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Full Length Research Paper

Genetic variability studies between released varieties of cassava and central Kerala cassava collections using SSR markers

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Twelve released varieties of cassava and 24 central Kerala collections were assessed at the genomic DNA level with 36 SSR primers for genetic diversity study. The minimum number of SSR primers that could readily be used for identification of the 36 cassava genotypes was also determined. For the genetic diversity study, the similarity coefficients generated between released varieties and central Kerala varieties ranged from 40 to 95% and two separate DNA cluster groups were formed at 0.60 coefficients using "numerical taxonomy" and "multivariate analysis system software package". The similarity index for released varieties ranged from 60 to 93% and in the case of central Kerala varieties it ranged from 70 to 98%. The mean fixation index (F) for released varieties was 0.0688 and that for central Kerala collections was 0.1337, indicating an overall conformance to Hardy-Weinberg equilibrium. Principal component analysis helped in identifying primers which contributed much to the variation present in the population and reduce the cost and time of research for genetic diversity and genotype identification studies for cassava genetic improvement programs.

Key words: Cassava, genetic diversity, genotypes, microsatellites, principal component analysis, similarity index, simple sequence repeats primers.

INTRODUCTION

The genus *Manihot* originates from Latin America where 98 species are found (Rogers and Appan, 1973). *Manihot esculenta* Crantz (cassava) was initially introduced to Africa 400 years ago, where its cultivation for food spread throughout tropical and subtropical regions. The second *Manihot* species present in Africa, *M. glaziovii* Mueller Von Argau, was introduced 200 years ago as a source of rubber, although its distribution was less extensive (Jones, 1959). Cassava, which is generally propagated vegetatively, is one of the major sources of food in Africa (Cock, 1982). The roots, which are an excellent source of carbohydrates, have a very low protein content. In addition, the roots have a high content of cyanogenic glucosides (de Bruijn, 1971) which often necessitates extensive processing before cassava is edible. Cassava has the advantage of being well adapted to a wide range of environmental stresses. It grows very well in less fertile soil in contrast to many other crops that are highly vulnerable to environmental stresses during critical stages of plant development (Ugorji, 1998). Current economy advancement has also turned cassava into a cash crop, since several items are processed from it, which find various end uses. One of the best methods to increase cassava production to serve as the main food security and cash crop in Africa and developing countries is by the development of better varieties that are resistant to diseases, pests, and drought (Ugorji, 1998).

Genetic improvement of cassava is to a certain extent limited by a poor knowledge of genetic diversity within the

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Table 1a. Morphological characters of CTCRI released varieties of cassava.

Characteristics	RV1	RV2	RV3	RV4	RV5	RV6	RV7	RV8	RV9	RV10	RV11	RV12
Plant type	E.B	E.B	E.B	E.B	E.B	E.B	E.B	E.B	E.B	EB	MH	T
Stem colour	D.G	L.G	G	D.S	D.G	R.B	G	RB	GB	BW	LG	GG
Leaf colour	L.S	L.B	Gr	L.B	S	L.S	L.P	S	LP	LS	LS	LG
Leaf type	B	B	B	B	B	D.G	B	B	B	B	M	M
Petiole colour	D.G	L.G	G	D.G	P	P	L.P	LP	LG	P	P	DG
Flowering	F	F	F	F	F	SF	S F	F	F	SF	F	F
Tuber shape	C	Fu	C	Fu	C	C	C	Co	Co	Co	Co	C
Tuber skin colour	L.B	G.B	Cr	Br	L.B	Br	L.B	B	B	LB	B	SW
Tuber rind colour	Cr	Cr	L.P	Cr	Cr	Cr	Cr	P	Cr	Cr	LY	W
Tuber flesh colour	W	W	W	L.Y	W	W	W	W	LY	Cr	LY	W
Tuber neck	A	A	A	A	L.N	S.N	A	A	A	A	A	A

EB-Erect branching, DG-dark green, LS- light sepia, B- brown, F- flowering, Co-conical, C- cylindrical, LB- light brown, Cr-cream, W-white, A-absent, lg-light green, Fu- fusiform, GB- greyish brown, Gr- grey, G-green, LP-light pink, DS-dark sepia, S-sepia, P-pink, LY-light yellow, LN-long neck, SN-small neck, SF- shy flowering, RB-reddish brown, M-medium, MH-medium height, T-tall, GG-grayish green and SW- silvery white.

species. Isoenzymes have been used as a method to estimate genetic diversity within cassava, but low polymorphism was detected and the technique was not reproducible (Hussain et al., 1987; Ramírez et al., 1987; Lefèvre and Charrier, 1993). 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). Studies were conducted earlier to study the variability of cassava in Kerala using simple sequence repeats (SSR) markers (Sree Lekha and Pillai., 2008, 2010). DNA-based molecular markers such as RAPDs, nuclear RFLPs (restriction fragment length polymorphism) and microsatellites (= SSR markers) 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, SSRs or microsatellites have become a tool of choice for investigating important crop germplasm (Hokanson et al., 1998). SSR markers have been confirmed to be the most informative and appropriate for cassava (Mba et al., 2000). Perera et al. (2001) also supported SSR markers as the most informative for plants.

Valuable attributes of all SSR markers are co-dominance (many alleles are found among closely related individuals), technical simplicity, sensitivity, analytical simplicity (data are unambiguously scored, and highly reproducible) and are high abundance (markers are uniformly dispersed throughout genome as frequently as every 10 kb and therefore are ideal tools for many genetic applications. Microsatellites are short stretches of tandemly repeated, 1 to 5 nucleotide sequences, such as (G-A) n. They are ubiquitously present in eukaryotic genomes and are highly polymorphic (Tautz 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 was to: 1) quantify the genetic variability and diversity available in the land races of central Kerala and released varieties and 2) to assess the minimum number of SSR primers that could readily be used for the identification of 36 cassava genotypes in order to reduce the time and cost of research studies.

MATERIALS AND METHODS

Plant material

Twelve varieties of cassava which were released from our institute CTCRI to the farmers and twenty four cassava cultivars that were collected from central part of kerala were selected for this study. The varieties were planted at the CTCRI farm and were evaluated for plant type; stem colour, leaf colour, leaf type, petiole colour, flowering, tuber shape, skin colour, rind colour and flesh colour (Table 1a and b).

DNA extraction

DNA was extracted according to Dellaporta et al. (1983). Plants 3 to 4 weeks old were selected and approximately 2 g of fresh and young leaf tissue was used for DNA extraction. After crushing the fresh leaf tissue in a porcelain pestle using liquid nitrogen, 5 ml of extraction buffer was added then incubated for 30 min at 60°C.

Table 1b. Morphological characters of Central Kerala varieties of cassava.

Characteristics	CK1	CK2	CK3	CK4	CK5	CK6	CK7	CK8	CK9	CK10	CK11	CK12
Plant type	EB	EB	EB	EB	EB	EB	EB	EB	EB	EB	EB	EB
Stem colour	W	W	LP	W	D	W	W	LB	LB	W	LB	W
Leaf colour	LP	LP	LP	LP	LP	P	LP	P	G	P	G	G
Leaf type	B	B	M	B	M	B	B	B	B	B	B	M
Petiole colour	R	R	G	R	R	P	G	G	P	LP	P	P
Flowering	F	F	F	F	F	F	F	F	F	F	SF	SF
Tuber shape	C	C	C	C	C	C	C	Co	C	C	C	C
Tuber skin colour	LB	LB	B	L	B	B	LB	LB	LB	LB	LB	LB
Tuber rind colour	C	C	C	C	C	C	C	P	LP	C	P	C
Tuber flesh colour	W	W	W	W	C	W	W	W	C	C	W	W
Tuber neck	A	A	A	A	A	A	A	A	A	A	A	A

Table 1b. Contd.

Characteristics	CK13	CK14	CK15	CK16	CK17	CK18	CK19	CK20	CK21	CK22	CK23	CK24
Plant type	EB	EB	EB	EB	EB	EB	EB	EB	EB	EB	EB	EB
Stem colour	W	W	W	W	W	DB	W	W	LB	D	LB	W
Leaf colour	G	LP	P	G	G	G	LP	G	LP	P	G	G
Leaf type	B	B	B	B	B	B	B	B	M	B	B	B
Petiole colour	P	P	P	P	P	P	P	P	G	P	P	P
Flowering	F	SF	SF	F	F	F	F	F	F	F	F	SF
Tuber shape	Co	Fu	Fu	C	C	C	C	C	C	Fu	C	C
Tuber skin colour	LB	LB	LB	LB	LB	LB	B	LB	LB	LB	LB	B
Tuber rind colour	LP	C	C	C	C	C	C	LP	C	P	C	C
Tuber flesh colour	W	W	C	W	W	W	C	W	W	W	W	W
Tuber neck	A	A	A	A	A	A	L	MN	LN	MN	LN	LN

EB-Erect branching, W-white, G-green, B-broad, P-pink, F-flowering, Co-conical, LB- light brown, LP- light pink, MN- medium neck, P-pink, G-green, DB-dark brown, SF-small flower, Fu-fusiform, C-cylindrical, B-brown, A-absent and LN-long neck.

After incubation, 2.5 ml of 5 M potassium acetate was added and mixed well by inversion and incubated on ice for 20 min. The sample was centrifuged at 10,000 rpm for 10 min at 4°C. After centrifugation the supernatant was recovered and isopropanol was added to 2/3 of the previous volume by inverting slowly until the DNA precipitated. The precipitated DNA was centrifuged for 10 min at 10,000 rpm at 4°C. The supernatant was discarded and 1 ml of TE (10 mM Tris HCl and 1 mM EDTA; pH 8) was added and the nucleic acid was gently resuspended. Then 10 µl of RNase (Bangalore Genie, Bangalore, India) was added at 10 mg/ml per sample and incubated at 37°C for 1 h. Thereafter, 100 µl of 3 M sodium acetate and 2 ml of 95% ethanol was added to precipitate DNA and mixed by inversion, then centrifuged for 10 min at 10,000 rpm at 4°C.

To the DNA pellet, 500 µl of 70% ethanol was added. After centrifugation, the DNA was resuspended in 1 ml TE. Between 500 µg and 1 mg of high quality DNA was obtained from each extraction and quantified by UV absorption at 260 nm using a Shimadzu UV-260 spectrophotometer. DNA was also quantified by 0.8% agarose gel electrophoresis after staining with ethidium bromide (EtBr).

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 2. 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 by agarose gel electrophoresis. Approximately 10 µl of the amplified products and a 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.

Table 2. Sequence of SSR primers used for amplification.

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

NA- Not available.

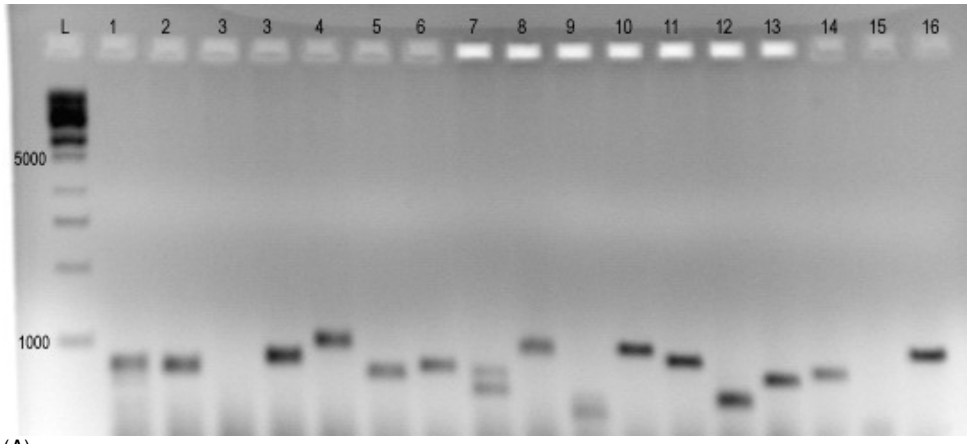
The gels were stained in an EtBr solution (1 mg/L) for 15 min, rinsed in double distilled water for 15 min and observed under a Gel Doc System for DNA fragment analysis (Syngene).

Genetic diversity study

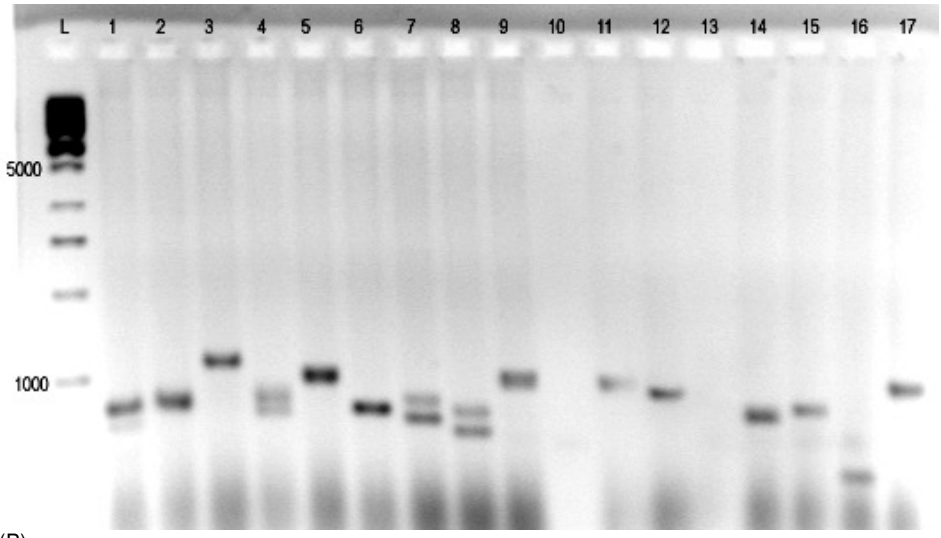
Allelic frequencies of SSR markers were used to estimate the percentage of polymorphic loci (*P*), mean number of alleles per locus (*A*), effective number of alleles (A_E), and observed heterozygosity (H_E) (Hedrick, 2004) using the computational program POPGENE 32 (Yeh and Yang, 1999). DNA bands were 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. Loci were considered to be polymorphic if more than one allele was detected. Wright's fixation index (*F*) was estimated using the formula:

$F = 1-(Ho/He)$

To quantify the lack of or excess heterozygosity, out-crossing rate (*t*) was estimated using $t = (1-F)/(1+F)$ (Weir, 1996). The portioning of genetic diversity within and among cassava cultivars was analyzed using *F*-statistics (Nei, 1973) according to the equations



(A)



(B)

Figure 1. Representative gels showing SSR marker profile of 17 (Lanes 1 to 17) released varieties (A) or central Kerala accessions (B). Lane M: 1-kb molecular weight marker.

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 groups of primers which contributed to the variation among the genotypes and to identify groups of lines which showed a similar response to primers. PCA removes any intercorrelation that may exist between genotypes by transforming the original variables into a few hypothetical components.

New PCAs 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 released varieties and the central Kerala cassava collections.

RESULTS

Genetic diversity in cassava was evaluated using 12 released varieties and 24 central Kerala varieties of cassava with SSR primers. The primers utilized were highly informative. Each band produced by the primers was distinct and reproducible. The polymorphic bands produced were efficient in assessing genetic diversity among the cultivars. Band size ranged from 0.2 to 0.3 kb and the number of scorable bands per primer ranged from 1 to 2. SSR primers used in DNA amplifications resulted in scorable PCR bands or loci (Figure 1a and b).

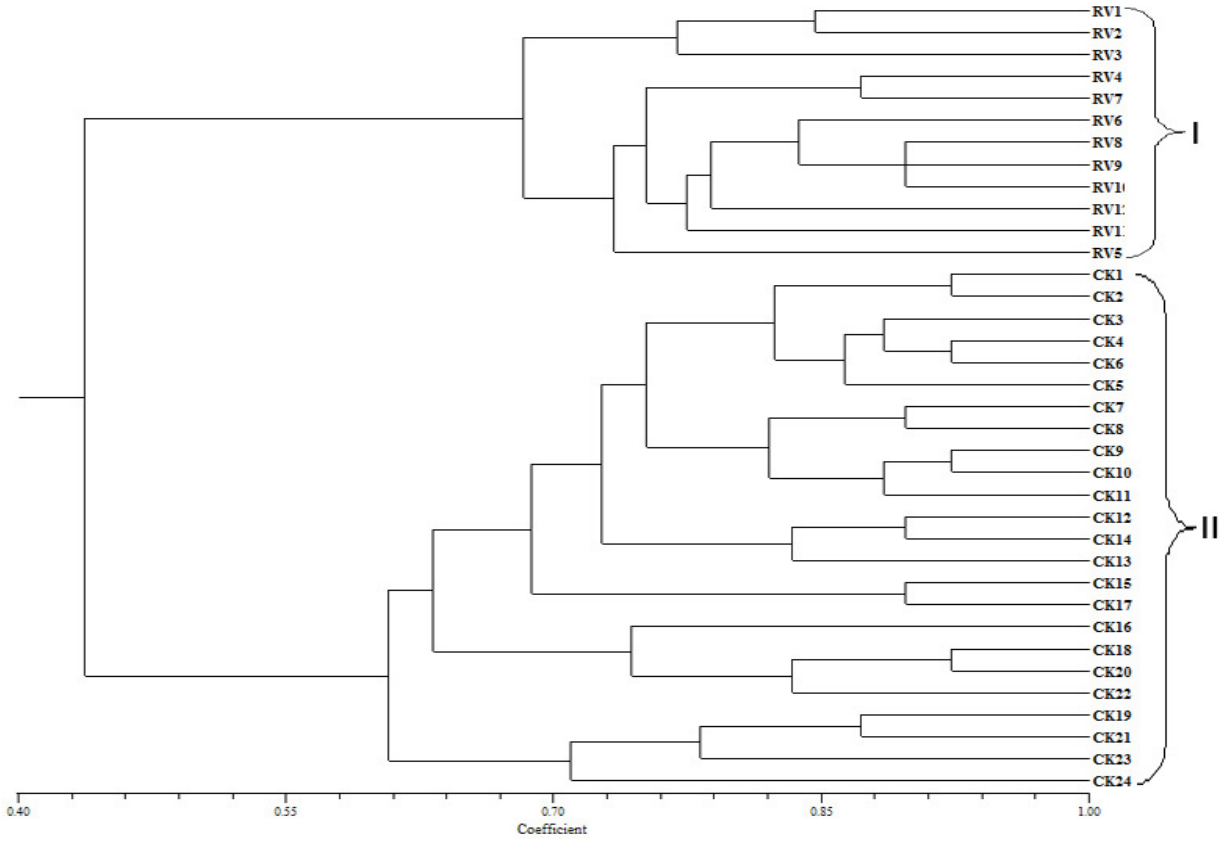


Figure 2. Unweighed Pair Group Method with Arithmatic Average (UPGMA) dendrogram of 12 accessions of released variety of cassava collections based on SSR data. The dendrogram was constructed from the matrix of Dice's similarity coefficients.

Based on SSR bands amplified by 36 primers, a total of 282 clear and scorable bands were detected for both released varieties and central Kerala collections using the 36 SSR primers and used for analysis using NTSYS software. The similarity matrix coefficient generated by the 282 SSR loci based on the NTSYS analysis ranged from 0.75 to 1.00 coefficients; the dendrogram obtained using UPGMA analysis in NTSYS software package revealed 6 distinct DNA cluster groups at 0.82 similarity coefficient units (Figure 2). Both released varieties of cassava and central Kerala varieties formed a distinct group and there was no overlapping of these two varieties. When the binary data from the 12 released varieties were treated alone in NTSYS, 10 DNA cluster groups were generated among the 12 released varieties (Figure 3) at 0.82 similarity coefficient units based on these morphological characters. Similarity index based on presence or absence of a specific band showed that the genetic similarity between varieties in this region varied from 60 to 93%. In cluster 1 vars. RV1 and RV2 were present: they have a dark green stem and light sepia leaf colour.

Cluster II includes var. RV3, which has special characters such as grey leaves. Cluster III included vars. RV4 and RV7. RV4 has a dark sepia stem colour and a fusiform-shaped tuber. RV7 has particular character such as light pink petioles and leaves. Only var. RV5 formed cluster IV; it has a long neck which is absent from accessions in other clusters. Cluster V consists of vars. RV6, RV8, RV9 and RV10, all of which have a reddish-brown stem and common characters such as a conical tuber. Var. RV12 was present in cluster VI. It is resistant to cassava mosaic disease (CMD) unlike all other varieties which are susceptible to CMD (Figure 4). Cluster VII consists of var. RV11 which is a medium height plant. Twenty four varieties collected from central Kerala were grouped into 6 clusters (Figure 5) at 0.82 similarity coefficient units based on there morphological characters; the genetic similarity between varieties in this region varied from 70 to 98%. Cluster I consisted of the major varieties, none of which had a neck. CK13 is the only variety in cluster II; it has special characters like narrow leaves. Cluster III consists of vars. CK19, CK20, CK21, CK22, CK23 and CK24, all of which have a small

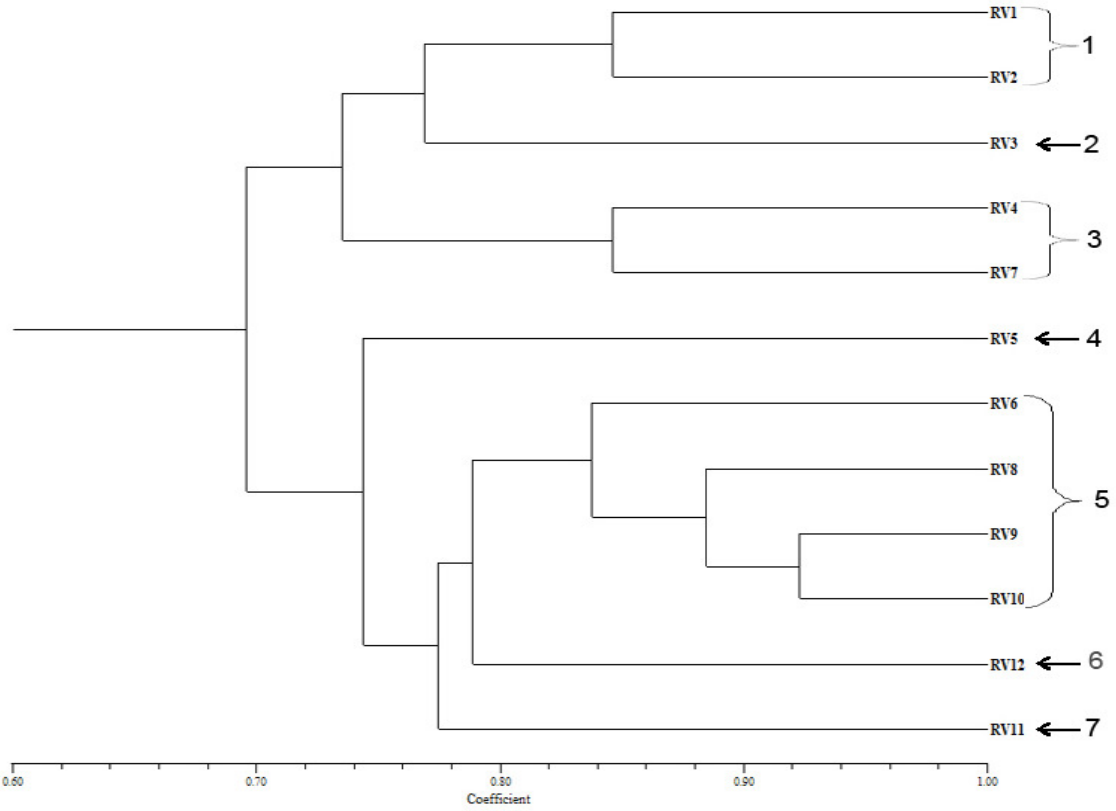


Figure 3. Unweighed Pair Group Method with Arithmatic Average (UPGMA) dendrogram of 24 accessions of cetral kerala collections based on the SSR data. The dendrogram was constructed from the matrix of Dice's similarity coefficients.

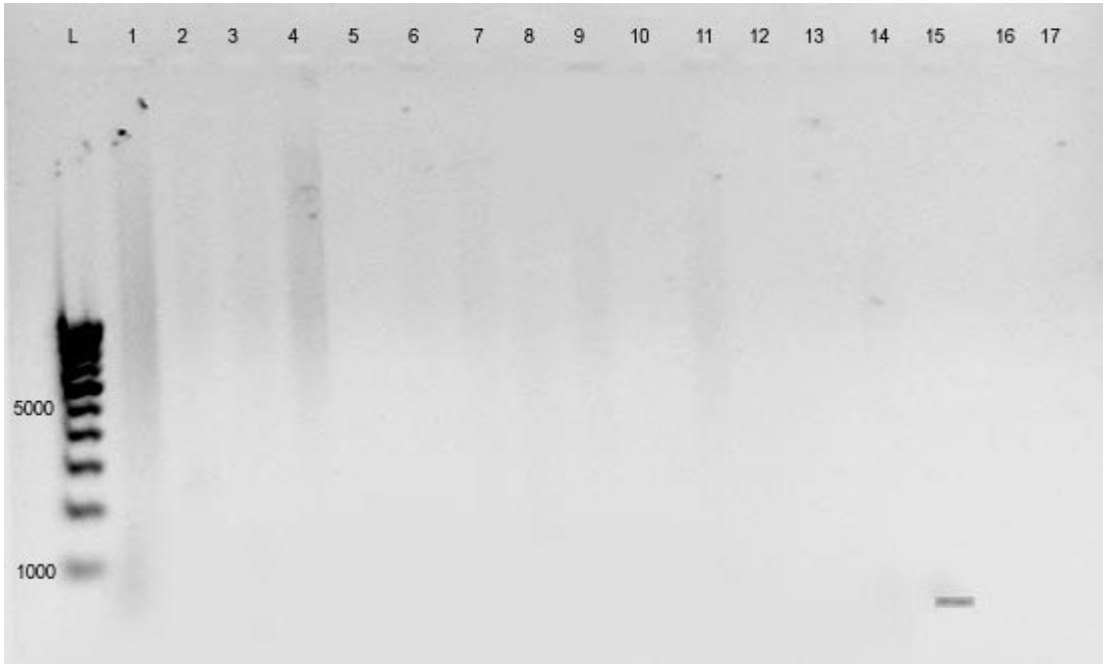


Figure 4. Representative gel showing CMD-resistant variety (Lane 15).

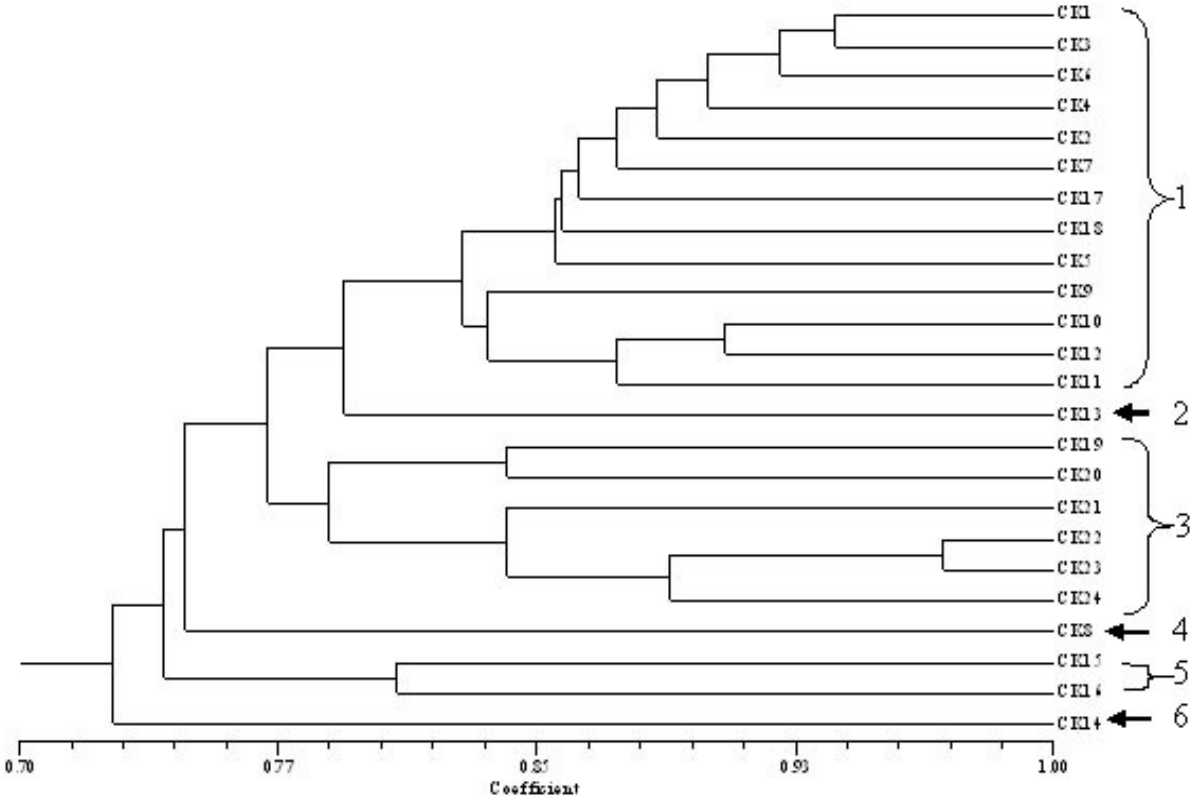


Figure 5. Unweighed Pair Group Method with Arithmetic Average (UPGMA) dendrogram of 24 accessions of central Kerala collections based on the SSR data. The dendrogram was constructed from the matrix of Dice's similarity coefficients.

or long neck. CK8 is the only variety present in cluster IV and it is an early cooking variety. CK15 and CK16 are grouped in cluster V and both have the same place of origin. Cluster VI consist of CK14 and it is an early maturing variety which matures in six months.

The binary data generated from the 36 cassava cultivars were also subjected to PCA using SAS. The first three principal components contributed 28.16, 16.76 and 8.11%, respectively of the total variation present in the data. A scatter diagram of the first two principal components (Figure 6) shows the relationship between the primers. PCA helped to identify primers which contributed much to the variation present in the population.

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 could differentiate the genetic diversity in the cassava accessions. The genetic diversity of cassava was revealed by the percentage of polymorphic loci (P), mean number of alleles per locus (A_O), effective number of alleles (A_E), observed heterozygosity (H_O), and expected mean heterozygosity (H_E). Each band obtained by SSR was treated as a gene locus and the homozygosity and heterozygosity for each loci was determined (Table 3a and b). The genetic analysis of released varieties of cassava accessions revealed that 100% heterozygosity was present in different accessions. The number of polymorphic loci and the percentage of polymorphic loci was 39 and 100%, respectively. The A_O, A_E, H_O and H_E were 2.000, 1.3486, 0.2407 and 0.2584, respectively (Table 4a). On the other hand, the collection of central Kerala cassava accessions revealed low percentage heterozygosity in different accessions except for the homozygous gene locus which expressed only in one allele at a time. The A_O, A_E, H_O and H_E were 1.7838, 1.5120, 0.2934 and 0.3386, respectively (Table 4b).

The aforementioned data shows that new alleles are formed in a cassava population by random and natural processes of mutation and recombination while the frequency of occurrence of an allele changes regularly as a result of mutation, genetic drift and selection in released varieties of cassava.

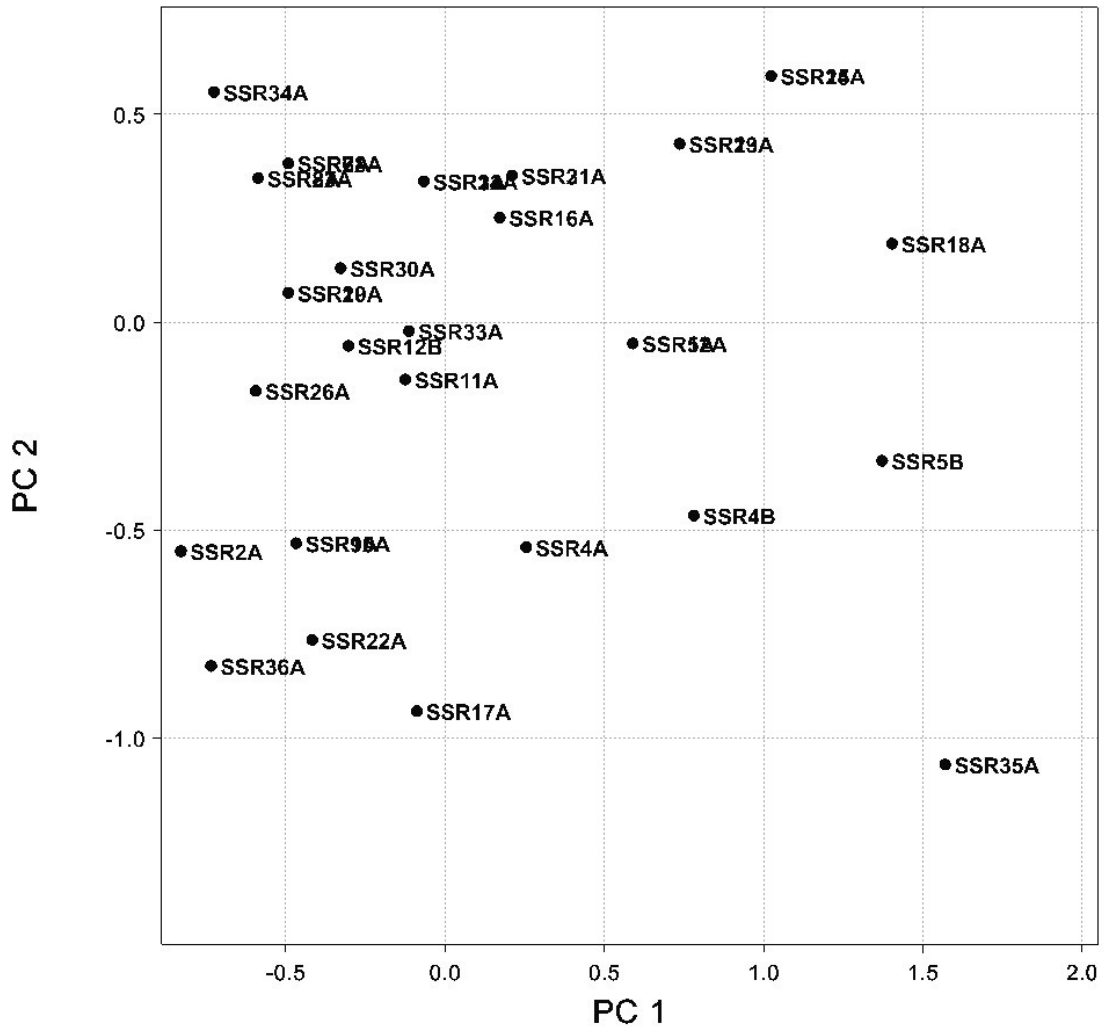


Figure 6. PCA of the studies varieties and accessions.

DISCUSSION

Understanding 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 11 different morphological traits studied in the present study revealed the existence of a high level of morphological diversity among the cassava accessions, providing scope for improvement through hybridization and selection. Morphological traits have commonly been used to express genetic diversity in cassava (Lefevre and Charrier, 1993; Haysom et al., 1994; Raghu et al., 2007; Sree Lekha and Pillai., 2008, 2010), although a number of genetic marker systems have also been used for the assessment of genetic diversity of cassava germplasm. These include isozyme markers (Sarria et al., 1992), RFLP (Angel et al., 1992), RAPD (Tonukari et al., 1997; Ugorji, 1998) and SSR (Fregene et al., 2001; Sree Lekha and Pillai., 2008, 2010) markers and low or medium genetic diversity has always been observed. In the present study there was generally high genetic diversity between released varieties and the central Kerala collections, as shown by the dendrogram. There is no relationship between these two varieties even though they are collected from same place.

The released varieties and the central Kerala varieties both form a distinct group and there is no overlapping of these two varieties observed in the dendrogram generated by NTSYS. The high differentiation between released varieties and central Kerala varieties suggests limited germplasm exchange between these two collections. SSR variation found within the released varieties of cassava was measured in terms of

Table 3a. Allelic frequencies of polymorphic loci studied in 12 cultivars of released cassava.

Locus	Allele	Allelic frequency	Locus	Allele	Allelic frequency	Locus	Allele	Allelic frequency
SSR1-A	0	0.9167	SSR12-A	0	0.8333	SSR24A	0	0.9167
	1	0.0833		1	0.1667		1	0.0833
SSR2-A	0	0.7500	SSR12-B	0	0.9167	SSR25A	0	0.7500
	1	0.2500		1	0.0833		1	0.2500
SSR3-A	0	0.9167	SSR13-A	0	0.8333	SSR26A	0	0.7500
	1	0.0833		1	0.1667		1	0.2500
SSR4-A	0	0.8333	SSR14-A	0	0.7500	SSR27A	0	0.9167
	1	0.1667		1	0.2500		1	0.0833
SSR4-B	0	0.7500	SSR15-A	0	0.9167	SSR28A	0	0.9167
	1	0.2500		1	0.0833		1	0.0833
SSR5-A	0	0.8333	SSR16-A	0	0.9167	SSR29A	0	0.8333
	1	0.1667		1	0.0833		1	0.1667
SSR5-B	0	0.5833	SSR17-A	0	0.8333	SSR30A	0	0.9167
	1	0.4167		1	0.1667		1	0.0833
SSR6-A	0	0.9167	SSR18A	0	0.3333	SSR31A	0	0.9167
	1	0.0833		1	0.6667		1	0.0833
SSR7-A	0	0.9167	SSR19A	0	0.9167	SSR32A	0	0.9167
	1	0.0833		1	0.0833		1	0.0833
SSR8-A	0	0.9167	SSR20A	0	0.9167	SSR33A	0	0.8333
	1	0.0833		1	0.0833		1	0.1667
SSR9-A	0	0.9167	SSR21A	0	0.9167	SSR34A	0	0.8333
	1	0.0833		1	0.0833		1	0.1667
SSR10-A	0	0.9167	SSR22A	0	0.8333	SSR35A	0	0.5000
	1	0.0833		1	0.1667		1	0.5000
SSR11-A	0	0.9167	SSR23A	0	0.9167	SSR36A	0	0.7500
	1	0.0833		1	0.0833		1	0.2500

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 (78.38%) in the central Kerala collections of cassava were lower than those from released varieties of cassava (100%). These results shows high level of polymorphism when compared to studies conducted by Okogbenin et al. (2006) and Sreelekha et al. (2010) in old and new collections of cassava collected from India. The distribution of species observed in the dendrograms (Figures 2, 3 and 5) is coherent and clearly shows that the SSR and analytical methods used in this study are powerful tools for studying the genetic diversity of *Manihot* species.

In this study the dendrograms clearly separate the released varieties from the accessions of the central

Table 3b. Allelic frequencies of polymorphic loci studied in 24 cultivars of central Kerala cassava.

Locus	Allele	Allelic frequency	Locus	Allele	Allelic frequency	Locus	Allele	Allelic frequency
SSR1-A	0	0.3913	SSR12-A	0	0.4167	SSR24-A	0	0.8333
	1	0.6087		1	0.5833		1	0.1667
SSR2-A	0	0.1667	SSR13-A	0	****	SSR25-A	0	0.0417
	1	0.8333		1	1.0000		1	0.9583
SSR3-A	0	0.7083	SSR14-A	0	0.7917	SSR26-A	0	0.7917
	1	0.2917		1	0.2083		1	0.2083
SSR4-A	0	0.4583	SSR15-A	0	0.4583	SSR27-A	0	0.1667
	1	0.5417		1	0.5417		1	0.8333
SSR5-A	0	0.2500	SSR16-A	0	****	SSR28-A	0	****
	1	0.7500		1	1.0000		1	1.0000
SSR6-A	0	****	SSR17-A	0	0.9167	SSR29-A	0	0.6250
	1	1.0000		1	0.0833		1	0.3750
SSR7-A	0	0.6522	SSR18-A	0	****	SSR30-A	0	0.3750
	1	0.3478		1	1.0000		1	0.6250
SSR7-B	0	0.5833	SSR19-A	0	0.4167	SSR31-A	0	****
	1	0.4167		1	0.5833		1	1..0000
SSR8-A	0	0.3750	SSR20-A	0	****	SSR32-A	0	0.1250
	1	0.6250		1	1.0000		1	0.8750
SSR9-A	0	0.5417	SSR21-A	0	0.7917	SSR33-A	0	0.2917
	1	0.4583		1	0.2083		1	0.7083
SSR10-A	0	0.5000	SSR22-A	0	0.7917	SSR34-A	0	0.3333
	1	0.5000		1	0.2083		1	0. 6667
SSR11-A	0	0.2917	SSR23-A	0	****	SSR35-A	0	0.2083
	1	0.7083		1	1.0000		1	0.7917
						SSR36-A	0	0.9583
							1	0.0417

Kerala collections. This clear partition into two groups is consistent with the concept that the two set of collections represent two different genetic entities. In a previous study in cassava with EST-SSR markers there was a marked separation between cultivated cassava accessions from their wild varieties (Raji et al., 2009). Studies conducted by Moyib et al. (2007) showed no differentiation between the improved varieties and Nigerian collections while Kizito (2006) showed no differentiation between the cassavas collected from different districts of Uganda. The clustering pattern shown by released varieties showed much higher diversity than the central Kerala collections. The mean fixation index (*F*) for released varieties was 0.0688 and

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

	Released varieties	Central Kerala varieties
P	100	78.38
Ao	2.00	1.78
A_E	1.35	1.51
H_O	0.24	0.29
H_E	0.26	0.34
F	0.068	0.13
t	0.396	0.43

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

that for central Kerala collection was 0.1337, indicating an overall conformance to Hardy-Weinberg equilibrium. The estimated *F* value, used to quantify an excess or deficiency of heterozygotes, was substantially higher than the mean value expected (0.05 or 5%), and positive, indicating an excess of homozygotic individuals. The excess of heterozygotes in released varieties 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, 1996).

The out-crossing rate (*t*) based on fixation indices for released varieties was 0.3964 and that for the central Kerala collection was 0.4332, which is higher than the value in released varieties. da Silva et al. (2001) reported an out crossing rate of 0.69 to 1.00 among 8 ethno-varieties of cassava from Brazil. The population genetic analysis data further provides ample evidence for the fact that recombination events that have occurred in the central Kerala 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 stresses. 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 of additive genetic variance, upon which progress in plant breeding depends. The differences in allele frequencies seen among landraces in this study are probably due to genetic drift effects subsequent to mutation. The unique and broad diversity of cassava landraces found in both collections reveals an invaluable germplasm resource for cassava improvement targeted to the region. The high level of differentiation between land races from both released varieties and central Kerala collections may represent a heterotic pool and provide an opportunity for the systematic exploitation of hybrid vigor in cassava. The two collections in the present study gave different views of the amount of genetic variation and genetic relationships.

The study of population genetics is increasingly important as we struggle to maintain healthy, wild and domestic populations and ecosystems, not only for cassava. 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 provides a data-base for cassava breeders informed about choices in selection of parental accessions for use in a breeding program based on genetic diversity. The hierarchical clustering illustrated in a dendrogam is usually reflected in a PCA scatter plot. PCA analysis provides information about associations of accessions, which are useful to formulate better breeding strategies. It also helps to identify primers which contributed much to the variation present in the population. The results of this study, thereby, established a collection of 9 highly polymorphic SSR primers (SSRY26, SSRY11, SSRY12, SSRY10, SSRY30, SSRY16, SSRY31, SSRY22 and SSRY32) that could be readily used for genotype identification and genetic diversity studies in both released varieties and collections from the central part of Kerala. Therefore, application of few highly polymorphic SSR markers is possible for genetic variation studies in cassava and has thus great application for genetic studies on cassava in collections from around the world. This reduces the stress of applying many SSR primers for the identification of cassava cultivars in Kerala (and elsewhere) and hence, saves time and also cuts the cost of research studies for genetic diversity studies.

Cluster analysis and PCA-based scatter plots showed great similarity among Brazilian cassava landraces (Siqueira et al., 2009). Lokko et al. (2009) also reported significant diversity within clusters among African land

races of cassava through PCA analysis.

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