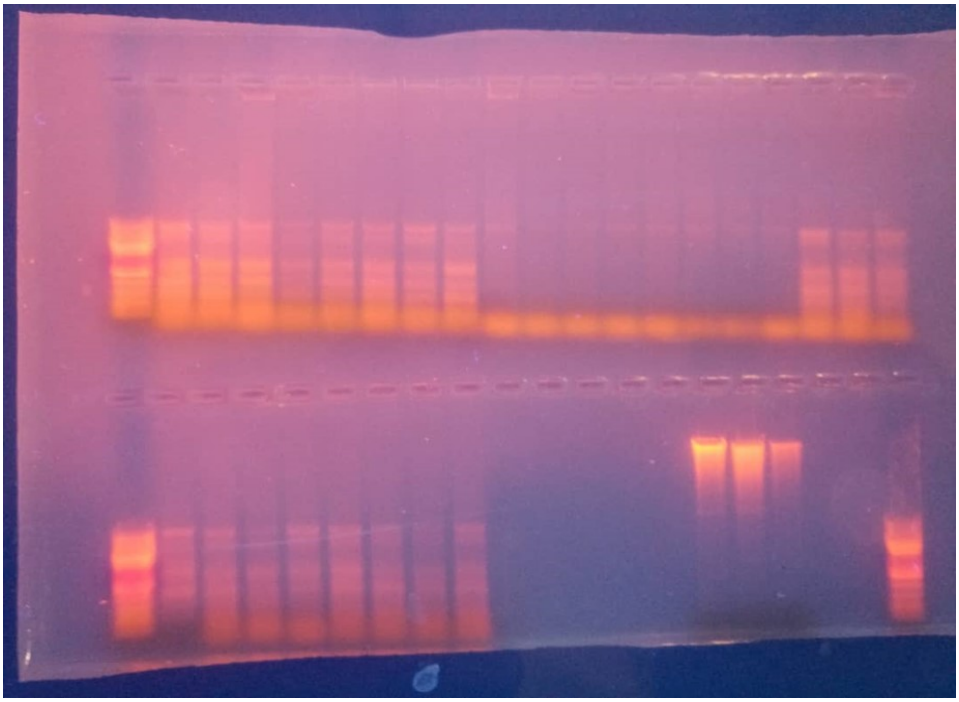

Figure 1. *Plate 3C showing SSR primer IBR-242 image on visual gel.*

Extraction of DNA

DNA was extracted from 500 mg of young leaves collected from each accession using the cetyl trimethyl ammonium bromide (CTAB) method according to Chipungu *et al.* (2017) with minor modifications. DNA samples were stored at -20°C and the genomic DNA were run on a 0.8% agarose gel which was stained by ethidium bromide solution and visualized using UV illumination and documented by Gel documentation system (Alpha Imager). The DNA was quantified by spectrophotometry. The final DNA concentration of each template stock was adjusted to 50 ng/µl.

Simple sequence repeat amplification

DNA samples were quantified and a total of 50 ng of total genomic DNA from each of the samples was used for polymerase chain reactions (PCR). Five pairs of SSR primers (Table 2) were used for the sweet potato DNA amplification reactions. The final volume of reaction mixture of 20 µl containing 10X buffer, 10 mM dNTPs each, 2µM primer, 3 U/µl Taq DNA Polymerase, 50 ng/µl DNA, and ddH2O was used for the PCR. The amplification conditions were as follows: 94°C for 5 min, denaturation at 94°C for 1 min; annealing at between 50.0 and 66.0°C (depending on the annealing temperature of the primer); polymerization at 72°C for 2 min; repeated step 2 to 4 for 30 cycles, and a final extension at 72°C for 5 minutes (Ghislain *et al.*, 2009; Martín *et al.*, 2014). Each reaction was included as a control without the DNA and each primer-template combination was tested. PCR amplifications were performed using a Gene Amp 7200 thermocycler (Applied Biosystems, Foster City, CA, USA) using five SSR primers according to (Cobos and Martin, 2008). Amplification products were analyzed using Gel documentation for the five pairs of SSR primers.

Agarose gel electrophoresis of the PCR products

Agarose of 1% was prepared in 100 ml IxTAE, and microwaved to dissolve the agarose powder and cooled down (50°C), while 2 µl ethidium bromide (10 mg/µl) was added to the melted agarose and mixed well by gentle shaking, the comb was prefixed in the gel and poured into the gel tray. The flask was allowed to cool enough to hold with bare hand and the gel casted was immersed into electrophoresis tank containing 0.5x TAE buffer. The comb in the gel was removed to expose the wells formed.

The reaction of each of the five SSR primers PCR product of DNA fragment was separated using electrophoresis by pipetting 10ul of the amplified PCR product added to 5ul of loading dye and loaded into the wells of the gel. A standard DNA molecular size marker (1 kb plus DNA) was also loaded to determine the band size. The gel electrophoresis was run for 1hr at 100V. After electrophoresis, the gels were visualized on transilluminator UV light, and image captured using Alpha gel documented system.

Scoring and data analysis

Each accession was scored on the gel for the presence and absence of polymorphic band for each primer (Figure 1). The data were entered into a binary matrix as discrete variables, 1 for presence and 0 for absence of the character and this data matrix was subjected to further analysis. The Excel file containing the binary data was imported into NT Edit of NTSYS-pc 2.02J. The band scoring data were used to calculate genetic similarity based on Jaccard's similarity coefficients (Jaccard, 1908). In addition, the Jaccard's similarity coefficient was used to perform cluster analysis based on the Unweighted Pair-Group Means average (UPGMA) method and in constructing a dendrogram (Rohlf, 2000). Polymorphism information content (PIC) were calculated according to Anderson *et al*. (1993) using the following simplified formula: PICi = 1- ∑ p2ij where pi is the frequency of the j^{th} allele for marker i^{th} summed across all alleles for the locus

RESULTS AND DISCUSSION

Characteristics of the sweetpotato microsatellites (SSR) primers

The five SSR primers used in the characterization of the thirty sweetpotato accessions, the primer sequences, primer source, the base pairs and annealing temperature are presented in Table 2. The size range of the amplified products differed according to the primers used, but all were in the range of 90bp-200bp while the annealing temperature of the primer ranged from 57 to 60. However, marker IBR-16 and IBR-242 revealed the highest annealing temperature (60°C) while marker IBR-286 revealed the lowest annealing temperature (57°C).

Polymorphism of microsatellites used for the characterization of the sweetpotato accessions

The DNA analysis of the thirty sweetpotato accessions presented in Table 3 revealed that the five primers detected a high level of polymorphism among the sweetpotato accessions used in the study. The primers detected a total of 18 alleles and the number of alleles per locus ranged from 4 for IBR-19, IBR-286, IBR-297 and 3 for IBR-16 and IBR-242 with an average of 3.67 alleles per locus.

Polymorphic information content (PIC)

The polymorphic information content (PIC) of the markers varied from 0.35 to 0.72 with an average of 0.497. Marker IBR-19 revealed the highest PIC of 0.72, while marker IBR-297 had the lowest PIC of 0.35. Observed heterozygosity ranged from 0.32 to 0.89 with a mean of 0.675 across the five SSR loci. The highest observed heterozygosity was in marker IBR-19 with a value of 0.89, while the lowest was 0.32 in marker IBR-297 (Table 4). However, from the DNA analysis, it is clear that all these accessions are actually different from each other. As DNA markers, SSRs are more advantageous over many other markers, as they are highly polymorphic, highly abundant, genetically co-dominant, and analytically simple.

Table 3. Amplified fragments of sweetpotato accessions used in the study.

SSR Markers	Number of polymorphic alleles	Number of monomorphic alleles
IBR -19	4	0
IBR -16	3	0
IBR -242	3	0
IBR-286	4	0
IBR-297	4	0
Mean	3.67	0

Table 4. Polymorphism of microsatellites used for the characterization of the accessions.

SSR Markers	Polymorphic information content values	Observed Heterozygosity
IBR -19	0.67	0.89
IBR -16	0.72	0.78
IBR -242	0.55	0.84
IBR-286	0.69	0.57
IBR-297	0.35	0.32
Mean	0.497	0.675

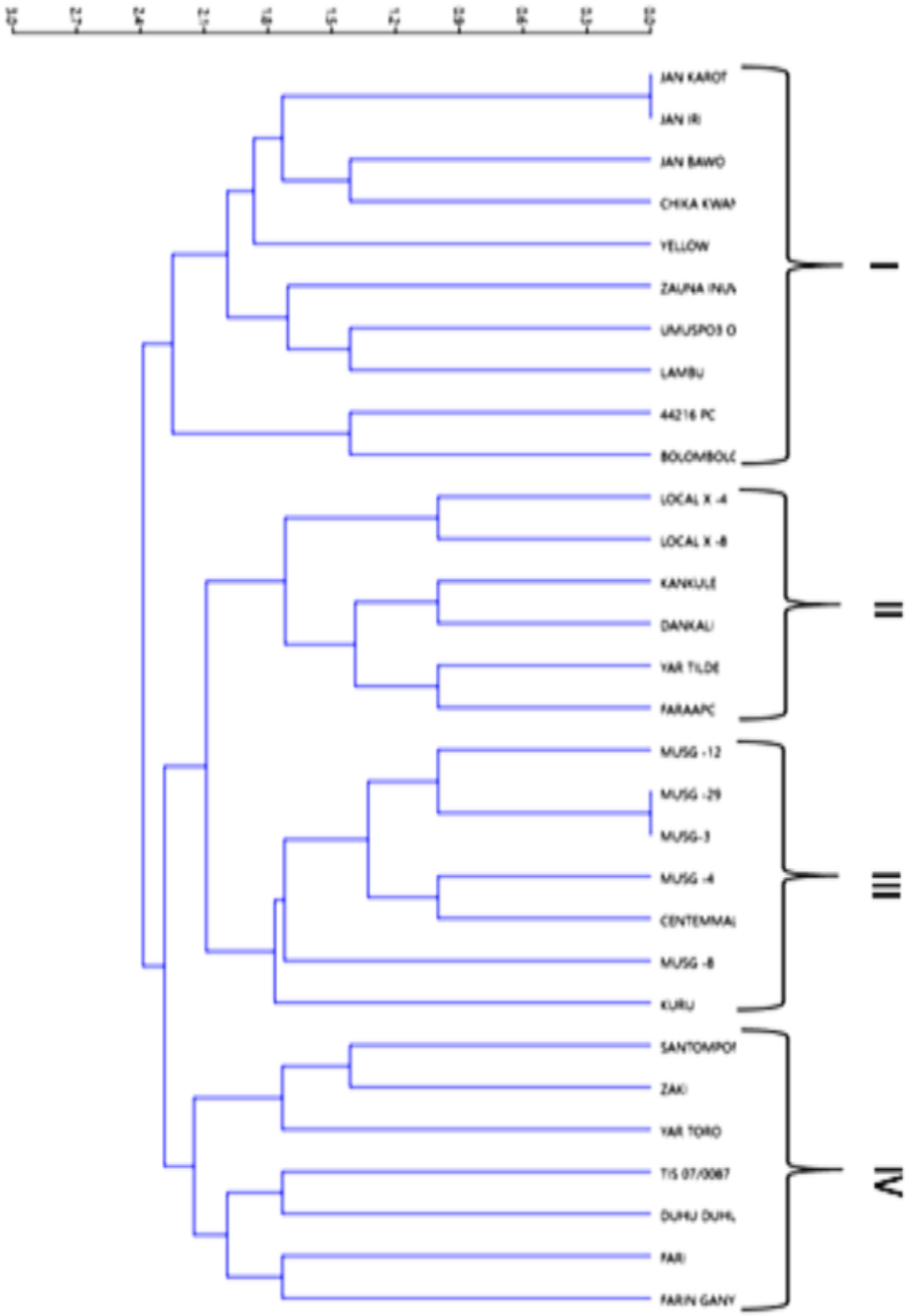

Figure 2. *Dendrogram of sweetpotato accessions based on molecular character using Jaccard's index genetic dissimilarity matrix.*

Cluster analysis

The Jaccard's similarity coefficient was used to perform cluster analysis based on the Unweighted Pair-Group Means average (UPGMA) method and NTSYS-pc software was used for analysis and the resulting clusters were used in constructing a dendrogram (Rohlf, 2000). Based on the UPGMA clustering algorithm from SSR marker, the thirty accessions were grouped into four main clusters (I, II, III and IV) with similarity coefficient of 0.00 to 3.00 (Figure 2). Cluster I had ten accessions and they were BOLOMBO, UMUSPO 3, YELLOW, JANKAROT, JANRI, CHIKA KWANDO, LAMBU, 44216 PC, JAN BAWO and ZAUNA INUWA. Cluster II had six accessions which were LOCAL-4, LOCAL-8, DANKALI, KANKULE, YARD TILDE and FAARAPC. Cluster III contained seven accessions which were MUSG-4, MUSG-12, MUSG-29, MUSG-3, MUSG-8, CENTEMMAL OP and KURU. Cluster IV had seven accessions which were ZAKI, SANTOMPONA, YARTORO, TIS 07/0087, DUHU DUHU, FARI AND FARIN GANYE. However, JANKAROT, JANRI clustered together at a similarity level of 0.00 in cluster 1 while MUSG-29, MUSG-3 clustered together at a similarity level of 0.00 in cluster 111.

Analysis of molecular variance (AMOVA)

To quantify the diversity level and the genetic relationship among the thirty sweet potato accessions, analysis of molecular variance (AMOVA) was done. AMOVA data showed that the majority 75.12% of the diversity were present within populations and 15.67% among the populations of the sweetpotato accessions.

Genetic variability

Extensive diversity exists among cultivated sweet potato both inter or intra variety accessions in terms of morphological, physiological, and agronomic traits. This is the basis for modern cultivar improvement by hybridization. The present study detected a wide range of diversity in cultivated sweet potato at the molecular level among the tested accessions, using the SSR primers. Evaluation of molecular genetic diversity is useful for conservation of genetic resources, identification of cultivars, and the selection of parents for hybridization. A high level of polymorphism was observed, with an average of four polymorphic bands per SSR primer. In the present study, five SSR primers detected a total of 18 alleles per locus which ranged from 3 to 4 with an average of 3.67 alleles per locus.

These results were comparable with Tumwegamire *et al.* (2011) who analyzed 92 African accessions with 26 SSR markers and found a mean value of 6.1 alleles per locus ranging from 2 to 11. Similarly, Gwandu *et al.* (2012) analyzed 57 sweet potato genotypes in Tanzania with 4 SSRs and found alleles from 11 to 22. The SSR marker analysis of the 23 loci showed a total of 255 alleles, ranging from 4 to 25 alleles per locus with a mean value of 11.08 alleles per locus (Rodriguez-Bonilla *et al.*, 2014). The high number of alleles found in sweet potato can be explained by the hexaploidy nature of sweet potato. High level of polymorphism was observed in this study. A measure of the amount of heterozygosity can be used as a general indicator of the amount of genetic variability in a population. Polymorphic information content (PIC) is a measure of the discriminatory capacity of a marker (Jia *et al.*, 2009). According to Heng-Sheng *et al.* (2012), a PIC value greater than 0.5 is high, and any marker with such value can be effectively used in genetic diversity studies. The polymorphic information content (PIC) of the markers varied from 0.35 to 0.72 with an average of 0.497. Marker IBR-19 revealed the highest PIC of 0.72, while marker IBR-297 had the lowest PIC of 0.35. The highest observed heterozygosity was in marker IBR-19 with a value of 0.89, while the lowest was 0.32 in marker IBR-297.The observed heterozygosity which ranged from 0.32 to 0.89 with a mean of 0.675 across the five SSR loci indicated a very high discriminating power of the SSR markers used. These values are greater than range and mean of 0 – 0.88, and 0.62 as reported by Somé *et al.* (2014). Based on the number of unique alleles and the PIC values, all the SSR markers that showed amplification were very effective in discriminating among the sweetpotato accessions. This is in agreement with the study conducted by Kiarie *et al.* (2016) who used ten SSR markers, and detected a total of 18 alleles with an average of 3 alleles per locus. A study conducted by Roullier *et al.* (2013) who used 369 landraces revealed 16 alleles at six SSR loci with a mean of 6.7 alleles per locus.

The high number of variants generated in this study could be attributed to the large number of accessions and the wide geographical sampling, (Zhang *et al.*, 2000). In sweetpotato, high levels of heterozygosity and genetic diversity could be explained by the out crossing and self-incompatible nature of the plant, (Zhang *et al.*, 1999). For instance, this self-incompatibility in the field might result in chance seedlings from crossings, providing another path to increase genetic diversity (Yada *et al.*, 2010). Random mutations that occur over time as a result of asexual propagation of sweetpotato using vines could explain the allelic diversity observed in the present study. This is in agreement with the findings of Zohary (2004); Roullier *et al.* (2011) and Roullier *et al.* (2013). Microsatellites have become one of the most widely used molecular markers in recent years. As DNA markers, SSRs are more advantageous over many other markers, as they are highly polymorphic, highly abundant, genetically codominant, and analytically simple. However, accessions that are usually difficult to be identified morphologically or agronomical because of their similar growing habits, plant height, leaf shape, pod shape, and so on, could be distinguished from one another by the SSR markers. Therefore, from the study SSR markers are very useful molecular markers in sweet potato genetic studies and in large scale breeding programs.

Genetic structure and analysis of molecular variance

The AMOVA results indicated that the high diversity revealed in the study was mostly distributed within the population for sweet potato accessions (75.12%) and (15.67%) among the populations. A similar result (64.4% variation within households) was obtained by Veasey *et al.* (2007) when studying morphological traits of the sweet potato accessions. This same pattern of a greater portion of variation being due to differences between accessions within households has also been reported for cassava (Sambatti *et al.*, 2000), as well as within groups or regions (Faraldo *et al.*, 2000; Cabral *et al.*, 2002). Furthermore, Zhang *et al.* (2004) found a highly significant variation of 86.7% within regions and 12.4% among regions when studying 75 sweet potato landraces from Latin America and Pacific areas with AFLP markers. Tseng *et al.* (2002) observed similar results with UPGMA clustering in the genotyping and assessment of genetic relationships among elite polycross breeding cultivars of sweet potato in Taiwan using SAMPL polymorphisms

Conclusion

In conclusion, SSR markers successfully evaluated the genetic relationships among the sweet potato accessions used in the study and generated a high level of polymorphism. The results of the present study will be useful for the management of germplasm, improvement of the current breeding strategies and for the release of new cultivar.

Conflict of interest

The authors declare no conflict of interest.

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