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GENETIC DIVERSITY AMONG FARMER-PREFERRED CASSAVA LANDRACES IN UGANDA

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ABSTRACT

Understanding of genetic diversity among a breeding population is an important requirement for crop improvement as it allows for the selection of diverse parental combinations and formation of heterotic pools for genetic gain. This study was carried out to determine genetic diversity within and among 51 farmer-preferred cassava (*Manihot esculenta*) landraces and 15 elite accessions grown in Uganda. Twenty six simple sequence repeat (SSR) markers used for genetic diversity assessment in this study revealed a total of 154 alleles, of which 24% were unique alleles present only in landraces. The genetic diversity and observed herozygosity in landraces were slightly higher than in elite accessions. Elite accessions clustered with some of the landraces indicating that there were some alleles in common. However, 58.8% of the landraces fell into 3 different clusters independent of the elite accessions. Including these landraces with unique alleles in cassava breeding schemes will increase the chances of producing farmer preferred adapted elite cultivars. The study also revealed genetic differentiation among accessions from different regions providing an opportunity for establishment of heterotic pools within a breeding programme.

Key Words: Alleles, *Manihot esculenta*, SSR markers

RÉSUMÉ

La compréhension de la diversité génétique est une condition importante dans la sélection de diverses combinaisons parentales et la formation des groupes héterotiques d'intérêt génétique pour l'amélioration des cultures. Cette étude était conduite pour déterminer la diversité génétique parmi 51 variétés locales de manioc (*Manihot esculenta*) préférées des fermiers et 15 accessions élites cultivées en Ouganda. Vingt six marqueurs simples à sequence répétée (SSR) utilisés pour l'évaluation de la diversité génétique ont révélé un total de 154 allèles, parmi lesquelles 24% étaient des allèles uniques présentes dans les races locales seulement. La diversité génétique et l'héterozygosité observées dans les races locales étaient légèrement supérieures que dans les accessions élites. Celles-ci s'étaient regroupées avec quelques variétés locales indiquant donc qu'elles avaient quelques allèles en commun. Par ailleurs, 58.8% des races locales ont formé trois différents groupes indépendamment des accessions élites. Inclure ces races locales à allèles uniques dans les systèmes d'amélioration du manioc permettra d'augmenter les chances de produire des cultivars élites préférés des fermiers. L'étude montre aussi que la différenciation génétique trouvée parmi les accessions de différentes régions est une opportunité offerte pour l'établissement des groupes héterotiques dans le programme d'amélioration.

Mots Clés: Allèles, *Manihot esculenta*, marqueurs SSR

INTRODUCTION

Cassava *(Mannihot esculenta* Crantz) is an important tuberous root crop widely cultivated in many parts of sub-Saharan Africa where it is a staple and food security crop for more than 200 million people (Legg, 1999). Its flexible planting and harvesting times (Mkumbira *et al.*, 2003) makes it an important subsistence crop for many communities in Africa. Evolution of cassava as a domesticated crop within Africa is not well documented despite its wide adaptability. There are many reports on landraces of cassava in sub-Saharan Africa but with limited studies on the genetic relatedness between landraces and elite accessions. Since introduction of the crop to Africa from Latin America by Arab and European traders, asexual propagation and human selection for resistance and adaptability to biotic and abiotic constraints are expected to have reduced cassava's genetic diversity (Fregene *et al.*, 2003). However, the comparable levels of genetic diversity observed in cassava landraces from Africa and several Neo-tropical countries (Brazil, Colombia, Peru, Venezuela, Guatemala, Mexico and Argentina) (Fregene *et al*., 2003), suggest that further diversification of the crop may have occurred within Africa. Cassava is an out-crossing crop and can result in production of volunteer seedlings which are subjected to natural and/or human selection, modifying diversity (Elias *et al.,* 2001; Kizito *et al*., 2005; Montero-Rojas *et al*., 2011). Besides, traditional farming practiced by majority of cassava farmers maintains or even increases genetic diversity through growing several varieties of the crop in a single field (Ferguson *et al*., 2011) that could promote gene flow through hybridisation (Elias, 2001; Resende *et al.*, 2004).

Traditional cassava farmers prefer a diversifying rather than a directional selection. They keep even low yielding or abiotic/biotic stress susceptible varieties at low frequencies instead of discarding them (Elias, 2001). There is thus high genetic diversity among crop populations maintained in farmers' fields. Human selection of many different varieties for diverse attributes such as farmer preferred agronomic and quality traits, resistance to pests and diseases, desirable plant architecture and other adaptation characteristics has been a key process in maintaining genetic diversity among cassava landraces (Raji *et al*., 2007). Many years of farmers' selection results in diverse landraces with genes required for adaptation to biotic and abiotic stresses in higher frequencies. Landraces are therefore, valuable for plant breeding because of their co-adapted gene complexes with tolerance to diseases and adaptation to specific ecological conditions. For example, a major dominant gene, CMD2, which confers resistance to cassava mosaic disease, has been reported in Nigerian landraces (Lokko *et al*., 2005). Including adapted landraces in breeding schemes broadens the genetic base of breeding programme and improves chances of producing progenies that meet farmers' needs.

Continuous germplasm exchange between different ethnic groups and farmers, through formal and informal distribution systems, make pedigree information limited and unreliable (Ajmone-Marsan *et al.*, 1992; Schut *et al*., 1997; Mignouna *et al*., 1998). It is important to assess agro-morphological and genetic diversity of cassava germplasm used by farmers. A prerequisite for genetic improvement of cassava is knowledge of the extent of genetic variation present between cultivars (Beeching *et al.*, 1993; Moyib *et al.*, 2007). Information on genetic diversity guides selection of divergent parents to broaden genetic base of a breeding population and produce progenies with heterosis (Manosh *et al*., 2008).

Selectable and neutral genetic markers are commonly used to assess genetic diversity among populations and accessions. Selectable markers (morphological and agronomical traits) respond to selection pressure and change after several years of natural and/or artificial selection (Yong-Jin *et al*., 2009). On the contrary neutral genetic markers are least subject to selection pressure and can accurately infer genetic diversity among populations and accessions (Chakravarthi and Naravaneni, 2006; Raji *et al*., 2009). Several neutral genetic markers have been used to study genetic diversity in cassava; RFLPs (Beeching *et al*., 1993), RAPDs (Mignouna and Dixon, 1997; Asante and Offei, 2003), AFLP markers (Fregene *et al*., 2000; Raji *et al*., 2009) and SSR markers (Kawuki *et al*., 2009; Sree Lehka

et al., 2010; Montero-Rojas *et al*., 2011). However, SSR markers remain competitive because of being multi-allelic, highly polymorphic, co-dominant and highly reproducible and provide rich genetic information with good genome coverage (Kawuki *et al*., 2009; Sree Lehka *et al*., 2010). The SSR markers are also affordable and amenable to most breeding procedures and thus, applicable in public breeding programmes which may not be able to afford expensive diversity assessment techniques. In this study, we report on the diversity and differentiation within and among cassava landraces and elite accessions grown in Uganda, assessed using genomic SSR markers.

MATERIALS AND METHODS

Plant material. Fifty four cassava accessions (thirty nine landraces and fifteen elite accessions) were collected in June 2009, from major cassava growing regions; Central, Eastern, Northern and Western regions of Uganda (Table 1). In these regions farmers practice traditional farming where stem cuttings are re-planted after harvest or are obtained from relatives' and neighbours' fields or from abandoned fields. Focus discussions were held with farmers to determine the use, origin and the preferred traits of their accessions. Cassava planting stakes of 20-30 cm in length from the 54 accessions obtained from farmers were planted in two rows with 5 plants per row for each genotype (5 X 2 plots) in a Randomised Complete Block Design (RCBD) with two replications in the open field at Makerere University Agriculture Research Institute Kabanyolo (MUARIK). After four months of establishment, young fully developed leaves were collected from each genotype for DNA extraction.

DNA extraction. DNA was extracted using standard procedures according to Dellaporta *et al*. (1983) with slight modifications. Freshly harvested young leaf (0.3 g) of each genotype was ground in liquid nitrogen using a pestle and a mortar. The fine powder was transferred to 1.5 ml eppendorf tube using a frozen spatula. Eight hundred microlitres of preheated (65^{o}C) extraction buffer and 50 µl of 20% SDS solution were added to each tube and the mixture homogenised for 30 seconds by intermittent inversion. The tubes were incubated at 65^{o}C for 15 minutes with intermittent inversions and incubated at room temperature for 5 min. Proteins and polysaccharides were precipitated by adding 250 µl of ice-cold 5M potassium acetate and mixed by inverting the tubes 5-8 times. The tubes were placed on ice for 20 minutes and centrifuged at 13,250 rcf in eppendorf centrifuge 5418 (Germany) for 10 minutes. The supernatant was transferred to a new eppendorf tube and 500 µl of ice-cold iso-propanol added and mixed by inverting gently 8-10 times to precipitate crude DNA. The mixture was incubated at -20^{o}C for 30 minutes and centrifuged at 13,250 rcf for 10 minutes.

The supernatant was poured off and last drops of isopropanol removed by placing eppendorf face down on paper towels for 30 minutes. After draining off isopropanol, 200 µl low salt TE was added to each sample followed by 3µl RNAse A (10 mg ml^{-1}) (Cat.EN0531 Fermentas) and incubated at 37^{o}C for 30 min. Two hundred microlitres of phenol:chloroform: isoamylalcohol (25:24:1) was added to each sample and inverted twice to mix and centrifuged at 13,250 rcf for 10 minutes. The upper layer was transferred to fresh eppendorf tube and 200 µl chloroform:isoamylalcohol (24:1) added. The tubes were inverted twice to mix and centrifuged at 13,250 rcf for 10 minutes. The upper aqueous layer (approximately 200 µl) was transferred to fresh tubes.To purify DNA, 500 µl ethanol: 3 M sodium acetate solution (30:1.5) was added to each sample and incubated for 5 minutes at -20^{o}C. The samples were centrifuged at 13,250 rcf for 5 minutes, the supernatant decanted and the pellet washed with 200 µl 70% ethanol. The samples were centrifuged at 13,250 rcf for 5 minutes, the supernatant decanted and DNA pellet air dried for 1 hr by placing the tubes face down on paper towels. The pellet was re-suspended in 100 µl low-salt TE buffer and stored at 4^{o}C. Also included in the analysis were the DNA samples from 12 Uganda landraces that had been preserved in the laboratory at Biosciences eastern and central Africa (BecA) (making a total of 66 DNA samples). The concentration and purity of 66 DNA samples were checked by NanoDrop UV spectrophotometry at A260 and A280, while the integrity of DNA was analysed by 1.5% agarose

TABLE 1. Regions in Uganda where cassava accessions used in the study were collected

Accession	Type	Region	Accession	Type	Region
Bamunanika	Landrace	Central	TME14	Elite	Eastern
Kabwa	Landrace	,,	TME204	Elite	,,
MH96_0686	Elite	,,	Yellow	Landrace	,,
MH97_2961	Elite	,,	ApacApac	Landrace	Northern
MM96_4271	Elite	,,	Bao	Landrace	,,
Mubende	Landrace	,,	Nyaraboke	Landrace	,,
Muwangazi	Landrace	,,	Bukalasa	Elite	Western
Nakati	Landrace	,,	Deruderu	Landrace	,,
Nase11	Elite	,,	Guaranda	Landrace	,,
Nase9	Elite	,,	Hoima1	Landrace	,,
Njule	Landrace	,,	Hoima3	Landrace	,,
Teleka	Landrace	,,	HoimaR21	Landrace	,,
Akena	Elite	Eastern	Kakumiro	Landrace	,,
Aladoalado	Landrace	,,	Kidimo	Landrace	,,
Buganda	Landrace	,,	Kwatamumpare	Landrace	,,
Ditu	Landrace	,,	Lugbara	Landrace	,,
EgabuK	Landrace	,,	Lyaholore	Landrace	,,
EgabuS	Landrace	,,	Maburu	Landrace	,,
Icilicili	Landrace	,,	Masindi4	Landrace	,,
Luderudu	Landrace	,,	Masindi5	Landrace	,,
Magana	Landrace	,,	MasindiR21	Landrace	,,
Mercury	Landrace	,,	Mukalasa	Elite	,,
Mufumbachai	Landrace	,,	Nyakakwa	Landrace	,,
Musita	Landrace	,,	Nyalanda	Landrace	,,
Nabunanyuza	Landrace	,,	Nyamutukura	Landrace	,,
Namukoni	Landrace	,,	Nyapamitu	Landrace	,,
Namulalu	Landrace	,,	Nyarare	Landrace	,,
Nase1	Elite	,,	Rugogoma	Landrace	,,
Nase12	Elite	,,	Rujumba	Landrace	,,
Nase3	Elite	,,	Rwaburaru	Landrace	,,
Omongole	Elite	,,	TimTim	Landrace	,,
Pilipili	Landrace	,,	Tongolo	Landrace	,,
Serere	Elite	,,	Tongolo2	Landrace	,,

gel electrophoresis in TBE buffer stained with ethidium bromide. The DNA samples were standardised to 50 ng μl^{-1} before PCR analysis.

Polymerase chain reaction (PCR). Polymerase chain reaction was carried out in a GeneAmp® PCR system 9700 thermal Cycler (Applied Biosystems, USA) using primers listed in Table 2. Ten microlitres of PCR reaction mix included 50 ng $\mu l^{-1}$of DNA; 1X buffer (10 mM Tris-HCL pH 8.0, 1 mM EDTA pH 8.0), 0.2 mM dNTPs, 2 mM MgCl2; 0.08 pmole of each of forward and reverse primers and 0.375 µl Taq polymerase (5U μl^{-1}) (BioLabs New England). PCR conditions included an initial denaturation of 3 min at 95 °C, followed by 35 cycles of 30 seconds at 95 °C; 1 min at the annealing temperature defined for each primer used (Table 2) and 1 min at 72 °C. The final extension included 30 min at 72 °C and final hold at 4°C**.** The PCR products were checked for amplification on 1.5% agarose gel electrophoresis stained with ethidium bromide in 1X TBE buffer at 80 V for 1 hour and visualised on trans-UV and photographed in UVP DIGIDOC-IT system (UVP BioImaging systems, USA). PCR products with high quality amplifications (high quality single bands) were subjected to capillary electrophoresis with ABI 3730 DNA genetic analyser for fragment

TABLE 2. SSR markers used to assess genetic diversity in 66 cassava accessions

Name	Type of repeat	Left primer (Forward 5' - 3')	Right primer (Reverse 5' - 3')	Annealing temperature (°C)	Product size
SSRY5	GA(38)	TGATGAAATTCAAAGCACCA	CGCCTACCACTGCCATAAAC	55	173
SSRY9	GT(15)	ACAATTCATCATGAGTCATCAACT	CCGTTATTGTTCCTGGTCCT	55	278
SSRY12	CA(19)	AACTGTCAAACCATTCTACTTGC	GCCAGCAAGGTTTGCTACAT	55	266
SSRY19	CT(8)CA(18)	TGTAAGGCATTCCAAGAATTATCA	TCTCCTGTGAAAAGTGCATGA	55	214
SSRY21	GA(26)	CCTGCCACAATATTGAAATGG	CAACAATTGGACTAAGCAGCA	55	192
SSRY38	CA(17)	GGCTGTTCGTGATCCTTATTAAC	GTAGTTGAGAAAACTTTGCATGAG	55	122
SSRY51	CT(11)CG CT(11) CA(18)	AGGTTGGATGCTTGAAGGAA	GGATGCAGGAGTGCTCAACT	55	298
SSRY52	GT(19)	GCCAGCAAGGTTTGCTACAT	AACTGTCAAACCATTCTACTTGC	55	266
SSRY59	CA(20)	GCAATGCAGTGAACCATCTTT	CGTTTGTCCTTTCTGATGTTC	55	158
SSRY63	GA(16)	TCAGAATCATCTACCTTGGCA	AAGACAATCATTTTGTGCTCCA	55	290
SSRY64	CT(13)CG CT(6)	CGACAAGTCGTATATGTAGTATTCACG	GCAGAGGTGGCTAACGAGAC	55	194
SSRY69	CT(18)ATT AT(2) CTTTCTT CTTT(2)CCTTCT	CGATCTCAGTCGATACCCAAG	CACTCCGTTGCAGGCATTA	55	239
SSRY100	CT(17)TT CT(7) CCCT	ATCCTTGCCTGACATTTTGC	TTCGCAGAGTCCAATTGTTG	55	210
SSRY102	GT(11)	TTGGCTGCTTTCACTAATGC	TTGAACACGTTGAACAACCA	55	179
SSRY110	GT(12)	TTGAGTGGTGAATGCGAAAG	AGTGCCACCTTGAAAGAGCA	55	247
SSRY135	CT(16)	CCAGAAACTGAAATGCATCG	AACATGTGCGACAGTGATTG	45	253
SSRY147	GA(16)	GTACATCACCACCAACGGGC	AGAGCGGTGGGGCGAAGAGC	45	113
SSRY148	GA(21)	GGCTTCATCATGGAAAAACC	CAATGCTTTACGGAAGAGCC	45	114
SSRY151	GA(126)	AGTGGAAATAAGCCATGTGATG	CCCATAATTGATGCCAGGTT	45	182
SSRY155	GA(136)	CGTTGATAAAGTGGAAAGAGCA	ACTCCACTCCCGATGCTCGC	55	158
SSRY161	CT(11)TT CT(21)CA(19)	AAGGAACACCTCTCCTAGAATCA	CCAGCTGTATGTTGAGTGAGC	55	220
SSRY169	GA(19)A(3)GAA(2)	ACAGCTCTAAAAACTGCAGCC	AACGTAGGCCCTAACTAACCC	55	100
SSRY171	TA(5)CATA GATA(8) GC GA(23)GTGA(2)	ACTGTGCCAAAATAGCCAAATAGT	TCATGAGTGTGGGATGTTTTTATG	55	291
SSRY181	GA(22)G(3)C GA(3) GGAA GA(4)	GGTAGATCTGGATCGAGGAGG	CAATCGAAACCGACGATACA	55	199
SSRY182	CA(17)N(31)GAGG GA(8)	GGAATTCTTTGCTTATGATGCC	TTCCTTTACAATTCTGGACGC	55	253
NS911		TGTTGTTCAGACGATGTCCAA	TTGAAGCAGTTATGAACCGT	50	127

segregation and allele calls were made using GENEMAPPER software v.3.7 (Applied Biosystems).

Data analysis. The data generated by Gene Mapper were analysed for genetic diversity parameters, including number of alleles per locus, allelic frequency, percent of polymorphic loci, observed heterozygosity, genetic differentiation and gene diversity (expected heterozygosity) obtained per locus and per group of accessions (landraces and elite), using Power marker software (Liu and Muse, 2005), GenAlEx (Peakall and Smouse, 2006) and Darwin (Perrier and Jacquemound-Collet, 2006). Cluster analysis for the accessions was performed on the similarity matrix using the Neighbour-Joining algorithm (Nei, 1973) and the results displayed as a dendrogram.

RESULTS

Genetic diversity parameters were assessed with 26 SSR markers across all the cassava accessions (landraces and elite accessions combined n=66) and the results are presented in Table 3. All (100%) SSR markers were polymorphic for both landraces and elite cassava accessions. A total of 154 polymorphic alleles across the groups and regions were observed. The number of alleles across loci and groups ranged between three and 11 with average number of alleles of 5.923. SSRY100 recorded the highest number of alleles followed by SSRY 69 (Table 3). The number of alleles in landraces ranged from 2 alleles in SSRY 155 to 10 alleles with SSRY 100 and SSRY 69 with mean number of alleles across all loci of 5.769 (Table 4). In elite accessions, the number of alleles ranged between 2 alleles (SSRY 147 and SSRY 5) and 8 alleles (SSRY64 and SSRY 100) with average number of alleles across loci of 4.5.

Of the 154 alleles revealed by 26 SSR markers across loci and groups, 41 (26.6%) were unique alleles either occurring only in landraces or in elite accessions. Thirty seven (90.2%) of the unique alleles occurred only in landraces (accounting for 24% of total alleles) and four (9.8%) occurred only in elite accessions. The SSRY 182 and SSRY 69 recorded the highest number (4) of unique alleles. Most of these unique alleles were 'rare' with allele frequencies of less than 0.05 (Table 5). For regions, accessions from Northern Uganda had no unique alleles while accessions collected from Eastern Uganda had higher number of unique alleles not presented in accessions from other regions (Table 5), but these differences are most likely a reflection of sample size than inherently more unique genotypes.

The average expected heterozygosity (gene diversity) (He) averaged across all the groups and loci ranged from 0.477 in SSRY 155 to 0.842 in SSRY 64 with an average of 0.667, while observed heterozygosity ranged from 0.273 to 0.985 in SSRY 59 and SSRY 148, respectively, with an average of 0.726. Polymorphic information content (PIC) of loci across the groups was highest in SSRY 64 (0.822) and lowest in SSRY 155 (0.377) with average of 0.611(Table 3).

Gene diversity among landraces was high ranging from 0.490 (SSRY 155) to 0.834 (SSRY 64) with a mean of 0.661. Landraces also had higher observed heterozygosity, ranging between 0.294 in SSRY 59 and 0.980 in SSRY148, with mean of 0.729, and higher PIC ranging between 0.370 in SSRY 155 and 0.813 in SSRY 64 with mean 0.604. Among elite accessions, gene diversity ranged from 0.291 (SSRY 155) to 0.824 (SSRY 64) with average of 0.637 while observed heterozygosity ranged from 0.2 in SSRY 59 to 1.0 in SSRY 148, SSRY 151 and SSR 5 with average of 0.720. The average PIC in elite varieties was 0.585 (Table 4).

Gene diversity was high in accessions collected from Eastern Uganda and was least in accessions collected from Northern Uganda. The genetic differentiation between the two groups (landraces and elite) as estimated by *Fst* (theta) averaged over all loci, was low (mean = 0.025). For regions, the *Fst* values between accessions collected from Central and Eastern regions was 0.012, Central and Northern was 0.048, Central and Western 0.012, Eastern and Northern 0.045, Eastern and Western 0.013, and between Northern and Western *Fst* was 0.035. The sample sizes used for different groups and regions were different and this could have affected the results (Table 4).

Principle coordinate analysis (PCoA) was calculated from dissimilarity coefficients and is graphically presented in Figure 1. The coordinates were calculated for the two first axes

TABLE 3. Genetic diversity parameters averaged across all groups and loci*

Marker	No. of Alleles	No. of unique alleles*	% alleles unique	He	Ho	PIC	F	Fst
SSRY9	7	1	0.143	0.649	0.606	0.620	0.074	0.007
SSRY102	3	0	0.000	0.574	0.424	0.484	0.268	0.076
SSRY169	6	2	0.333	0.626	0.364	0.591	0.425	0.011
SSRY51	4	0	0.000	0.740	0.485	0.691	0.351	0.025
SSRY64	9	1	0.111	0.842	0.909	0.822	-0.072	0.018
SSRY135	8	2	0.250	0.766	0.803	0.727	-0.041	0.018
SSRY148	6	2	0.333	0.717	0.985	0.667	-0.367	0.007
SSRY63	5	1	0.200	0.594	0.516	0.531	0.140	0.035
SSRY182	9	4	0.444	0.653	0.576	0.597	0.126	0.058
SSRY19	9	3	0.333	0.726	0.727	0.697	0.005	0.008
SSRY69	10	4	0.400	0.803	0.818	0.775	-0.011	0.041
NS911	5	0	0.000	0.664	0.846	0.623	-0.268	0.008
SSRY161	6	1	0.167	0.761	0.708	0.723	0.077	0.025
SSRY110	7	0	0.000	0.612	0.848	0.565	-0.379	0.030
SSRY52	4	0	0.000	0.574	0.600	0.520	-0.037	0.018
SSRY151	4	1	0.250	0.631	0.982	0.560	-0.550	0.020
SSRY155	3	0	0.000	0.477	0.615	0.377	-0.284	0.105
SSRY12	5	1	0.200	0.640	0.769	0.597	-0.195	0.013
SSRY21	7	3	0.429	0.778	0.937	0.743	-0.196	0.017
SSRY38	5	1	0.200	0.715	0.906	0.663	-0.260	0.002
SSRY147	3	1	0.333	0.507	0.970	0.386	-0.912	0.001
SSRY5	3	1	0.333	0.544	0.952	0.439	-0.745	0.002
SSRY181	5	1	0.200	0.684	0.800	0.627	-0.162	0.023
SSRY100	11	3	0.273	0.805	0.515	0.781	0.367	0.035
SSRY171	4	1	0.250	0.673	0.953	0.604	-0.409	0.003
SSRY59	6	3	0.500	0.575	0.273	0.484	0.531	0.046
Mean	5.923	1.423	0.240	0.667	0.726	0.611	-0.082	0.025

*He = expected heterozygosity (gene diversity), Ho = observed heterozygosity, Unique alleles = Alleles only in landraces, PIC = Polymorphic Information Content, F = fixation index/estimated inbreeding, Fst = genetic differentiation index

TABLE 4. Comparison of genetic diversity parameters averaged across loci and individuals within group and region variation of landraces and elite accessions

Group/region	Number of Alleles	Gene Diversity(He)	Heterozygosity(Ho)	PIC
Group				
Landrace (n=51)	5.769	0.661	0.729	0.604
Elite (n=15)	4.500	0.637	0.720	0.585
Region				
Central (n=12)	4.423	0.641	0.703	0.582
Eastern (n=24)	5.038	0.668	0.714	0.615
Northern (n=3)	3.038	0.571	0.782	0.503
Western (n=27)	5.038	0.653	0.740	0.593
Across groups/regions (n=66)	5.923	0.667	0.726	0.611

PIC = Polymorphic Information Content

TABLE 5. Allele frequencies of unique alleles by group and regions

Unique alleles by group				Unique alleles by regions				
Marker	Allele	Elite (n=15)	Landrace (n=51)	Marker	Allele	Central (n=12)	Eastern (n=24)	Western (n=27)
SSRY135	258	0.067		SSRY64	189	0.042		
SSRY182	229	0.067		SSRY135	258	0.083		
SSRY155	153	0.067		SSRY182	219	0.042		
SSRY100	241	0.033		SSRY38	72	0.042		
SSRY9	261		0.029	SSRY9	258		0.104	
SSRY169	83		0.088	SSRY135	247		0.021	
SSRY169	99		0.02	SSRY148	111		0.021	
SSRY64	189		0.01	SSRY182	220		0.021	
SSRY135	247		0.01	SSRY19	208		0.021	
SSRY135	255		0.01	SSRY69	220		0.021	
SSRY148	109		0.02	SSRY69	230		0.021	
SSRY148	111		0.01	SSRY110	243		0.063	
SSRY63	282		0.01	SSRY151	216		0.031	
SSRY182	219		0.01	SSRY155	153		0.042	
SSRY182	220		0.01	SSRY21	187		0.065	
SSRY182	226		0.049	SSRY100	218		0.083	
SSRY182	234		0.02	SSRY100	241		0.021	
SSRY19	208		0.01	SSRY59	156		0.021	
SSRY19	213		0.02	SSRY59	163		0.021	
SSRY19	214		0.01	SSRY135	255			0.019
SSRY69	214		0.01	SSRY148	109			0.037
SSRY69	220		0.01	SSRY63	282			0.019
SSRY69	230		0.01	SSRY182	234			0.037
SSRY69	238		0.01	SSRY19	214			0.019
SSRY161	216		0.02	SSRY69	214			0.019
SSRY151	216		0.011	SSRY69	238			0.019
SSRY12	263		0.1	SSRY161	216			0.038
SSRY21	187		0.031	SSRY110	251			0.037
SSRY21	191		0.021	SSRY147	111			0.019
SSRY21	193		0.052	SSRY181	188			0.037
SSRY38	72		0.01	SSRY100	237			0.037
SSRY147	111		0.01	SSRY171	289			0.037
SSRY5	105		0.057	SSRY59	142			0.019
SSRY181	188		0.02					
SSRY100	185		0.029					
SSRY100	202		0.039					
SSRY100	218		0.039					
SSRY171	289		0.02					
SSRY59	142		0.01					
SSRY59	156		0.01					
SSRY59	163		0.01					
Total number of alleles		4	37			4	15	14

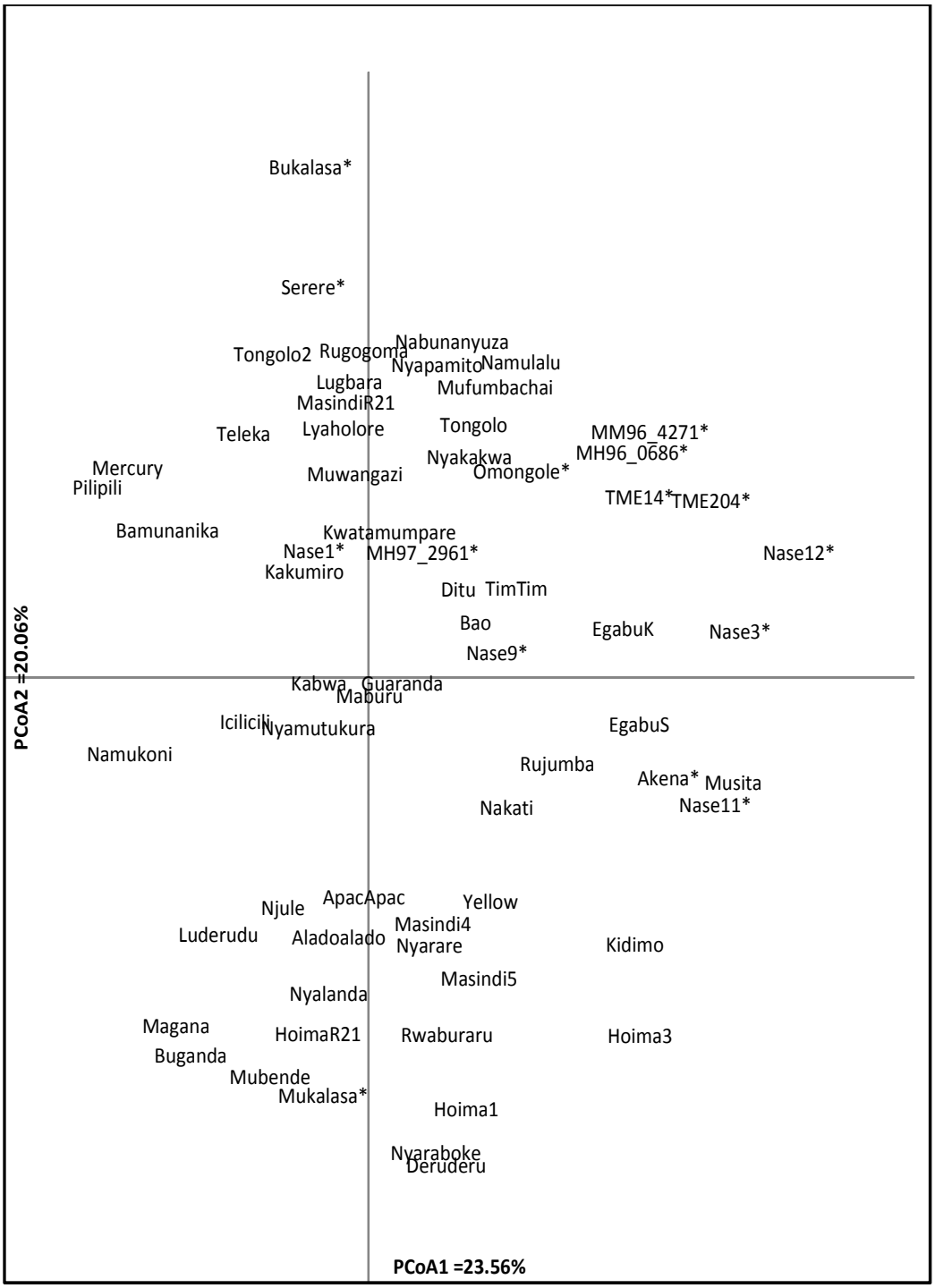

Figure 1. Principle coordinates analysis from dissimilarity coefficients of 66 cassava accessions (elite accessions are marked with asterisks).

with positive Eigen values. The two axes accounted for 43.62% of the total variation with the first axis (PCoA1) accounting for 23.56% and second (PCoA2) accounting for 20.06%. PoCA1 roughly separated landraces from elites with only *Mukalasa, Nase* 1 and *Bukalasa* being on the side of landraces. The PCoA2 put 12 (80%) of elite accessions on the upper cluster. The three elite accessions (*Akena, Nase* 11 and *Mukalasa*) that clustered with landraces in lower cluster may indicate that these accessions may have similar allele frequencies as landraces in the lower cluster. The PCoA however, showed loose clustering of both the elite and landraces because elite accessions clustered together with some landraces indicating that they share some alleles.

The dendrogram was constructed using the Neighbour Joining Method (NJ) (Nei, 1973) and separated the 66 accessions into four major clusters (Fig. 2). The primers did not fully discriminate the elite accessions from landraces. All elite accessions were included in cluster 1 (C1). The clusters C2, C3 and C4 contained only landraces. Clustering of accessions did not reflect regions from where they were collected. The dendrogram showed strong relationship between *Nyarare* and unknown *Masindi4* (which are actually duplicates), *Ditu* and *TimTim*, *Nyalanda* and *Icilicili,* and *Aladoalado* and *Njule*. The results from cluster analysis by Neighbour Joining Method (NJ) (Nei, 1973) were in general agreement with results from principle coordinates analysis at a level consistent with percent of variability accounted for by the PCoA.

DISCUSSION

Genetic diversity and differentiation among landraces and elite cassava accessions, and accessions according to region of origin was assessed with 26 SSR markers. Twenty six SSR markers are considered adequate to give reliable results on genetic diversity among and within populations of cassava (Fregene *et al*., 2003). Fregene *et al*. (2003) reported that PIC values increase exponentially with an increase in number of marker loci below a total of 30 markers. Kalinowski (2005) demonstrated that reliable estimates of genetic diversity can be obtained from less than 20 individuals per population with 16 polymorphic loci. Nei (1978) had earlier indicated that the reliability of genetic diversity results depends more on the number of loci than on the number of individuals sampled. Moyib *et al*. (2007) obtained comparable results when using between five and 16 polymorphic SSR markers with Nigerian cultivated cassava suggesting that application of few number of polymorphic SSR markers is possible for genetic variation studies in cassava. However in this study, the estimates of genetic differentiation varied widely at different loci (ranging between 0.01 and 0.105) suggesting that assessment of SSR diversity may require more than 16 SSR markers.

The SSR markers showed high mean PIC (61.1%) demonstrating their ability to discriminate between individual accessions. The higher the PIC of the marker, the more informative the marker is. Across groups and loci SSRY 64 was the most informative marker (PIC = 82.2%) while SSRY 155 (PIC = 37.7%) was the least informative. The PIC obtained in this study is within the range of the previous studies in cassava using SSR markers. Sree Lekha *et al*. (2010) obtained an extremely high mean percentage polymorphism (88.89%) among the Indian cassava while Adebola *et al*. (2009) reported slightly lower mean PIC (55%) with accessions collected from different African countries. Kawuki *et al*. (2009) reported PIC ranging from 0.358 to 0.759 with average of 0.571 when using cassava germplasm from Africa, Asia and America. The high levels of PIC obtained in these studies suggest that polymorphic SSR markers in cassava are generally very informative. The SSR markers provide greater discrimination among accessions making them appropriate for diversity assessments. The high PIC average value of SSRs is derived from their multi-allelism, rapid mutation rate and low probability of being affected by the narrowing influence of selection (Kawuki *et al.*, 2009).

Cassava accessions showed high mean (5.923) alleles per polymorphic locus across the accessions. The high number of alleles obtained is in agreement with a recent study conducted on cassava in Puerto Rico by Montero-Rojas *et al*. (2011) who found an average of 7.15 alleles per locus ranging between two and 14 alleles per locus. Fregene *et al*. (2003) reported average

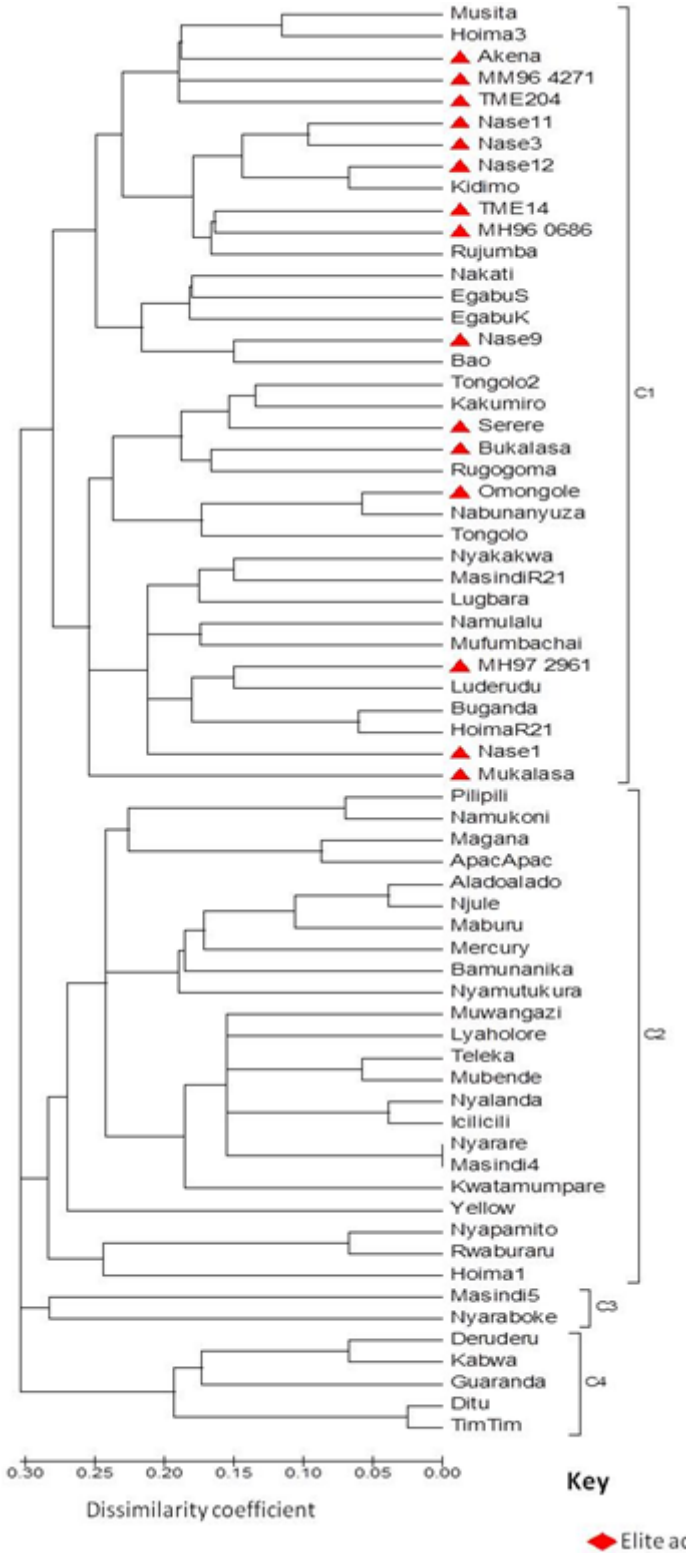

Figure 2. Dendrogram showing relationships among cassava accessions.

number of alleles of 6.0 for cassava landraces from Colombia and 5.2 for Brazilian cassava. Kawuki *et al.* (2009), working with three different populations, from Asia, Africa and American cassava reported number of alleles ranging between three and eleven. Similarly, Kizito *et al.* (2005) using landraces from Ugandan reported average of alleles of 5.229 per SSR locus. Therefore, the number of alleles among cassava accessions from Uganda is comparable to that from Puerto Rico, Asia, Colombia and Brazil.

Both expected (He) and observed heterozygosity (Ho) averaged across the landraces and elite accessions were high, 0.667 and 0.726, respectively. The He is an indication of the probability that two randomly selected alleles from an accession of germplasm in Uganda are different is 66.7%. Expected heterozygosity also known as "gene diversity" was introduced by Nei (1978) to explain the probability that two alleles arbitrarily selected are different. Similar high observed and expected heterozygosity in cassava has been reported. Raji *et al.* (2009) using cassava germplasm from various countries in Africa, reported average values of He and Ho of 0.630 and 0.730, respectively. Montero-Rojas *et al.* (2011) reported He of 0.709 and Ho of 0.671 with Puerto Rico cassava germplasm. However, the average values of He obtained in this study were higher than obtained by Kizito *et al.* (2005) of 0.532 among Ugandan landraces and Kawuki *et al.* (2009) of 0.553, 0.556 and 0.615 for Asian, African and American cassava germplasm, respectively. Cassava is clonally propagated by cuttings, and this together with human selection over time for stress tolerant, vigorous and well-adapted landraces that has occurred over time is expected to have reduced its genetic diversity (Fregene *et al.*, 2003). However, the high diversity obtained in this study is an indication that genetic diversity and diversification of the crop has been maintained. The out-crossing and heterozygous nature of cassava combined with the use of volunteer seedlings as new varieties maintains considerable genetic diversity in cassava (Fregene *et al.*, 2003; Raji *et al.*, 2009). High genetic diversity is desirable because it increases fitness and therefore, reduces the likelihood of local extinction (Futuyma, 2005).

Heterozygosity was slightly higher in landraces than elite varieties. Similarly, the number of alleles in landraces was higher than in elite accessions. High heterozygosity means high genetic variability suggesting that the landraces were slightly more variable than the elite germplasm. The relatively low genetic variability within the elite cultivars is a direct reflection of sharing of most of the alleles among themselves suggesting comparatively narrow genetic base. This may be attributed to limited number of germplasm resources available to cassava breeders during the initial development of elite cultivars. In addition breeding pressures for selected traits of economic importance and the vegetative propagation nature of cassava (Raji *et al.*, 2009) may be responsible for low genetic diversity observed in elite accessions. The high genetic diversity among landraces can be attributed to spontaneous recombination and farmer selection from volunteer seedlings as new varieties, a practice common in the traditional agricultural practice of slash and burn (Fregene *et al.*, 2003; Kizito *et al.*, 2005). Genetic diversity among landraces in a particular geographical region is also affected by different cultural traditions, inter-ethnic contacts and economic pressures (Mignouna and Dixon, 1997; Emperaire *et al.*, 2001; Raji *et al.*, 2007). The differences in heterozygosity obtained between landraces and elite groups may also have resulted from usage of different sample sizes (n) for different groups. Nevertheless, since many alleles were unique to the landraces, breeding programmes can benefit from including these genetic diverse landraces in breeding programmes to broaden or widen genetic base.

The genetic differentiation between landraces and elite cassava accessions as estimated by *Fst* averaged across all loci was generally low (*Fst*=0.025). Similarly, the inbreeding coefficient across landraces and elite accessions was negative (mean= -0.082) and not very different within groups (-0.118 for elite and -0.120 for landraces). The low overall genetic differentiation may indicate that there is some random mating and interbreeding among the groups or at least among their ancestors. Likewise, the negative coefficient of inbreeding is an indication that there is no inbreeding within landraces and elite

accessions. Assortative mating, which results in excess heterozygosity, could also contribute to the negative inbreeding coefficients within the groups and low genetic differentiation between the groups (Hartl and Clark, 1997; Hedrick, 1999).

The *Fst* among accessions from different regions were low ranging from 0.048 between accessions collected from Central and Northern regions to 0.012 between accessions collected from Central and Eastern, and from Central and Western regions. The low differentiation between accessions in Central, Eastern and Western regions may indicate that farmers in these regions exchange accessions among themselves. The slightly greater difference between the Northern region and others could be simply a function of the low number of accessions from the North, but could also indicate less exchange between it and other regions. Lesser exchange could be attributed to the fact that farmers in Northern Uganda prefer growing bitter types of cassava whereas in Central, Western and Eastern Uganda, farmers prefer growing sweet types. This differentiation should be confirmed in a further study using a large sample size.

The genetic differentiation between accessions from Northern region and Central region may suggest that accessions from these regions could be used as a basis for the developing heterotic pools (Fregene *et al.*, 2003). Heterosis is expressed when there are differences in allele frequencies (genetic diversity) between populations (Falconer and Mackay, 1996). Nevertheless, to confirm heterotic potential, progeny from various crosses need to be evaluated (Hallauer *et al.*, 1988). The study also revealed a wide range of coefficient of genetic similarity from 0.029 to 0.598 indicating that it is possible to identify different parental combinations with maximum genetic variability for introgression of desirable genes from diverse landraces into the available elite genetic base (Smith, 1984; Mohammadi and Prasanna, 2003). Magoon and Krishnan (1977), and Cowen and Frey (1987) reported that parents should be genetically diverse to provide allelic variation that can be used to create new favourable gene combinations and exploit heterosis.

The SSR markers revealed that thirty (58.8%) of the landraces had 37 unique alleles that were not present among the elite accessions. The unique alleles that were present only in landraces accounted for 24% of the total alleles across landraces and elite accessions. This information is important for cassava improvement and germplasm conservation. The unique alleles might be associated with farmer preferred traits in adapted landraces that are not present in elite accessions, a reason why farmers keep both elite and landraces in their fields. Including these landraces with unique alleles in breeding is likely to increase the chances of producing progenies with farmer preferred traits. Use of landraces to introgress tolerance to cassava mosaic disease has been reported (Lokko *et al*., 2005). The clustering revealed one duplicate of *Masindi4* and *Nyarare* landraces. The clustering further revealed misnaming of accessions among farmers. Farmers in different districts gave different accessions similar names or *vice versa* (two different accessions were given the same name under *Tongolo*, and *Egabu*). The misnaming by farmers is due to morphological similarities, perceived differences, use or characteristic trait. For example the two "*Tongolo*" varieties are bitter type and resemble each other in most morphological features except that "*Tongolo*2" has taller stems than *Tongolo*. Also in Masindi, a variety "*Lyahorole*" locally translated as "taste for yourself" is a name given to the variety due its good taste. Several authors (Mignouna and Dixon, 1997; Mignouna *et al*., 1998; Raji *et al*., 2007) reported that farmers exchange planting materials among themselves over a wide geographic range and different ethnic groups assign different vernacular names to similar varieties or similar names to different varieties.

CONCLUSION

The study revealed that landraces have unique alleles not represented in elite germplasm used in Ugandan breeding programme. This however, may be a result of the relatively small sample size used. These alleles could be associated with the farmer preferred traits or may suggest that the landraces have genes for adaptation to particular areas. Inclusion of such unique landraces in cassava breeding programmes would increase chances of producing farmer preferred or better

adapted elite cultivars. The high genetic diversity revealed in this study implies a high amount of additive genetic variance upon which progress in plant breeding depends. The study also revealed variable levels of differentiation between accessions from different regions of the country, particularly between the northern and central regions, although the northern region was only represented by three genotypes. This possibly reflects reduced germplasm exchange among these regions, and should be explored further using an increased sample size. The genetic differentiation among accessions from different regions provides an opportunity for the establishment of heterotic pools within in a breeding programme. Information on the genetic differentiation and genetic distances among individuals is an important tool for selection of genetically distinct cultivars to perform guided crosses. Lastly, the genetic diversity observed among landraces in this study and previous studies is an indication that cassava landraces in Uganda may be very useful for identification of new and unique alleles.

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