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MOLECULAR DIVERSITY OF ORANGE-FLESH SWEET POTATO (*Ipomoea batatas* [L.] Lam) GENOTYPES USING SIMPLE SEQUENCE REPEATS (SSR) MARKERS

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ABSTRACT

All breeding programs aimed at developing new and improved cultivars with desired traits primarily rely on information related to genetic diversity. Molecular characterisation of plant material has been fundamental for crop improvement, and genetic diversity has long been recognised as vital for the rational management and utilisation of crops. In this study, twenty-seven (27) genotypes of orange-fleshed sweet potato (*Ipomoea batatas* [L.] Lam) and ten (10) simple sequence repeat (SSR) markers were used to assess genetic diversity. The ten SSR markers proved highly informative in discriminating among the genotypes. These markers exhibited moderate average values for the number of alleles per locus (3.7), with a total of 282 alleles detected across all loci. Additionally, polymorphic information content (PIC) values were high (PIC > 0.50), indicating strong marker informativeness. Using the unweighted pair-group method with arithmetic averages (UPGMA) based on similarity matrices, the 27 genotypes were clustered into eight primary groups at a similarity coefficient of 0.84. The findings of this study demonstrate that SSR markers can effectively estimate genetic diversity and analyse phylogenetic relationships. Moreover, these markers can assist sweet potato breeders in selecting desirable quantitative traits within their breeding programs.

Keywords: Genotypes, Genetic Diversity, Molecular Markers, Simple Sequence Repeat, Sweet Potato

INTRODUCTION

Sweet potato (*Ipomoea batatas* (L.) Lam) is an important root crop grown in more than 115 countries in the world (FAO, 2019). Sweet potato worldwide production of 2020 was 89,487,835 tons, with area of production 7,400,472 ha (FAO, 2022). Asia is the largest (62.6%) producer, followed by Africa 32.2% (FAO, 2019). In West African production data, Nigeria comes first with 3,867,871 tons (FAO, 2022). The rainy and dry seasons support its production, but its growth is mostly supported by the rainy season in Nigeria. In sub-Saharan Africa, sweet potato is a major food crop renowned for its role in market value as well as in food security, nutritional and health benefits (Agili *et al.*, 2012 and Arancibia *et al.*, 2014). Generally, sweet potato ranks as the seventh most important food crop in terms of production worldwide, seventh in digestible energy production, sixth in dry matter production, and fifth economically (Loebenstein *et al.*, 2009). Its large, starchy, sweet-tasting, tuberous roots are most important root crop worldwide (Wadl *et al.*, 2018).

Storage roots of sweet potatoes contain 30% dry matter, of which 70% consists of starch, 10% sugars, and 5% proteins. In addition, storage roots contain significant quantity of phytochemicals, which have beneficial health effects, while roots and leaves contain β-carotene (provitamin A), ascorbic acid (vitamin C), vitamin B complex and phenolic compounds such as anthocyanins (Andrade, 2007 and Teow *et al.*, 2007). Kosieradzka *et al.* (2004) reported that purple and red-fleshed potatoes are rich in natural anthocyanins. The phytochemicals of sweet potato consist of high free-radical scavenging activity, which is important to prevent the chronic diseases and age-related neuronal degeneration (Teow *et al.*, 2007). The young leaves and shoots are sometimes eaten as vegetables. Orange, white, and creamy flesh sweet potato is most commonly grown and eaten. In orange- and purple-fleshed sweet potato, colours are due to the presence of anthocyanins and carotenoids of which β-carotene is most abundant. To increase the yield and quality, it is important to study on agro-morphological traits in sweet potato genotypes, but unfortunately, negligible work has been done on sweet potato in spite of fact that lot of variability exists in sweet potato for physiological and biochemical characters, which can be utilized for improving tuber yield coupled with high nutritive value (Wang *et al.*, 2018). Genetic diversity is essential for future research and the development of cultivars with the desired properties. To deal with the genetic development of sweet potato, it is necessary to understand the genetic diversity in examined germplasm.

To ascertain the genetic diversity of sweet potatoes, both morphological and molecular markers have been used (Nair *et al.*, 2017). OFSP varieties tolerate harsh growth conditions, are rich in vitamins (A, C, D, and E), and provide more edible energy than all other food staples. The limited diversity in available food crops has prompted the search for urgent alternatives to these major crops to ensure food security. Molecular markers have the benefit of being more trustworthy and not being impacted by environmental circumstances; besides, it is possible to do DNA testing at any stage of plant development (Rodriguez-Bonilla *et al.*, 2014). In addition to biochemical-based markers, In recent years, simple sequence repeat (SSR) markers or microsatellites have become the most widely used molecular marker technologies for the study of both animals and plants (Li *et al.*, 2009; Karuri *et al.*, 2010; Yada *et al.*, 2010). Multi-allelic, highly polymorphic, highly reproducible, codominant SSR markers give extensive genetic information with good genome coverage (Kawuki *et al.*, 2009; Sree *et al.*, 2010). SSR markers are inexpensive and adaptable to most breeding protocols, making them ideal for public breeding programs that cannot afford expensive diversity evaluation approaches (Turyagyenda *et al.*, 2012). The objective of this research was to employ the SSR markers to characterize the genetic diversity of orange-flesh sweet potato genotypes and determine the phylogenetic relationships among the twenty-seven genotypes

MATERIALS AND METHODS

Planting Materials

Twenty seven (27) Orange-flesh sweet potato fruit and vine were sourced from National Root Crops Research Institute (NRCRI) Umudike, Abia State, Nigeria, Landraces from farmer's field, Uganda varieties. The 27 potato genotypes in collection were used for this study (Table 1).

Table 1: Genotypes Used In the Study and Its Original Code

New code	Original code	Source	New code	Original code	Source
SP1	SOLO-1/144	Nigeria	SP15	Landrace	Nigeria
SP2	F2M5/3	Nigeria	SP16	Landrace	Nigeria
SP3	Ng-Jay	Nigeria	SP17	Landrace	Nigeria
SP4	MD	Nigeria	SP18	Landrace	Nigeria
SP5	F1M1/4	Nigeria	SP19	NASPOT-8	Uganda
SP6	ELINDA	Nigeria	SP20	NASPOT-9	Uganda
SP7	SOUL	Nigeria	SP21	NASPOT-10	Uganda
SP8	AI2IB	Nigeria	SP22	Carrot C	Uganda
SP9	TIS.87/0087/08	Nigeria	SP23	Landrace	Uganda
SP10	KWARA/00	Nigeria	SP24	Ejumula	Uganda
SP11	F1M4/11	Nigeria	SP25	Mayai	Uganda
SP12	SOLO-1/100	Nigeria	SP26	Resisto	Uganda
SP13	Landrace	Nigeria	SP27	Kakamega	Uganda
SP14	Landrace	Nigeria			

Genomic DNA Extraction

Genomic DNA was extracted from young leaves (4 to 6 weeks old) of the 27 genotypes of orange flesh sweet potato (OFSP), using a modified cetyl trimethylammonium bromide (CTAB) technique (Doyle and Doyle 1987). The DNA was extraction at Molecular laboratory, Centre for Dryland Agriculture, Bayero University Kano, Nigeria. The DNA samples obtained were quantified using NanoDrop (Cleaver scientific Ltd) for concentration and purity. SSR markers were used to examine molecular diversity of selected sweet potato genotypes. Ten SSR markers were selected from the sequence information available in the published literature by Koussao *et al.* (2014) and Rahman et al. (2023) were used for SSR marker analysis. The details of the 10 polymorphic primers with their sequence are presented in Table 2.

PCR Amplification and Standardization

PCR reaction was performed in a total volume of 15 µL containing 30 to 50 ng template DNA were used. Briefly, 1 µM concentration of each primer (forward and reverse), 8 µL master mix (2×Power Taq PCR MasterMix), 2 µL of template DNA and 3 µL nuclease-free water. PCR amplification for background marker was conducted in a thermo cycler (Cleaver Scientific Ltd), with a program of an initial denaturation at 94°C for 5 min, 11 cycles of 94°C /30 sec, 60°C /30 sec decreasing by 0.5°C per cycle, and 72°C/60 sec, followed by 30 cycles of 94°C/30 sec, 55°C/30 s, and 72°C/60 s, and a final extension at 72°C for 10 min. PCR products were separated on 3% agarose (MetaPhor™ Agarose Lonza) gel, 1X TBE buffer. The gel was stained with gel view and run at a constant voltage of 80V for 60 min, visualized under UV light, and analyzed using Bio-Imaging system (Cleaver scientific Ltd) bands were scored.

Molecular Data and Cluster Analysis

For SSRs, the polymorphism information content (PIC) value for each SSR locus were computed using the formula PIC = 1-∑pi2, where pi is the frequency of the ith allele in the set of 27 selected sweet potato genotypes (Weir, 1990). The POPGENE 1.31 program (Yeh *et al.*, 1999) was used to calculate the total. In all 27 selected sweet potato genotypes investigated, amplified fragments were evaluated for the presence (1) or lack (0) of the corresponding bands. Based on the binary data, a cluster and principal component analysis were performed (Rohlf, 2000).

Table 2: Primers Used In the Study and Their Sequence

Primers	Forward sequence	Reverse sequence
IbU6	GGGGTAGAGAGAAGAGAGTGAC	CCAGGTGAGAGTGTCTTTCAA
IbU4	GGCTGGATTCTTCATATTTAGC	GCTTAATGGATCAGTAACACGA
IbU13	GCAACCAATCTACAGCAAACTA	CAGATAAAGTCCCCATTTCTTC
IbU20	GGAGAGCAAGTGGAGAAAGTAT	ACTCCTAGACCCACAATTGAAC
IbL16	GTCTTGCTGGATACGTAGAACA	GGGAGAAGTAAGAGAACCGATA
IbL32	GGGATGAAGGAGAGAATGAGTA	TTGAAAACCTAGAGAGAAAGGG
IbL46	CTGAAATTAGGGATTGAAGAGG	TCCAATCACTCCTTGTTTTCTC
IBS12	CAGTTATCAATTCCCACCTACC	TTGCTGTGTTATAGGCTTTGTC
IBS24	AGTGCAACCATTGTAATAGCAG	TCCTTTCTTCATCATGCACTAC
IBS33	ATCTCTTCATACCAATCGGAAC	CAATGATAGCGGAGATTGAAG

RESULTS AND DISCUSSION

Co-dominant Gene Characterization

The investigation employed ten SSR primers due to their consistent and polymorphic DNA amplification patterns across genotypes. All markers were polymorphic, and microsatellite allele sizes were obtained at 37 loci (Table 3). The number of alleles per locus ranged from 3 to 6, with a mean of 3.7 alleles per SSR locus. The total observed number of alleles was 282, ranging from 124 (IbU20) to 375 (IbU6). The ten SSR markers demonstrated their effectiveness in

distinguishing between genotypes, with PIC values ranging from 0.520 for IbU20 to 0.820 for IbU6, averaging 0.689 (Table 3). All ten markers exhibited high polymorphic information content (PIC > 0.50). The high PIC values observed in this study indicate that the SSR markers used were highly informative. In this study, the average polymorphic information content (PIC) value was 0.689, ranging from 0.520 to 0.820. This value was higher than the average PIC value of 0.450 reported by Musa et al. (2024b) but lower than the average value of 0.727 reported by Koussao et al. (2014).

A PIC value above 0.5 suggests loci with high levels of polymorphism. The classification of PIC values into low, moderate, and high polymorphic loci has been established by several studies (Kalia et al., 2011; Ge et al., 2013; Gramazio et al., 2019; Musa et al.,2024a). Results from similar studies using SSR markers in sweet potato diversity analysis have been reported, with most differences attributed to sample size, the number of SSR markers used, and the source of materials. Moderate genetic diversity values were reported by Yada et al. (2010b) among 192 accessions using 10 SSR markers; Gichuru et al. (2006) also reported low diversity, and Soegianto et al. (2011) reported similarity ranging from 15% to 78% between Indonesian accessions. The low diversity has been attributed to the narrow geographic range of cultivar collection. High SSR-based diversity has been observed by Veasy et al. (2008), Shih et al. (2002), and Li et al. (2009).

Table 3: Microsatellite Loci Analysis

Locus	No. of Alleles per locus	Total Alleles	PIC
IbU4	3	330	0.713
IbU6	4	375	0.820
IbU13	5	321	0.786
IbU20	3	124	0.520
IbL16	4	212	0.740
IbL32	3	354	0.687
IbL46	3	222	0.579
IBS12	6	255	0.782
IBS24	3	315	0.676
IBS33	3	310	0.690
Mean	3.7	282	0.689

Cluster and Principal Component Analysis

The ten SSR markers were selected based on the Euclidean distances among the 27 sweet potato genotypes to construct a UPGMA dendrogram, as shown in Table 4 and Figure 1. The dendrogram classified the 27 genotypes into eight main groups with a similarity coefficient of 0.84, which was deemed most appropriate for a clear discussion, indicating a high level of variation among the sweet potato genotypes. Group I consisted of twelve genotypes the largest group including six from Uganda (SP20, SP21, SP22, SP25, SP26, and SP27) and six from Nigeria (SP1, SP3, SP4, SP9, SP13, and SP14). Group II included two Nigerian varieties (SP8 and SP15), while genotype SP19 from Uganda formed Group III. Cluster IV comprised seven genotypes from Nigeria: SP2, SP5, SP6, SP7, SP10, SP16, and SP17 (Figure 1).

Cluster V contained two genotypes, SP11 and SP12. Nigerian and Ugandan genotypes were well separated from the other groups and formed Groups VI, VII, and VIII, each consisting of a single genotype: SP23 (Uganda), SP24 (Uganda), and SP18 (Nigeria), respectively (Figure 1). The same trend of grouping was reported by other researchers characterizing Tanzanian sweet potato genotypes (Elameen et al., 2008; Tairo et al., 2008; Gwandu et al., 2012). These studies indicated that the eight main groupings of sweet potato may have resulted from the introductions of the crop come from different genetic pools (Figure 1). Similar results were obtained with the sweet potato collections from Brazil (Veasey et al., 2007), South Africa (Laurie et al., 2013) and Uganda (Yada et al., 2010).

Table 4: Molecular Diversity among the 27 Sweet Potato Genotypes Based On Binary Data

Cluster	Number of genotypes	Genotypes	Origin
I	12	SP1, SP9, SP14, SP13, SP21, SP22, SP3, SP20, SP26, SP27, SP4, SP25	Nigeria and Uganda
II	2	SP8, SP15	Nigeria
III	1	SP19	Uganda
IV	7	SP2, SP7, SP16, SP6, SP5, SP17, SP10	Nigeria
V	2	SP11, SP12	Nigeria
VI	1	SP23	Uganda
VII	1	SP24	Uganda
VIII	1	SP18	Nigeria

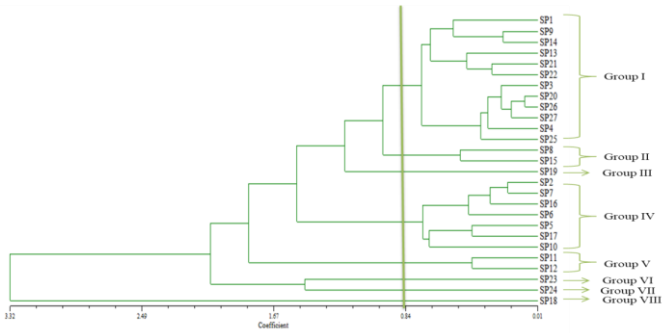

Figure 1: Dendrogram Demonstrating the Genetic Associations among 27 Sweet Potato Genotypes using 10 SSR Markers

The two-dimensional graphical representation (2-D plot) generated from the PCA of the SSR data (Figure 2) also supports the clustering pattern observed in the UPGMA dendrogram. The genotypes farthest from the centroid were SP18, SP23, and SP24, while the other genotypes were relatively close to the centroid. The graph shows that most genotypes are clustered closely together, except for a few, such as SP18, SP23, and SP24, as indicated by the eigenvectors. The variation percentages for PC1 and PC2 are 46.36% and 24.40%, respectively, with PC1 accounting for the largest portion of the total variation (Figure 2). The high level of genetic diversity observed in sweet potato accessions may result from spontaneous mutations, which are common in this species, as well as from selection, regional, and environmental factors. These influences make populations of this species a significant genetic resource (He et al., 2006). Several studies have employed SSR markers to investigate the genetic diversity of sweet potato.

For example, Koussao et al. (2014), Sulaiman et al., (2020) and Rahman et al. (2023) have reported on sweet potato diversity using genotypes from various countries, which may share ancestry or exhibit similar morphological traits. The principal component analysis (PCA) provided eigenvalues for all principal component axes in the ordination of genotypes, with the first axis accounting for the majority of the variation between genotypes (Figure 2). The results of the PCA further supported the assessment of the clustering. Genetically, Falconer and Mackay (1981) reported that clusters of associated genotypes with high yield will be useful for future breeding programs.

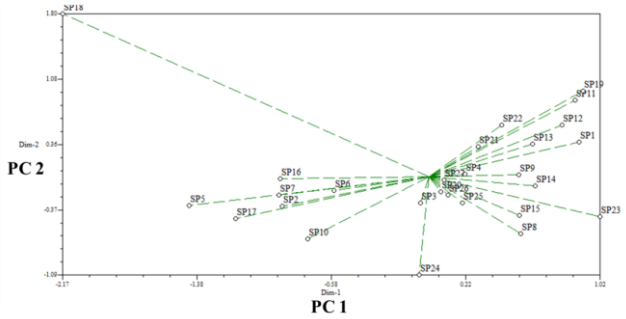

Figure 2: Two-dimensional Graph Principal Component Analysis Showing the Relationship among the 27 Sweet Potato Genotypes

CONCLUSION

The findings of the present study reveal that orange-fleshed sweet potato germplasm from Nigeria and Uganda exhibits moderate to high genetic diversity based on molecular assessment methods. A total of 10 SSR markers were analyzed, and the results were used to select cultivars for future research. To achieve valuable outcomes, new cultivars are needed, which may be developed by introducing or collecting new germplasm. The efforts of this study provide fresh insights into the classification of orange-fleshed sweet potatoes and will support genetic research and breeding programs aimed at improving the existing germplasm of this root crop.

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