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Genetic diversity assessment of Tanzanian sweetpotato genotypes using simple sequence repeat markers

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ABSTRACT

Genetic diversity assessment of 48 Tanzanian sweetpotato genotypes was conducted using nine polymorphic simple sequence repeat markers to determine genetic relationship and select unique parents which could be used for future breeding. Genetic diversity parameters, cluster analysis, and analysis of molecular variance were calculated to determine genetic diversity and relationships. Results showed that the SSR markers used had the mean PIC of 0.78, allelic richness per locus ranged from 4–17 with a mean of 10.0 and the number of effective alleles varied from 2.2–6.1 with a mean value 3.5. The un-weighted pair group method with arithmetic mean allocated the germplasm collection into three major genetic clusters. The greatest genetic distance was identified between the genotypes sourced from Kagera, Temeke, Mkuranga and Kisarawe areas of Tanzania. The study identified genetically unrelated and complementary sweetpotato genotypes such as Ex-Ramadhani, Kibakuli, Mkombozi, Mjomba mkwe, Ex-Halima-3 and Kabuchenji which are recommended for future breeding programmes.

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1. Introduction

Sweetpotato is an important root crop serving as food, feed and raw material globally. Its role as a cash crop is significantly increasing due to the crop's high yield potential and ability to grow in a wide range of environments (Chiona, 2009; Wang et al., 2011). Most agricultural practices have greatly improved crops through selection and breeding (Messeguer, 2003). Targeted selection for specific traits such as high yields has narrowed genetic diversity among modern cultivars compared to farmers' varieties (Ulukan, 2009).

Genetic diversity analyses give better understanding on the extent of variation available between and within germplasm collections (Tumwegamire et al., 2011). Genetic diversity is a precondition for successful plant breeding (Ulukan, 2009). Several approaches have been used in crop genetic diversity analysis including morphological, agronomical, biochemical and DNA-based markers (Mohammadi and Prasanna, 2003). The choice of approach depends on objectives, required information and resources. Molecular markers have become important tools in genetic diversity analysis of sweetpotato for enhancing efficient sweetpotato breeding (Buteler et al., 2002; Hu et al., 2004; Wang et al., 2011; Zhao et al., 2013). Molecular techniques used in sweetpotato genetic diversity studies include randomly amplified polymorphic DNAs (Gichuki et al., 2005), amplified fragment length polymorphisms (Elameen et al., 2008) and simple sequence repeat (SSR) markers (Karuri et al., 2009). The SSR markers have been widely used in genetic diversity analysis of sweetpotatoes. Previous studies by Yada et al. (2010) and Rodriguez-Bonilla et al. (2014) showed that SSR markers revealed the highest level of polymorphism due to the co-dominance nature and high numbers of alleles per locus. These markers are powerful and have the ability to discriminate genotypes including those related by pedigree.

In Tanzania, sweetpotato is an important food crop supporting millions of people. It is the second most important root crop after cassava. Sweetpotato yields in Tanzania ranges from 3–6 t ha^{-1}, lower than the yield potential of 15–27 t ha^{-1} (Kapinga et al., 1995; Sebastiani et al., 2007). Average area harvested for the last ten years was 500,000 ha with mean yield of 3.83 t ha^{-1} (FAOSTAT, 2015). Sweetpotato productivity could be enhanced through the effective selection of locally adapted and farmers' preferred genotypes and targeted breeding. This requires genetic diversity analysis using effective molecular tools such as SSR markers.

There are limited sweetpotato genetic diversity studies conducted in Tanzania. Tairo et al. (2008) and Elameen et al. (2011) used agro-morphological parameters to study the diversity present within Tanzanian sweetpotato germplasm. Elameen et al. (2008) and Gwandu et al. (2012) used amplified fragment length polymorphism and SSR markers, respectively to analyze the genetic diversity of sweetpotato germplasm. Gwandu et al. (2012) specifically analyzed the genetic diversity among elite sweetpotato genotypes for resistance

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to sweetpotato virus disease (SPVD) and dry matter content. The author reported a relatively high level of genetic variation within the studied germplasm. However, most farmers grow landraces and have limited access to elite sweetpotato varieties. Systematic genetic grouping of sweetpotato genotypes well-adapted to diverse geographical locations may offer a unique genetic resource base. The use of polymorphic SSR markers could efficiently assist genetic grouping of sweetpotato germplasm and consequently reduce the timeline for developing sweetpotato cultivars in the country. Therefore, the objective of this study was to determine the genetic relationship within Tanzania sweetpotato germplasm and select unique parents for breeding using SSR markers.

2. Materials and methods

2.1. Plant materials, DNA extraction, SSR amplification and polymerase chain reaction

A total of 48 agronomically useful and morphologically distinct sweetpotato genotypes (Table 1) were selected from the 144 germplasm collected from the lake and eastern zones of Tanzania. The selection of genotypes was based on agro-morphological attributes and their reaction to sweetpotato virus disease (SPVD).

DNA samples of the sweetpotato genotype were collected on FTA cards. The sap was extracted from fresh tender leaves of five plants per genotype grown at Sugarcane Research Institute (SRI) — Kibaha in 2013/2014. Genotyping was conducted at Incotec laboratory, South Africa. All samples were used in bulked amplification, using DNA from five individual leaf samples. A single punch of each card per submission was taken and homogenized in the Finnzymes dilution buffer (Kit). Two micro-liters of each bulked sample was used in the polymerase chain reaction (PCR).

The PCR products were fluorescently labeled and separated by capillary electrophoresis on an ABI 3013 automatic sequencer (Applied Biosystems, Johannesburg, South Africa); analysis was performed using GeneMapper 4.1. A total of nine polymorphic SSR markers were used for this study (Table 2). Markers were selected based on their polymorphic information content (PIC) values which ranged from 0.52 to 0.81 and their history from previous related studies (Table 2).

2.2. Data analysis

2.2.1. Genetic diversity analysis

Genotypic data were subjected to analyses with various measures of genetic diversity within and among genotypes using FSTAT version 2.9.3 and GenAlex software version 6.5 (Goudet, 2001; Peakall and Smouse, 2012). Genetic diversity parameters such as total number of alleles per locus, number of effective alleles per locus, Shannon's Information Index, and gene diversity were determined using the protocol of Nei and Li (1979). Other genetic parameters such as differentiation, gene flow and polymorphic information content (PIC) were estimated using GenAlex software. Based on Euclidian distances, analysis of molecular variance (AMOVA) was conducted using GenAlex software to partition total genetic variations into, within and among districts and agro-ecologies of germplasm collection so as to quantify the diversity level and genetic relationship among genotypes.

2.2.2. Cluster analysis

The SSR marker alleles were converted to binary data scored as either presence or absence of the band for all the 48 sweetpotato clones and treated as dominant marker. To evaluate the results of SSR markers, each amplified fragment was considered as one locus. The genetic dissimilarity matrix of the 48 sweetpotato clones was calculated using Jaccard's coefficient (Jaccard, 1908).

Cluster analysis was done based on neighbor-joining algorithm using the un-weighted pair group method using arithmetic average (UPGMA) in DARwin 5.0 software (Perrier and Jacquemoud-Collet,

Table 1
Description of sweetpotato genotypes used in the study.

Sr. no.	Genotypes	Zone	District	DMC (%)	Yield (t ha^{-1})	Root flesh color	Reaction to SPVD
1	Ex-Kazimzumbwe-4	Eastern	Kisarawe	33.75	2.5	2	2
2	Ex-Halima-1	Eastern	Mkuranga	36.25	8.9	2	1
3	Ex-Miale-1	Eastern	Mkuranga	35.00	8.5	2	2
4	Ex-Kibuta-1	Eastern	Kisarawe	35.5	6.0	1	2
5	Ex-Maneromango-1	Eastern	Kisarawe	36.25	6	1	2
6	Ex-Kazimzumbwe-3	Eastern	Kisarawe	34.40	6.5	2	2
7	Shangazi	Eastern	Kilosa	37.50	4.0	4	4
8	Ex-Kibuta-2	Eastern	Kisarawe	35.00	5.0	2	1
9	Ex-Kazimzumbwe-2	Eastern	Kisarawe	33.75	4.0	3	2
10	Mwanatata	Lake	Kagera	37.50	4.5	3	2
11	Ex-Halima-2	Eastern	Mkuranga	36.25	7.0	1	1
12	Ex-Maneromango-2	Eastern	Kisarawe	36.25	5.5	1	2
13	Ex-Miale-2	Eastern	Kilombero	36.25	8.9	1	2
14	Gairo	Eastern	Kilombero	36.25	4.6	3	3
15	Mbutu	Eastern	Bagamoyo	35.00	3.5	1	3
16	Ex-Madina	Eastern	Kisarawe	31.25	7.6	3	1
17	Ex-Msimbu-2	Eastern	Kisarawe	36.90	2.5	1	2
18	Ex-Msimbu-4	Eastern	Kisarawe	33.75	4.0	4	1
19	Berene	Lake	Kagera	32.50	6.0	1	1
20	Ex-Ungindoni	Eastern	Temeke	35.60	4.0	2	1
21	Ex-Msimbu-3	Eastern	Kisarawe	34.40	5.0	3	2
22	Mkombozi	Lake	Kagera	32.50	9.0	4	3
23	Ex-Kibugumo	Eastern	Temeke	36.25	6.0	3	1
25	Kabuchenji	Lake	Kagera	38.75	7.0	2	1
26	Ex-Halima-3	Eastern	Mkuranga	33.75	6.5	1	1
27	Ex-Mengwa-3	Eastern	Kisarawe	41.25	3.0	1	2
28	Mjomba mkwe	Eastern	Kisarawe	32.50	4.0	4	1
29	Ex-Kiboda-2	Eastern	Temeke	36.25	1	2	2
30	Liponjwa	Eastern	Mkuranga	34.40	3	1	2
31	Ex-Sungwi	Eastern	Kisarawe	34.40	8.7	3	1
32	Kikabeji	Lake	Kagera	35.60	7.5	2	1
33	Sekondari	Lake	Kagera	32.50	3.0	2	1
34	Matako mapana	Eastern	Bagamoyo	37.50	6.5	1	2
35	Ex-Ramadhani	Eastern	Kisarawe	37.50	2.0	1	1
36	Mchikichini	Eastern	Temeke	32.50	6.0	3	2
37	Mkwakwa	Eastern	Kisarawe	38.75	5.5	2	2
38	Kigambile nyoko	Lake z	Kagera	38.75	3.0	4	2
39	Ex-Kiboda-4	Eastern	Temeke	36.25	3.0	3	2
40	Ex-Berene	Lake	Kagera	36.25	6.5	3	1
41	Ex-Msimbu-1	Eastern	Kisarawe	41.25	7.0	1	3
42	Ex-Kiboda-1	Eastern	Temeke	40.00	3.5	2	2
43	Kandoro	Eastern	Kisarawe	34.40	5.5	1	2
44	Ex-Yohana	Eastern	Kisarawe	34.40	6.0	1	1
45	Ex-Mwanza	Lake	Kagera	38.75	5.5	3	1
46	Ex-Bwana	Eastern	Kisarawe	38.75	6.5	3	1
47	Ex-Kazimzumbwe-1	Eastern	Kisarawe	36.25	4.5	2	3
48	Binti Jongo	Eastern	Mkuranga	33.75	6.5	2	1

Root flesh color: 1 = white, 2 = cream, 3 = yellow and 4 = orange.
SPVD: 1 = no visible symptoms, 2 = mild symptoms (a few local lesions on a few leaves), 3 = moderate symptoms (mosaic symptoms on leaves), 4 = severe symptoms (mosaic symptoms with plant stunting) and 5 = very severe symptoms of purpling/yellowing or mosaic on leaves, severe leaf distortion, reduced leaf size and severe stunting.

Table 2
Details of SSR markers used to genotype 48 sweetpotato genotypes collected from Tanzania.

Name	Dye	Primer 5′-3′	Primer reverse 5′-3′
IB-R03	PET	GTAGAGTTGAAGAGCGAGCA	CCATAGACCCATTGATGAAG
1B-S07	FAM	GCTTGCTTGTGGTTCGAT	CAAGTGAAGTGATGGCGTTT
IB-R12	NED	GATCGAGGAGAAGCTCCACA	GCCGGCAAATTAAGTCCATC
IB-R16	VIC	GACTTCCTTGGTGTAGTTGC	AGGGTTAAGCGGGAGACT
1B-R19	PET	GGCTAGTGGAGAAGGTCAA	AGAAGTAGAACTCCGTCACC
IB-CIP13	NED	CGTGCTTGAGGTCTGAGTAGAA	TTCCCTAGAAGCTGCGTGAT
SSR 07	PET	TTTTCAACGACAAGCCTCTTGC	TCAAAGGTCCGCATGGAAATC
SSR 09		AAGTTAATCTAAGGTGGCGGGG	CGTCGATTCCAGTCTAATCCAA TCC
690524	VIC	AAGGAAGGGCTAGTGGAGAA GGTC	CAAGGCAACAAATACACACACA CG

Source: Karuri et al., 2009; Gwandu et al., 2012.

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