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Assessing Genetic Diversity of Indian Cassava: A Comparison of Old and New Collections Using Microsatellite Markers

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ABSTRACT

In this study the variability and diversity in cassava (*Manihot esculenta* Crantz) at the molecular level was assessed using SSR (simple sequence repeat) markers. 60 accessions of old collections and 50 accessions of new collections from different parts of South India were compared to study their genetic diversity. 36 sets of SSR markers, available in the public domain and known to generate polymorphic bands, were used for amplification. There were significant differences in genetic diversity between old and new collections. The similarity index among the old varieties ranged from 80 to 100% and from 68 to 98% in the new varieties. 34 loci or 34.34% of the loci were polymorphic in old collections and 59 or 93.65% of the loci were polymorphic in the new collection. The Shannon's Information Index for old varieties was 0.2778 and for new varieties it was 0.4967 for older ones. Wright's mean fixation index was higher than the mean value expected (5%) indicating an excess of homozygotic individuals in old collections. The presence of new recombination events by random and natural processes of mutation and recombination might have resulted in high genetic diversity in new collections.

Keywords: dendrogram, heterozygous, homozygous, simple sequence repeat, variability

INTRODUCTION

Cassava (*Manihot esculenta* Crantz, Euphorbiaceae) is a tropical root crop that originated in South America and spread throughout the world by Portuguese sailors in the 16th century (Rogers 1993). Since then, cassava has gradually become an economically important crop in developing countries. Today cassava ranks fourth among the major sources of carbohydrates in the tropics; where it is an important food for over 500 million people (FAO 2001; Montagnac *et al*. 2009). A considerable amount of genetic variation exists in cassava germplasm due to its heterozygous nature (Siquera *et al*. 2009). It is used as a subsidiary food and also as a raw material for the starch industry (Srinivas 2007). Cassava was introduced in Kerala during 17th century through Portuguese sailors. Because of its high adaptability to rainfed conditions and low-fertility soils, it became a subsidiary food and a famine reserve crop. Planned introduction was made by local rulers in the 19th century. Later, more varieties were introduced through international organizations. Even though only a few varieties were introduced initially, many natural recombinants have evolved over the years and good variability is available in the field. Studies have been conducted earlier to assess the variability based on biometrical characters as well as RAPD (randomly amplified polymorphic DNA) markers (Pillai 2002; Pillai *et al.* 2004). DNA-based molecular markers such as RAPDs, nuclear RFLPs (restriction fragment length polymorphism) and microsatellites were used to develop the cassava molecular genetic map (Fregene *et al*. 1997). There is a wide range of molecular techniques available to assess genetic variability of a species. Due to their co-dominant inheritance, robustness and amenability to high throughput, simple sequence repeats (SSRs) or microsatellites have become a tool of choice for investigating important crop germplasm (Hokanson *et al*. 1998). Microsatellites are short stretches of tandemly repeated, 1-5 nucleotide sequences, such as (G-A) n. They are ubiquitously present in eukaryotic genomes and are highly polymorphic (Tautz *et al*. 1989). Conservation of microsatellite flanking sequences allows the design of primers for PCR amplification. In cassava, SSR markers have been used to search for duplicates in the CIAT (International Centre for Tropical Agriculture, Cali, Colombia) core collection (Chavarriaga-Aguirre *et al*. 1999) and to analyze variation in natural populations of putative progenitors of cassava (Olsen and Schaal 2001). At present more than 500 SSR markers are available in cassava which will provide genetic tags for various phenotypes in cassava. The objective of the present study is to quantify the genetic variability and diversity available in the land races of old and new collections of cassava in South India.

MATERIALS AND METHODS

Plant material

60 morphologically distinct cassava accessions of old collections and 50 accessions of new collections from different parts of south India (States Kerala and Tamilnadu) were selected for studying the variation in morphological characters and SSR allele diversity.

DNA extraction

Extraction of DNA was carried out according to Dellaporta *et al.* (1983). Plants 3-4 weeks old were selected and around 2 g of fresh and young leaf tissue was used for DNA extraction. Between 500 µg and 1 mg of high purity DNA was obtained from each extraction and quantified by UV absorption at 260 nm using a spectrophotometer (Shimadzu UV-260). The extracted DNA was dissolved in TE buffer, and treated with 3 µl RNase A, 10 mg ml^{-1}) (Bangalore Genie, Bangalore, India), incubated at 37°C for 10 min, and stored at -20°C until use. DNA was also quantified by means of 0.8% agarose gel electrophoresis followed by ethidium bromide staining. A 1 kb DNA ladder (Bangalore Genie) was run parallel as DNA size marker.

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Original Research Paper

PCR assay and gel analysis

A set of 36 SSR markers developed at CIAT (Chavariagga-Aguirre *et al*. 1998; Mba *et al*. 2001) were used for the genetic variability study. The SSR markers used in the present study are listed in **Table 1**. The reaction mixture (25 µl) consisted of 10X buffer, 100 mM each of dNTPs, 600 mM $MgCl_2$, 600 pM of each forward and reverse primer (all from Banglore Genei), 0.5 U *Taq* polymerase (Finnzymes, Finland) and 25 ng of template DNA. PCR was carried out in a thermal cycler (MJ Research PTC-100, USA), under the following conditions; an initial denaturation at 94°C for 4 min followed by 40 cycles of 94°C for 1 min each, 35°C for 1 min and 72°C for 2 min and a final extension at 72°C for 5 min. The amplified DNA fragments were separated through agarose gel electrophoresis. Approximately 10 µl of the amplified products and 1 kb molecular ruler were run for 2 h at 80 V on a 3% (w/v) agarose gel. PCR products from DNA bulks of the different accessions were each loaded into one lane. The different accessions were adjacent on each gel to enable the identification of different alleles, even in closely related accessions. The gels were stained in an EtBr solution (1 mg/L for 15 min, rinsed in ddH_2O for 15 min and observed under a Gel Doc System for DNA fragment analysis (Syngene).

Genetic data analysis

Allelic frequencies of SSR markers were used to estimate the percentage of polymorphic loci (*P*), mean number of alleles per locus (*A*), effective number of alleles (A_E), and observed heterozygosity (H_E) (Hedrick 2004) using the computational program POPGENE 32 (Yeh and Yang 1999). Loci were considered polymorphic if more than one allele was detected. Wright's fixation index (*F*) was estimated using the formula $F = 1\text{-}(Ho/He)$ to quantify the lack or excess of heterozygosity. Out-crossing rate (*t*) was estimated using $t = (1\text{-}F)/(1+F)$ (Weir 1996). The portioning of genetic diversity within and among the cultivar of cassava was analyzed using *F*-statistics (Nei 1973) according to the equations of Weir and Cockerham (1984). Cluster analysis of the SSR data was performed separately with the assistance of the SIMQUAL programme of NTSYS software, version 2.10 (Applied Biostatistics Inc., Setauket, NY, USA). Similarity matrices were generated using DICE and simple matching coefficients. An unweighted pair grouping by mathematical averaging (UPGMA) cluster analysis was produced from similarity matrices constructed for SSR data and resulting dendrograms were compared. Principal component analysis (PCA) was applied to identify group of primers which contributed to the variation among the genotypes and to identify group of lines which showed similar response to primers. The PCA removes intercorrelation that may exist between genotypes by transforming the original variables into few numbers of hypothetical components. The new PCA are orthogonal to each other (Smith 1991). Statistical analysis was done using SAS v. 8, 1999. A scatter diagram was plotted for the 36 primers using the scores obtained from first two principle components in the case of both old and new collections of cassava.

RESULTS

The morphological features of the cassava varieties are summarized in **Tables 2A** and **2B**. 36 primers were used to perform the amplification reactions, which generated distinct bands. Polymorphic DNA bands were scored for the presence (1), absence (0) or ambiguous (9) for each accession by visual inspection. To ensure accurate scoring, all markers were scored twice from two different gels. SSR primers used in DNA amplifications resulted in scorable PCR bands or loci (**Fig. 1A, 1B**). Band size ranged from 0.2 to 0.3 kb and the number of scorable bands per primer ranged from one to two. The primers utilized were highly informative.

The SSR fragments observed in the 60 accessions of old collections showed a low degree of polymorphism and 50 accessions of new collections showed a high degree of polymorphism within the populations. Each band produced by the primers was distinct and reproducible. The polymor-

Table 1 Sequence of SSR primers used for amplification.

No.	Sequence (5′-3′)	Product size
1	GGTAGATCTGGATCGAGGAGG	199
	CAATCGAAACCGACGATACA	
2	CGACAAGTCGTATATGTAGTATTCACG	194
	GCAGAGGTGGCTAACGAGAC	
3	ACTGTGCCAAAATAGCCAAATAGT	291
	TCATGAGTGTGGGATGTTTTTATG	
4	AGTGGAAATAAGCCATGTGATG	182
	CCCATAATTGATGCCAGGTT	
5	AACTGTCAAACCATTCTACTTGC	266
	GCCAGCAAGGTTTGCTACAT	
6	TGTCCAATGTCTTCCTTTCCTT	196
	CTTTTTGCCAGTCTTCCTGC	
7	TGTGACAATTTTCAGATAGCTTCA	211
	CACCATCGGCATTAAACTTTG	
8	CAACAATTGGACTAAGCAGCA	192
	CCTGCCACAATATTGAAATGG	
9	AGGTTGGATGCTTGAAGGAA	298
	GGATGCAGGAGTGCTCAACT	
10	CATTGGACTTCCTACAAATATGAAT	143
	TGATGGAAAGTGGTTATGTCCTT	
11	GGAAACTGCTTGCACAAAGA	270
	CAGCAAGACCATCACCAGTTT	
12	AGTGCCACCTTGAAAGAGCA	247
	TTGAGTGGTGAATGCGAAAG	
13	CGTTGATAAAGTGGAAAGAGCA	158
	ACTCCACTCCCGATGCTCGC	
14	CAGGCTCAGGTGAAGTAAAGG	226
	GCGAAAGTAAGTCTACAACTTTTCTAA	
15	AAGGAACACCTCTCCTAGAATCA	220
	CCAGCTGTATGTTGAGTGAGC	
16	GTACATCACCACCAACGGGC	113
	AGAGCGGTGGGGCGAAGAGC	
17	AAGACAATCATTTTGTGCTCCA	290
	TCAGAATCATCTACCTTGGCA	
18	ACCACAAACATAGGCACGAG	268
	CACCCAATTCACCAATTACCA	
19	AACGTAGGCCCTAACTAACCC	100
	ACAGCTCTAAAAACTGCAGCC	
20	TCGAGTGGCTTCTGGTCTTC	225
	CAAACATCTGCACTTTTGGC	
21	TCAAACAAGAATTAGCAGAACTGG	187
	TGAGATTTCGTAATATTCATTTCACTT	
22	GCAATGCAGTGAACCATCTTT	158
	CGTTTGTCCTTTCTGATGTTC	
23	GGCTGTTCGTGATCCTTATTAAC	122
	GTAGTTGAGAAAACTTTGCATGAG	
24	ATAGAGCAGAAGTGCAGGCG	287
	CTAACGCACACGACTACGGA	
25	TCTCCTGTGAAAAGTGCATGA	214
	TGTAAGGCATTCCAAGAATTATCA	
26	CATGCCACATAGTTCGTGCT	203
	ACGCTATGATGTCCAAAGGC	
27	ACAATTCATCATGAGTCATCAACT	278
	CCGTTATTGTTCCTGGTCCT	
28	TTCCAGACCTGTTCCACCAT	279
	ATTGCAGGGATTATTGCTCG	
29	CGATCTCAGTCGATACCCAAG	239
	CACTCCGTTGCAGGCATTA	
30	CCAGAAACTGAAATGCATCG	253
	AACATGTGCGACAGTGATTG	
31	GCTGAACTGCTTTGCCAACT	130
	CTTCGGCCTCTACAAAAGGA	
32	TGAGAAGGAAACTGCTTGCAC	272
	CAGCAAGACCATCACCAGTTT	
33	TTGGCTGCTTTCACTAATGC	179
	TTGAACACGTTGAACAACCA	
34	CCTTGGCAGAGATGAATTAGAG	163
	GGGGCATTCTACATGATCAATAA	
35	ATCCTTGCCTGACATTTTGC	210
	TTCGCAGAGTCCAATTGTTG	
36	ACAATGTCCCAATTGGAGGA	NA
	ACCATGGATAGAGCTCACCG	

Table 2A List of 60 old collections of cassava accessions used in the present study and their distinctive morphological characters.

Sl.no	Place of collection	Colour of stem	Apical leaf color	Petiole color	Lob shape	Apical pubescence	Tuber skin color	Tuber rind color	Tuber flesh color	Neck length	Flower	Periderm color
OC1	CT	SG	G	GP	L	A	LB	C	W	N	A	C
OC2	NT	SG	P	P	L	A	C	C	W	N	P	C
OC3	CT	G	LP	P	L	A	LB	C	W	N	A	W
OC4	Che	G	P	GP	E	A	C	C	W	SN	P	C
OC5	Che	LG	LP	P	E	A	DB	C	C	LN	A	LP
OC6	Che	LG	LG	LP	GP	E	P	DB	C	W	MN	P
OC7	Che	SG	LP	P	E	A	LB	C	W	N	P	LP
OC8	Che	LB	LP	P	E	A	DB	P	W	MN	P	W
OC9	T	G	LP	GP	E	P	DB	LP	C	NN	P	W
OC10	Che	G	LP	GP	L	P	LB	C	C	NN	A	C
OC11	Anc	BL	P	GP	E	A	DB	P	W	LN	A	P
OC12	Anc	G	P	GP	E	A	LB	C	W	MN	A	C
OC13	Anc	G	P	GP	L	P	LB	LP	W	NN	A	LP
OC14	Anc	SG	LP	GP	E	A	LB	C	W	M	P	LP
OC15	KK	G	LP	P	E	A	C	C	C	SN	A	P
OC16	KK	G	LP	P	E	A	LB	C	W	SN	P	P
OC17	T	LP	GP	E	P	LB	C	W	LN	P	P	P
OC18	G	G	LP	GP	E	A	C	C	W	LN	P	P
OC19	Q	G	LP	GP	E	A	C	C	C	MN	P	P
OC20	Q	G	LP	P	L	A	LB	C	W	LN	P	C
OC21	T	G	LP	GP	E	A	C	C	W	S	P	C
OC22	T	G	LP	GP	E	A	DB	P	W	L	P	C
OC23	NT	G	LP	P	L	A	LB	C	W	S	A	P
OC24	Q	G	LP	GP	L	A	LB	C	W	S	A	P
OC25	Q	G	LP	GP	E	A	LB	LP	W	S	P	P
OC26	PK	G	LP	P	E	P	LB	C	C	A	A	P
OC27	Q	G	P	GP	L	P	DB	P	W	S	A	C
OC28	PK	LB	P	GP	E	A	DB	C	W	A	P	P
OC29	KK	LG	P	GP	E	A	DB	P	W	A	A	P
OC30	CT	G	P	GP	L	P	DB	LP	W	S	P	C
OC31	KK	G	LP	GP	E	A	LB	LP	W	A	P	P
OC32	Q	G	LP	GP	E	A	LB	C	C	S	A	P
OC33	Q	LB	P	P	L	A	DB	P	C	S	P	LP
OC34	Q	G	LP	P	L	P	DB	LP	W	S	A	P
OC35	Che	SG	G	G	E	A	C	C	W	S	A	P
OC36	CT	G	LP	GP	E	A	LB	C	C	S	P	P
OC37	T	SG	G	GP	E	A	C	C	C	L	A	P
OC38	KK	SG	LP	GP	E	A	DB	C	C	S	A	C
OC39	T	G	LP	GP	E	A	LB	C	C	S	A	P
OC40	K	S	LP	GP	E	A	LB	C	C	S	A	P
OC41	Ka	LB	LP	GP	L	P	LB	C	W	S	A	C
OC42	Ka	LG	LP	P	E	A	DB	P	W	S	A	C
OC43	Ka	G	LP	GP	E	A	C	C	N	S	A	P
OC44	Q	G	LP	P	E	A	C	C	N	S	A	P
OC45	Q	LB	LP	P	E	P	DB	C	C	S	A	C
OC46	Q	G	P	G	L	A	DB	LP	C	S	A	P
OC47	T	G	LP	P	E	A	LB	C	C	L	P	P
OC48	Q	G	LP	P	E	A	LB	C	C	L	P	C
OC49	T	SG	P	P	E	A	LB	P	W	S	P	P
OC50	PK	LB	G	P	E	A	DB	LP	W	S	P	P
OC51	PK	G	LP	GP	E	A	DB	LP	W	S	A	C
OC52	PK	LB	LP	P	L	A	DB	P	W	S	P	C
OC53	Mad	G	LP	GP	E	A	C	LP	N	S	P	P
OC54	T	G	P	GP	E	A	DB	P	N	S	P	P
OC55	T	LG	LP	P	E	A	DB	P	C	S	P	C
OC56	Mys	G	LP	P	E	A	LB	C	C	S	P	P
OC57	Kili	G	LP	P	E	A	C	C	C	S	P	C
OC58	Nil	G	LP	P	E	A	LB	C	W	S	P	P
OC59	T	SG	LP	GP	E	A	C	C	W	S	P	P
OC60	KK	G	LP	P	E	A	C	C	C	S	P	P

OC-Old collections

CT-Central Travancore, NT- North Travancore, Che- Chennai, T- Trivandrum, Anc- Anchal, KK- Kayamkkulam, Q- Quilon, PK- Palakkad, K- Kollam, Ka- Kattakada, Mad- Madurai, Mys- Mysore, Kili- Kilimannoor, Nil- Nilamel

*LB- Light brown, SG- Slightly green, DB- Dark brown, LP- Light pink, G- Green, P- Pink, GP- Greenish pink, C- Cream, W- White

**L- Long, E- Elliptical, S- Small, L- Long

***A- Absent, P- Present

phic bands produced were efficient in assessing the genetic diversity among the cultivars. Based on SSR bands amplified by 36 primers, genetic distances among the cassava accessions were calculated and a dendrogram was constructed for both old and new collections by UPGMA method (**Fig. 2A, 2B**). The UPGMA dendrogram based on genetic distance indicated that genetic similarity value ranged from 80 to 100% in the case of old collections and 68 to 98% in the case of new collections.

60 varieties of cassava collected from old collection

Table 2B List of 50 new collections of cassava accessions used in the present study and their distinctive morphological characters.

Sl.no	Place of collection	Colour of stem	Apical leaf color	Petiole color	Lob shape	Apical pubescence	Tuber skin color	Tuber rind color	Tuber flesh color	Neck length	Flower	Periderm color
NC1	T	LB	LG	GP	E	A	DB	LP	C	S	A	LG
NC2	T	SG	G	GP	E	A	LB	C	W	L	A	LG
NC3	T	LG	LP	GP	E	A	DB	C	C	L	A	G
NC4	T	G	P	GP	E	P	LB	C	W	A	A	G
NC5	T	LB	P	GP	E	A	LB	C	W	A	A	LG
NC6	T	G	G	G	E	P	LB	LP	W	S	A	G
NC7	T	G	LP	GP	E	P	DB	C	Y	S	A	G
NC8	T	G	LP	GP	E	P	LB	W	W	S	A	G
NC9	T	LB	LP	P	E	A	LB	C	Y	S	A	G
NC10	T	G	LP	P	E	A	LB	C	W	A	A	G
NC11	T	LG	LP	GP	E	A	DB	W	W	S	P	G
NC12	T	G	LP	GP	E	A	DB	W	W	S	A	G
NC13	T	G	LP	GP	E	A	LB	LP	W	A	P	G
NC14	NK	LB	LP	GP	E	A	LB	P	W	A	A	LG
NC15	T	G	G	G	E	A	DB	C	C	S	A	G
NC16	T	LG	LP	GP	E	P	BD	LP	W	S	A	G
NC17	T	LG	LP	GP	E	A	LB	C	Y	S	A	DG
NC18	T	G	G	GP	E	A	DB	P	W	S	A	G
NC19	Id	LB	LP	GP	E	A	DB	P	W	S	A	LG
NC20	T	G	LP	GP	E	A	LB	P	W	S	P	G
NC21	Id	LG	P	P	E	A	DB	C	C	S	P	DG
NC22	Id	LG	LP	GP	L	A	DB	P	Y	S	A	DG
NC23	T	LB	LP	P	E	A	DB	P	C	S	A	LG
NC24	T	DB	LP	P	E	A	DB	P	W	A	A	LG
NC25	Tri	LB	LP	P	E	A	DB	P	C	S	A	LG
NC26	T	LB	LP	P	E	A	DB	P	C	S	A	LG
NC27	Id	LB	LP	GP	E	A	LB	LP	C	A	A	LG
NC28	Id	G	LP	GP	E	A	DB	LP	W	S	P	LG
NC29	T	G	P	P	L	A	LB	P	W	S	A	G
NC30	T	DB	LP	P	E	A	LB	C	W	S	A	LG
NC31	Tri	DB	P	P	E	A	DB	C	Y	S	A	LG
NC32	Tri	LB	LP	GP	E	A	LB	C	W	A	A	LG
NC33	ER	G	LP	GP	E	A	LB	P	W	A	A	G
NC34	ER	LB	LP	GP	E	A	DB	C	W	A	A	LG
NC35	ER	LG	P	GP	E	A	DB	C	W	S	A	G
NC36	ER	SG	LP	GP	L	A	LB	C	W	L	A	LG
NC37	MO	LG	P	GP	E	A	DB	C	C	A	A	DG
NC38	ER	LB	LP	GP	E	P	LB	P	W	A	A	LG
NC39	Tri	LG	LP	GP	E	A	DB	C	W	S	A	G
NC40	Tri	G	LP	P	E	P	C	C	W	S	A	G
NC41	Tri	G	LP	P	E	A	LB	C	W	S	A	G
NC42	Tri	G	LP	P	E	A	DB	P	W	A	A	G
NC43	Pal	LB	LP	GP	E	A	LB	LP	W	S	A	G
NC44	Pal	G	LP	GP	E	A	DB	C	W	A	A	LG
NC45	Cal	LB	LP	GP	E	A	LB	C	C	A	A	G
NC46	Cal	LB	LP	GP	E	A	LB	LP	W	S	A	LG
NC47	Cal	LG	LP	GP	E	A	LB	P	W	S	A	LG
NC48	Pa	SG	LP	GP	E	A	DB	W	W	L	A	DG
NC49	Ma	G	LP	P	E	A	C	C	C	S	A	G
NC50	Da	G	LP	P	E	A	LB	C	C	S	A	G

NC- New collection
*T- Trivandrum, NK- Northern Kerala, ID- Idukki, Tri- Trichur, ER- Erode, Pal- Palakkad, Cal- Calicut, Ma- Mavelikara
**LB- Light brown, SG- Slightly green, DB- Dark brown, LP- Light pink, G- Green, P- Pink, GP- Greenish pink, C- Cream, W- White
***L- Long, E- Elliptical, S- Small, L- Long
****A- Absent, P- Present

were grouped into two clusters based on their origin and morphological characters (**Table 3A**). Cluster 1 consists of 4 accessions which were collected from Chennai, Tamilnadu State. Cluster II which was the major group collected from both Tamil Nadu State and different parts of Kerala State. The second cluster was further subdivided into 6 groups. Group 1 which consisted of only one accession having hairs in apical stem, which was also collected from Chennai. Accessions 6, 7 and 8 belonging to group 2 have same apical leaf color light pink, apical pubescence absent and tuber flesh color white. Accession numbers 11 and 12 are in group 3 and are collected from Anchal, Kerala. They have the common characters like apical leaf color pink, petiole color greenish pink, lob shape elliptical, apical pubescence absent and flower absent.

Major accessions come under group 4 which is collected from different parts of Kerala. This group is further divided into several subgroups based on their characters. Accession numbers 13,14,15, 16, 17, 18, 23, 24, 31, 32 and 34 have 100% similarity and they have common characters like stem color green, apical leaf color light pink, lob shape elliptical, tuber rind color cream and periderm color pink. Accession numbers 27, 28, 29 and 30 have 100% similarity and were collected from northern part of Kerala. They have same apical leaf color pink, petiole color greenish pink, tuber skin color dark brown and tuber flesh color white. Accession numbers 25 and 26 have 100% similarity and common characters are green stem, apical leaf color light pink, elliptical lob shape, tuber skin color light brown and periderm color pink. Accession numbers 19 and 20 have

Table 3A List of varieties in old collections in different clusters.

Cluster No	Sub group	No of varieties
I		4- (OS-1,2,3,4)
II	(I)	1- (OS-5)
	(II)	3- (OS-6,7,8)
	(III)	2- (OS-11,12)
	(IV)	27- (OS-13,14,15,16,17,18,23,24,31,32,34,27,28,29,30,33,25,26,19,20,21,22,35,36,37,38,39)
	(V)	21- (OS-40,47,49,43,42,41,50,48,46,51,53,52,44,45,54,60,57,58,56,59,55)
	(VI)	2- (OS-9,10)

Table 3B List of new collections in different clusters.

Cluster No	Sub group	No of varieties
I	(1)	9- (S-1, 2, 3, 26, 18, 20, 19, 21, 22)
	(11)	13- (S- 5, 30 ,9, 6, 23, 29, 24, 38, 39, 40, 35, 36, 37)
	(111)	9- (S-17, 28, 33, 31, 41, 42, 34, 25, 27)
	(1V)	2- (S- 4, 8)
	(V)	4- (S- 10, 11, 32, 12)
	(V1)	8- (S- 43, 44, 45, 46, 47, 48, 49, 50)
II		5- (S-7, 13, 15, 16, 14)

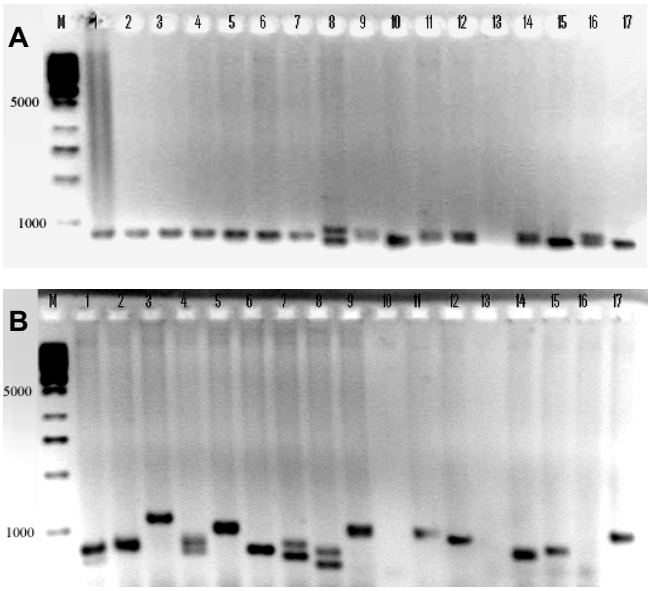

Fig. 1 Representative gel showing SSR marker profile of 17 accessions in (**A**) old and (**B**) new accessions. Lane M = 1 kb molecular weight marker; Lanes 1-17: SSR pattern of old (**A**) or new (**B**) cassava accessions.

common characters like apical leaf color light pink, no hairs in apical stem, tuber rind color cream and cylindrical in shape. Accession numbers 35 and 36 have 100% similarity having common characters like elliptical lob shape, apical pubescence absent, tuber rind color cream, small neck and periderm color pink. Accession numbers 37, 38 and 39 have 100% similarity and have the same petiole color greenish pink, elliptical lob shape, apical pubescence absent, tuber rind color cream and flower absent.

The fifth group is also further divided into sub groups. Accession numbers 40, 41, 42, 43, 46, 47, 48 and 49 have 100% similarity and they have common origin Kattakada, Kerala. Accessions 51, 52 and 53 have same origin Palakkad, Kerala and have common characters like apical leaf color light pink and tuber rind color light pink. Accessions 44 and 45 originated in Quilon, Kerala and have same rind color, cream. Accession no 54, 56, 57, 58, 59 and 60 have 100% similarity and have apical leaf color light pink, rind cream, flower present and apical pubescence absent. Group 6 consist of accession numbers 9 and 10 both are originated in Tamilnadu State having green stem, apical leaf color light pink, flesh color cream, apical pubescence present.

50 accessions of new collections are also grouped into two clusters (**Table 3B**). Cluster II consisted of five accessions, and were collected from Trivandrum district, Kerala

Table 4 Genetic variation parameters of both old and new accessions.

	Old accessions	New accessions
P	34.34	93.65
Ao	1.3434	1.9365
A_E	1.2252	1.5834
H_O	0.1329	0.3347
H_E	0.1838	0.3684
F	0.2769	0.0914
t	0.5662	0.8325

P- Percentage of polymorphic loci, A_O- mean number of allele per locus, A_E- mean effective number of alleles, H_O-mean observed heterozygosity, H_E- mean expected heterozygosity, *F*-Wright's fixation index, *t*- out crossing rate

State. Cluster I was the major group and was further subdivided into 6 groups. The first group consisted of nine accessions and their place of origin is Trivandrum. The second group which was collected from Palakad and Trichur, Kerala consisted of 13 accessions. The 3rd group collected from northern part of Kerala consisted of nine accessions. The fourth group consisted of only two varieties; their place of origin was Trivandrum. These two varieties are similar in apical leaf color, periderm color, tuber skin color and of leaf lob shape. The four accessions which grouped together to form the 5th group were collected from Erode, Tamilnadu. The sixth group consisted of eight accessions and was collected from Palakad and Calicut, Kerala. In this group accession Ci 48 and Ci 49 showed 100% similarity. These two varieties are similar in all the characters studied like place of collection, stem color, lob shape, apical pubescence, tuber skin color, tuber rind color, tuber flesh color, neck length, flower and periderm color. The results of PCA analysis of old and new collections in the present study is presented in **Fig. 3A** and **Fig. 3B**, respectively.

Population genetic analysis

Population genetic analysis in different cassava accessions was done using POPGENE software. Each band produced was treated as a locus and variations among the alleles were calculated. The SSR markers used in the study were able to differentiate the genetic diversity in the cassava accessions. The genetic diversity of cassava was revealed by percentage of polymorphic loci (*P*), mean number of alleles per locus (*A*), effective number of alleles (A_E), observed heterozygosity (H_O), and expected mean heterozygosity (H_E). Each band obtained by SSR procedure was treated as a gene loci and the homozygosity and heterozygosity for each loci was determined (**Table 4**). The genetic analysis of old collections of cassava accessions revealed that a low percentage of heterozygosity in that category keeping aside the homozygous gene locus which express only one of the allele at a time (**Table 5A**). The number of polymorphic loci and per-

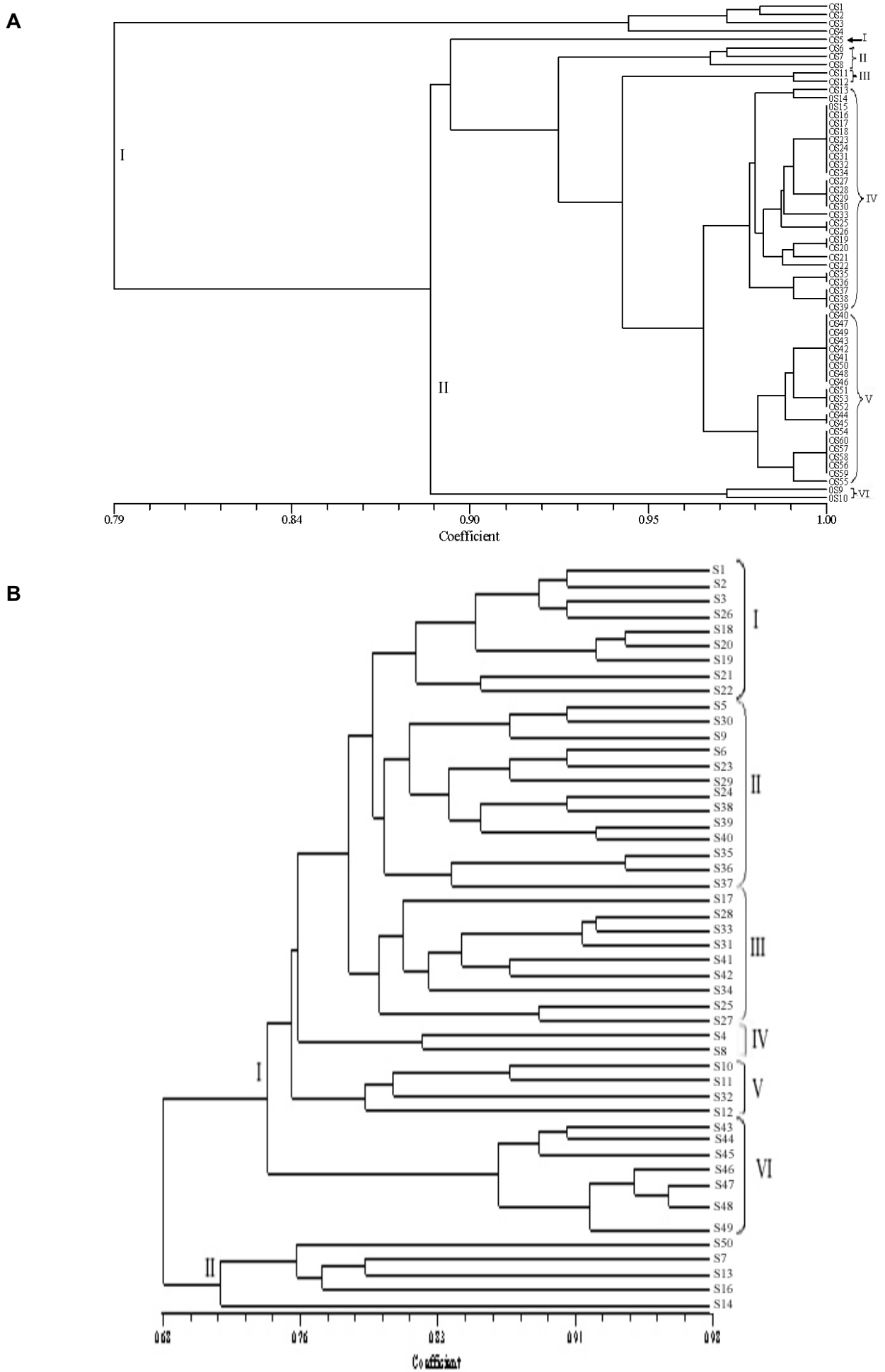

Fig. 2 UPGMA dendrogram of (**A**) 60 accessions of old cassava collections or (**B**) 50 accessions of new cassava collections based on the SSR data. The dendrogram was constructed from the matrix of Dices similarity coefficients.

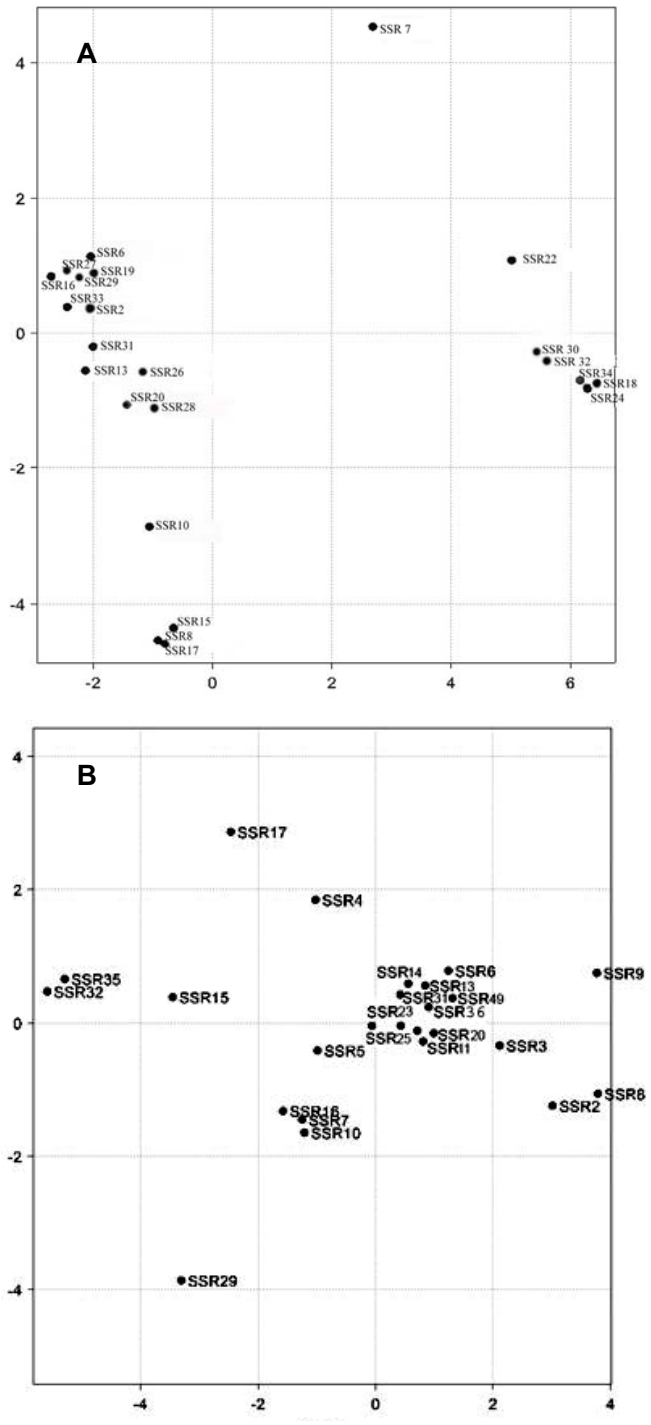

Fig. 3 Scatter diagram of the old (**A**) and new (**B**) collections of cassava. X-axes = PC 1; Y-axes = PC 2.

centage of polymorphic loci was 34 and 34.34%, respectively. The observed mean number of alleles (A_O), effective number of alleles (A_E), observed heterozygosity (H_O), and expected mean heterozygosity (H_E) were 1.3434, 1.2252, 0.1329 and 0.1838, respectively. On the other hand, new collections of cassava accessions revealed a high percentage of heterozygosity in different accessions keeping aside the homozygous gene locus viz. SSR 1-A, SSR 6-A and SSR 37-A (**Table 5B**). The Shannon's Information Index (I) for gene diversity was 0.2778 and 0.4967 for old and new collections respectively, reflecting genetic diversity in agreement with cluster analysis data. The above data shows that new alleles are formed in cassava population by the random and natural process of mutation and recombination and the frequency of occurrence of an allele changes regularly as a result of mutation, genetic drift and selection in new collections.

DISCUSSION

Understanding the genetic diversity in tuber crops is important as it is the first step in harnessing their phenotypic variability for crop improvement. Morphological traits are useful tools for preliminary evaluation, because they offer a fast and useful approach for assessing the extent of diversity. The estimation of descriptive statistics of eleven different morphological traits studied in the present study revealed the existence of higher level of morphological diversity among the cassava accessions, providing scope for improvement through hybridization and selection. Morphological traits have previously been used to express genetic diversity in cassava (Lefevre and Charrier 1993; Haysom *et al*. 1994). Meanwhile, a number of genetic marker systems have also been used for the assessment of genetic diversity of cassava germplasm. They include allozymes (Ocampo *et al.* 1992), random amplified polymorphic DNA (RAPDs) (Tonukari *et al.* 1997), amplified fragment length polymorphisms (AFLPs) (Second *et al*. 1997), simple sequence repeats (SSRs) (Chavarriaga-Aguirre *et al*. 1998; Fregene *et al*. 2003) and Diversity array technology (DArT) (Xia *et al*. 2005).

In the present study, the high level of SSR variation found within the new collections of cassava was measured in terms of percentage of polymorphic loci, alleles per locus, or genetic diversity. They are indicative of high genetic differentiation within populations. The results showed that the level of polymorphism P (34.34%) and mean observed heterozygosity H_O (0.1329) derived from old samples were lower than those from new collections of cassava (93.65% and 0.3347, respectively). The level of polymorphism in both old collections and new collections also provided differing views of the amount of genetic variation in the cassava collections. In a recent study, Siqueira *et al.* (2009) reported a high level of diversity (P = 88-100%; *Ho* = 0.265) among 42 landraces of cassava from different regions of Brazil revealed through SSR marker study. Meanwhile, studies conducted by Fregene *et al.* (2003) have reported an average heterozygosity of 0.5358 ± 0.1184 in cassava from different countries from Africa and the Neotropics.

The clustering pattern in the case of old collection showed very poor diversity than the new collections. A possible explanation for the differences found among these dendrograms might be based on the period of collection. This might stem from the fact that only few varieties were available in old time and farmers had given different names to the same varieties in course of time. The mean fixation index (F) for old collections was 0.2769 and that for new collections was 0.0914, indicating an overall conformance to Hardy-Weinberg equilibrium. The F value estimated to quantify the excess or deficiency of heterozygotes was substantially higher than the mean value expected (0.05 or 5%), indicating an excess of homozygotic individuals. The F value was significantly greater than zero and positive, indicating an excess of homozygotes. The excess of heterozygotes observed in this study may be the result of farmer selection during the domestication process, but an accumulation of somatic mutations can also contribute to the number of heterozygous genotypes (Birky Jr. 1996).

The out-crossing rate (t) based on fixation indices for old collection is 0.5662 and that for new collection is 0.8325, which is much higher than the value in old collection. da Silva *et al.* (2001) reported an out crossing rate from 0.69-1.00 among 8 ethnovarieties of cassava from Brazil. The population genetic analysis data further provides ample evidence for the fact that recombination events have occurred in the new cassava accessions could be due to natural selection. Apart from maintaining a high level of genetic diversity, the formation of new varieties also serves as an insurance against crop failure due to biotic and abiotic stress. The unique diversity suggests that the germplasm might have genes, in high frequencies, for adaptation to the area, while the high genetic diversity implies a high amount

Table 5A Allelic frequencies of polymorphic loci studied in 60 cultivars of cassava, sample size (N = 60).

Locus	Allele	Allelic frequency	Locus	Allele	Allelic frequency	Locus	Allele	Allelic frequency	Locus	Allele	Allelic frequency
SSR1-A	0	****	SSR9-C	0	****	SSR18-B	0	0.2649	SSR27-C	0	****
	1	1.0000		1	1.0000		1	0.7351		1	1.0000
SSR1-B	0	****	SSR10-A	0	0.3244	SSR19-A	0	0.2649	SSR28-A	0	****
	1	1.0000		1	0.6756		1	0.7351		1	1.0000
SSR1-C	0	****	SSR10-B	0	0.3244	SSR19-B	0	0.4956	SSR28-B	0	****
	1	1.0000		1	0.6756		1	0.5044		1	1.0000
SSR2-A	0	0.1873	SSR10-C	0	****	SSR19-C	0	****	SSR28-C	0	0.3504
	1	0.8127		1	1.0000		1	1.0000		1	0.6496
SSR2-B	0	0.2294	SSR11-A	0	****	SSR20-A	0	0.4189	SSR29-A	0	0.3974
	1	0.7706		1	1.0000		1	0.5811		1	0.6026
SSR2-C	0	****	SSR11-B	0	****	SSR20-B	0	****	SSR29-B	0	****
	1	1.0000		1	1.0000		1	1.0000		1	1.0000
SSR3-A	0	****	SSR12-A	0	****	SSR20-C	0	****	SSR29-C	0	0.3746
	1	1.0000		1	1.0000		1	1.0000		1	0.6254
SSR3-B	0	****	SSR12-B	0	****	SSR21-A	0	0.2962	SSR30-A	0	0.2962
	1	1.0000		1	1.0000		1	0.7038		1	0.7038
SSR3-C	0	****	SSR12-C	0	****	SSR21-B	0	0.2294	SSR30-B	0	****
	1	1.0000		1	1.0000		1	0.7706		1	1.0000
SSR4-A	0	0.2649	SSR13-A	0	0.1325	SSR21-C	0	****	SSR31-A	0	****
	1	0.7351		1	0.8675		1	1.0000		1	1.0000
SSR4-B	0	****	SSR13-B	0	****	SSR22-A	0	****	SSR31-B	0	****
	1	1.0000		1	1.0000		1	1.0000		1	1.0000
SSR4-C	0	****	SSR13-C	0	****	SSR22-B	0	****	SSR31-C	0	0.2294
	1	1.0000		1	1.0000		1	1.0000		1	0.7706
SSR5-A	0	****	SSR14-A	0	****	SSR23-A	0	0.1873	SSR32-A	0	****
	1	1.0000		1	1.0000		1	0.8127		1	1.0000
SSR5-B	0	****	SSR14-B	0	****	SSR23-B	0	****	SSR32-B	0	0.2649
	1	1.0000		1	1.0000		1	1.0000		1	0.7351
SSR6-A	0	0.1873	SSR14-C	0	****	SSR23-C	0	****	SSR33-A	0	****
	1	0.8127		1	1.0000		1	1.0000		1	1.0000
SSR6-B	0	0.1873	SSR15-A	0	****	SSR24-A	0	****	SSR33-B	0	****
	1	0.8127		1	1.0000		1	1.0000		1	1.0000
SSR6-C	0	0.1873	SSR15-B	0	****	SSR24-B	0	****	SSR33-C	0	0.2649
	1	0.8127		1	1.0000		1	1.0000		1	0.7351
SSR7-A	0	0.5774	SSR15-C	0	****	SSR25-A	0	****	SSR34-A	0	0.1325
	1	0.4226		1	1.0000		1	1.0000		1	0.8675
SSR7-B	0	0.5774	SSR16-A	0	****	SSR25-B	0	0.39740.60	SSR34-B	0	0.1325
	1	0.4226		1	1.0000		1	26		1	0.8675
SSR7-C	0	****	SSR16-B	0	****	SSR25-C	0	****	SSR35-A	0	****
	1	1.0000		1	1.0000		1	1.0000		1	1.0000
SSR8-A	0	0.2649	SSR16-C	0	****	SSR26-A	0	****	SSR35-B	0	0.3746
	1	0.7351		1	1.0000		1	1.0000		1	0.6254
SSR8-B	0	0.2649	SSR17-A	0	****	SSR26-B	0	****	SSR36-A	0	****
	1	0.7351		1	1.0000		1	1.0000		1	1.0000
SSR8-C	0	0.2649	SSR17-B	0	0.2294	SSR26-C	0	****	SSR36-B	0	****
	1	0.7351		1	0.7706		1	1.0000		1	1.0000
SSR9-A	0	****	SSR17-C	0	****	SSR27-A	0	****	SSR36-C	0	****
	1	1.0000		1	1.0000		1	1.0000		1	1.0000
SSR9-B	0	****	SSR18-A	0	0.2649	SSR27-B	0	****			
	1	1.0000		1	0.7351		1	1.0000			

**** monomorphic allele

of additive genetic variance, upon which progress in plant breeding depends.

The high level of differentiation between land races represent a heterotic pool and provide an opportunity for the systematic exploitation of hybrid vigor in cassava. Out crossing within cassava as well as possible genetic introgression from other species could account for the variation. The two collections in the present study gave different views of the amount of genetic variation and genetic relationship. The study of population genetics is increasingly important as we struggle to maintain healthy, wild and domestic populations and ecosystems. Moreover, information on the population's effective population size, heterozygosity levels and inbreeding coefficients for particular individuals can be used to design relocation or planned breeding programs which will help to maximize the genetic variation in successive generations. The current study provide a data base for cassava breeders informed about choices in selection of parental accessions for use in breeding program based on genetic diversity.

In the present study, PCA analysis of old samples showing less variation and that of new samples are varied and showed more variation. The hierarchical clustering illustrated in dendrogam is usually reflected in PCA scatter plot. The PCA analysis provides information about associations of accessions, which are useful to formulate better strategies for breeding. It also helps in identifying primers which contributed much to the variation present in the population. Results of the present study also establish that the primers used in new samples could readily be used for genotype identification and genetic diversity studies in the cultivated cassava. Cluster analysis and principal component-based scatter plotting showed greater similarity among landraces of cassava in Brazil (Siqueira *et al.* 2009). Lokko *et al.* (2009) also reported significant diversity within clusters among land races of cassava through PCA analysis.

Table 5B Allelic frequencies of polymorphic loci studied in 60 cultivars of cassava, sample size (N=50).

Locus	Allele	Allelic frequency	Locus	Allele	Allelic frequency	Locus	Allele	Allelic frequency
SSR1-A	0	****	SSR15-A	0	0.4738	SSR26-C	0	0.5151
	1	1.0000		1	0.5262		1	0.4849
SSR2-A	0	0.5714	SSR16-A	0	0.5714	SSR27-A	0	0.8571
	1	0.4286		1	0.4286		1	0.1429
SSR3-A	0	0.4949	SSR17-A	0	0.1429	SSR27-B	0	0.6389
	1	0.5051		1	0.8571		1	0.3611
SSR3-B	0	0.9897	SSR17-B	0	0.6999	SSR28-A	0	0.7954
	1	0.0103		1	0.3001		1	0.2046
SSR4-A	0	0.5345	SSR17-C	0	0.6220	SSR28-B	0	0.7423
	1	0.4655		1	0.3780		1	0.2577
SSR4-B	0	0.6851	SSR18-A	0	0.9258	SSR29-A	0	0.8207
	1	0.3149		1	0.0742		1	0.1793
SSR5-A	0	0.5151	SSR18-B	0	0.2020	SSR29-B	0	0.5714
	1	0.4849		1	0.7980		1	0.4286
SSR5-B	0	0.6999	SSR19-A	0	0.9689	SSR29-C	0	0.6227
	1	0.3001		1	0.0311		1	0.3773
SSR6-A	0	****	SSR19-B	0	0.6389	SSR30-A	0	0.8571
	1	1.0000		1	0.3611		1	0.1429
SSR7-A	0	0.5714	SSR20-A	0	0.7825	SSR31-A	0	0.3780
	1	0.4286		1	0.2175		1	0.6220
SSR7-B	0	0.8207	SSR21-A	0	0.3499	SSR31-B	0	0.3499
	1	0.1793		1	0.6501		1	0.6501
SSR8-A	0	0.6547	SSR22-A	0	0.1429	SSR32-A	0	0.8921
	1	0.3453		1	0.8571		1	0.1079
SSR9-A	0	0.3780	SSR22-B	0	0.9258	SSR32-B	0	0.3194
	1	0.6220		1	0.0742		1	0.6806
SSR10-A	0	0.2020	SSR23-A	0	0.3499	SSR33-A	0	0.2857
	1	0.7980		1	0.6501		1	0.7143
SSR10-B	0	0.9368	SSR23-B	0	0.9476	SSR33-B	0	0.4738
	1	0.0632		1	0.0524		1	0.5262
SSR11-A	0	0.2857	SSR23-C	0	0.3499	SSR34-A	0	0.8571
	1	0.7143		1	0.6501		1	0.1429
SSR12-A	0	0.3194	SSR24-A	0	0.9476	SSR35-A	0	0.2020
	1	0.6806		1	0.0524		1	0.7980
SSR12-B	0	0.9689	SSR24-B	0	0.4518	SSR35-B	0	0.2474
	1	0.0311		1	0.5482		1	0.7526
SSR13-A	0	0.1429	SSR25-A	0	0.8806	SSR36-A	0	0.4041
	1	0.8571		1	0.1194		1	0.5959
SSR14-A	0	0.4949	SSR26-A	0	0.3499	SSR37-A	0	****
	1	0.5051		1	0.6501		1	1.0000
SSR14-B	0	0.7284	SSR26-B	0	0.8921			
	1	0.2716		1	0.1079			

**** monomorphic allele

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