Journal of Applied Horticulture, 16(3): 215-221, 2014

Molecular and agromorphological assessment of cashew (*Anacardium occidentale* L.) genotypes of India

R. Dasmohapatra[1], S. Rath[1], B. Pradhan[2] and G.R. Rout[3]*

*[1]Department of Fruit Science, [2]Department of Plant Breeding & Genetics, [3]Department of Agricultural Biotechnology , College of Agriculture, Orissa University of Agriculture & Technology, Bhubaneswar- 751 003, Odisha, India, *E-mail: grrout@rediffmail.com*

Abstract

Morphological and PCR based molecular markers were used to assess the genetic diversity of cashew (*Anacardium occidentales* L.) genotypes of India. Wide genetic variation was observed in respect to nut yield, nut weight, shelling percentage, plant height, trunk girth of the potentially superior genotypes. A wide variation was noticed with regards to fruit quality, colour of fruits, nut yield, nut weight, shelling percentage and apple weight. Twenty RAPD primers and 14 ISSR primers were used to detect the genetic variability among and between the genotypes. One hundred eighty-eight polymorphic bands and 31 monomorphic bands were observed by using both RAPD and ISSR primers. Twenty RAPD primers yielded 19 monomorphic and 84 polymorphic bands with percent of polymorphism was 81.55%. Of a total 116 ISSR bands generated by using 14 ISSR primers, 104 bands (89.65 %) were found to be polymorphic. Cumulative data generated from these two markers precisely arranged genotypes into 14 clusters. It was also noted that the var. BBSR-1 and Vengurla-7 were grouped into a single cluster and phenotypically they are similar with each other. Two dimensional scaling by principal component analysis indicates that some of the genotypes are out grouped. The major bands having 300 - 600 bp generated with PCR based markers can be used for identification of genotypes. This information will be useful for cashew improvement program as well as to assess the variety purity certification program.

Key words: Cashew, genetic similarity, molecular marker, genetic variability

Abbreviations: ISSR- Inter Simple Sequence Repeat, NaCl- sodium chloride, PCR- Polymerase Chain Reaction, RAPD- Randomly Amplified Polymorphic DNA

Introduction

Cashew (*Anacardium occidentale* L.) is an important tropical tree belonging to the family Anacardiaceae and native to tropical America, West indies to Brazil and Peru. It was introduced into India by the Portuguese 500 years ago (Rao and Swamy, 1994). The cashew tree is also pantropical, especially in coastal areas. Major producers of cashew nuts are India, Tanzania, Mozambique and Kenya. Cashew yields are reported to range from 0-50 kg/tree, with an average yield of 800-1000 kg/ha. Genetic improvement of cashew nut yield and quality is still not much researched. Genetic improvement is limited by the lack of knowledge of genetic diversity of the indigenous germplasm in both India and other countries. Moreover, breeding of cashew is mostly based on traditional methods of selection of useful traits such as nut size, nut weight, sex ratio, length of panicle and yield performance (Asceno, 1986 a, b; Mneney *et al.*, 2001). Broadening the genetic bases by introducing new alleles present in exotic germplasm and a systematic exploitation of heterosis have been suggested as means to overcome the selection (Masawe, 1994). Identification of parental lines to exploit heterosis and to introduce valuable characters into the cashew breeding programme will require more reliable information about the level of genetic similarity of gene pools available around the world (Mneney *et al.*, 2001). Varietal identification is based on morphological traits. This approach has, however, limited usefulness since the variation in morphological traits is influenced by environmental factors. Therefore, polymorphic DNA markers are needed for identification and estimation of genetic variability among the genotypes. Molecular markers are highly heritable and exhibit polymorphism to discriminate genotypes. Genetic relationships in plant species is an important component of crop improvement program. PCR based markers have been used to detect the DNA polymorphism among the cashew genotypes for identification, diversity analysis, mapping population of cashew genotypes (Samal *et al.*, 2003; 2004; Thimmappaiah *et al.*, 2009). ISSR markers generated from single primer PCR reactions are scattered evenly throughout the genome, inherited in a dominant or co-dominant Mendelian fashion and they circumvent the challenge of characterizing SSR loci that microsatellites require (Bajpai *et al.*, 2008). There are very scanty reports available on genetic variability and mapping study of cashew nut by using two molecular markers and in combination with the qualitative and quantitative characteristics (Archak *et al.*, 2003; Samal *et al.*, 2004; Aliya and Awopetu 2007; Cavalcanti and Wilkinson 2007; Thimmappaiah *et al.*, 2009). The present investigation is therefore, aimed to estimate the phylogenetic analysis among the 25 genotypes of India using the phenotypic attributes coupled with molecular markers.

Materials and methods

Plant material: Morphological data included plant height; trunk girth, canopy spread, nut yield, nut weight, shelling percentage and apple weight collected on 25 cashew genotypes maintained

at the centre of germplasm conservation field unit under All India Coordinated Research Project on Cashew, OUAT, Bhubaneswar. The germplasm included the collections from different parts of India. The important morphological observations made on the basis of 10 plants in each variety are presented in Table 1. Semi-mature leaf samples were collected from 10 individuals of each variety and kept in the ice box and brought to the laboratory for molecular experiment.

DNA extraction: Semi mature leaves (2.0 g) were ground to a fine powder in liquid nitrogen in a mortar and pestle. The fine powder was resuspended in preheated 10 mL DNA extraction buffer [(1 M Boric acid (pH 8.0), 2mM EDTA, 1.4 M NaCl, 1.5% hexadecyl-trimethyl-ammonium bromide (CTAB), 0.2% β-mercaptoethanol (v/v)] (Doyle and Doyle 1990). DNA amplification was performed by visualizing DNA bands under UV light, after electrophoresis on 1.0% (w/v) agarose gel. The resuspended DNA was then diluted in sterile distilled water to 5 ng/µL concentration for use in amplification reactions (Samal *et al.,* 2003).

RAPD analysis: Thirty 10-mer primers, corresponding to kits A, C, K, M and N from Operon Technologies (Alameda, California, USA) were initially screened using five varieties to determine the suitability of each primer for the study. Out of thirty, twenty decamer primers were selected on the basis of distinct bands and polymoprphic amplified product within the varieties. Polymerase Chain Reaction (PCR) were carried out in a final volume of 25µL containing 20ng template DNA, 100 µM each deoxynucleotide triphosphate, 20 ng of decanucleotide primers (M/S Operon Technology Inc., Alameda, CA 94501, USA), 1.5 mM $MgCl_2$, 1 x Taq buffer [10mM Tris-HCl (pH- 9.0), 50 mM KCl, 0.01% gelatin] and 0.5 U Taq DNA polymerase (M/S Bangalore Genei, India). The amplification was achieved in a thermal cycler (Peqlab, Germany) programmed for a 4 min denaturation step at 94 °C, followed by 45 cycles of denaturation at 94 °C for 1 min, annealing at 37 °C for 1 min and initial extension at 72 °C for 2 min, finally at 72 °C for 10 min. The amplified products were separated alongside a molecular weight marker (1kb ladder, MBI Fermentas, USA) by electrophoresis on 1.2% agarose gels run in 0.5x TAE (Tris Acetate EDTA) buffer, stained with ethidium bromide and visualized under UV light. Gel photographs were scanned through Gel Documentation System (UV-Tech, United Kingdom) and the amplification product sizes were evaluated using the software Quantity One.

ISSR analysis: The reaction mixture composition and reaction conditions were empirically determined. Optimization of primer concentration and annealing temperature of each primer were also carried out. Primer screening was carried out with 25 ISSR primers synthesized by M/S Bangalore Genei, India. Out of which, 14 primers both anchored and non-anchored were

Table. 1. Phenotypical characteristics of 25 genotypes of cashew (*Anacardium occidentale* L.)

	Varierty	Selection /Hybrid	Parentage	Nut yield (kg/plant)	Nut weight (g)	Shelling (%)	Apple weight (g)
1	BBSR-1	Selection	WBDC-5 (Vengurla 36/3)	1.58	4.6	30	50
2	Jagannath	Hybrid	Bhubaneswar cluster -2 x VTH 711/4	3.30	8.6	35	62
3	Balabhadra	Hybrid	Bhubaneswar cluster -1 x H 2/16	3.69	7.4	33	53
4	BPP- 4	Selection	9/8 Epurupalem	1.08	5.6	25	30
5	BPP- 6	Selection	T No. 56	0.72	5.8	26	34
6	BPP- 8	Hybrid	T. No. 1 x T No. 39	3.93	7.2	29	55
7	Ullal-1	Selection	8/46 Taliparmba	0.59	6.6	31	35
8	Ullal-3	Selection	5/37 Manchery	0.74	7.5	30	37
9	Ullal-4	Selection	2/77 TuniAndhra	0.96	7.2	30	40
10	Chintamani-1	Selection	8/46 Thaliparmba	1.23	6.4	30	24
11	NRCC-2	Selection	2/9 Dicherla	1.87	9.0	32	40
12	Vengurla-1	Selection	Ansur--1	0.98	6.2	30	30
13	Vengurla-4	Hybrid	Midnapore Red x Vetore 56	1.34	8.0	30	42
14	Vengurla – 7(H255)	Hybrid	Vengurla -3 x M 10/4 (VRI – 1)	2.28	10.0	31	60
15	Madakkathara -1 (BLA-39-4)	Selection	T No. 39 of Bapatala	0.66	6.0	27	25
16	Madakkathara -2 (NDR-2-1)	Selection	Neduvellur Material	0.80	7.0	26	30
17	Dhana (H-1608)	Hybrid	ALGD – 1 x K-30-1	2.22	8.0	30	48
18	Kanaka (H-1598)	Hybrid	BLA 139-1 x H-3-13	1.13	6.0	31	40
19	Priyanka (H-1591)	Hybrid	BLA 139-1 x K-30-1	1.32	9.8	28	62
20	Vengurla -6 (H-68)	Hybrid	Vetore56 x Ansur-1	1.43	7.4	30	60
21	Amrutha (H-1597)	Hybrid	BLA 139-1 x H-3-13	0.78	8.0	31	40
22	K-22-1	Selection	Kottarakkara-22 (Layer -23)	1.23	6.0	27	65
23	Bhaskara	Selection	Selection from forest plantation of Gondengram in Goa	3.36	7.7	27	50
24	Vridhachalam-3	Selection	M 26/2 Edayanchavadi material	1.98	7.0	29	40
25	Jhargram-1	Selection	T.No. 16 of Bapatala	1.05	6.0	30	50
	LSD (*P*=0.05)			0.45	0.5	2	5
	C.V.			17.06	4.51	5.17	7.40

selected having good amplification and reproducibility. These primers were used for the ISSR analysis with 25 varieties of cashew. The PCR reactions were performed as per the method followed in RAPD analysis except using annealing temperature. The amplified products were separated on 1.5% agarose gel with 0.5x Tris-borate-EDTA buffer by electrophoresis. The gels were stained with ethidium bromide and the bands were visualized and separated under UV light. The size of the amplified products was estimated using 1Kb ladder as marker.

Data analysis: Only clear distinct and reproducible bands produced by RAPD and ISSR primers were scored as present (1) or absent (0) with all the varieties studied. Each amplification fragment was named by the source of the primer, kit number and its approximate size in base pairs. Bands with similar mobility to those detected in the negative control, if any, were not scored. The discriminating power of primers was assessed by calculating percentage of polymorphism, polymorphic information content (PIC) and marker index (MI). The PIC content of primers was estimated (Powell *et al.*, 1996) and the marker index for each primer was calculated by the formula:

PIC = 1- $\sum fi^2$

Where '*fi*' is the frequency of ith allele.

Marker index (MI) = PIC x number of polymorphic bands.

Jaccard's coefficient of genetic similarity between all possible pairs of accessions was analyzed (Sneath and Sokal, 1973). Similarity coefficient values estimated were used to construct a dendrogram using UPGMA and Principal Co-ordinate Analysis (PCA) was also carried out by the NTSYS-pc ver. 2.02 (Rohlf, 2005).

Results and discussion

Phenotypic variation was observed within twenty five genotypes with regards to nut yield, nut weight, shelling percentage and apple weight (Table 1). The maximum and minimum plant height was observed in variety Chintamani-1 and Ullal-1, respectively with CD value (0.65). The nut yield and weight were significantly different between the 25 cashew genotypes (Table 1). The shelling percentage varied from 26 to 35 % in different genotypes. The colour of the apple and apple weight also differed in different genotypes of cashew (Fig. 1). Identification of genotype and its genetic relationships in cashew is difficult due to lack of standard technique for identification using morphological and floral traits. There were a lot of variation among the genotypes due to their origin as a result of cross pollination. Further, these phenotypic characters are generally influenced by environmental factors and the growth state of the plant. Both RAPD and ISSR markers were used to assess the genetic variability between the 25 cashew genotypes. On the basis of primer screening, 20 RAPD and 14 ISSR primers showed good amplification (Table 2). Some of the primers showed amplification but the intensity of the fragments was very low. Some of the primers did not show any amplification. The reproducibility of the amplification products was tested on template DNA from ten independent extractions of ten clones of each genotype. The amplification profiles of 25 cashew genotypes with 20 random primers produced 103 consistent amplified DNA bands, ranging from 100 to 2500 bp; out of which 19 were monomorphic. The maximum and minimum number of amplification fragments was produced in primer OPC 18 and OPN-06 respectively. The pattern of RAPD profiles produced by the primer OPC 18 and OPC 04 are shown in Fig 2A. The polymorphic information content (PIC) varied from 0.32 (OPC 12) to 0.90 (OPC 08) with an average PIC of 0.64 and the marker index (MI) varied from 0.54 (OPK 20) to 8.80 (OPK 18) with an average of 2.95. Among the 9 fragments amplified by primer OPA 07, 2 unique bands of 300 bp and 900 Kb were present in variety 'BPP-6', 'Ullal-1" and 'Ullal-4' which clearly distinguished it from other genotypes. Similarly, another

Fig. 1. Fruits of 25 cashew genotypes

Table. 2. RAPD & ISSR primers used to generate the polymorphism among 25 genotypes of *Anacardium occidentale*

Primer Code	Sequence (5'-3')	Total number of bands	Polymorphic band	Polymorphism (%)	Band Size Range (bp)	Average PIC value	Marker index (MI)
RAPD							
OPA-04	AATCGGGCTG	5	4	80.00	300-1000	0.68	2.72
OPA-07	GAAACGGGTG	9	9	100.00	200-1500	0.77	6.93
OPA-02	TGCCGAGCTG	3	2	66.67	100-1000	0.54	1.08
OPA-06	GGTCCCTGAC	4	3	75.00	200-1000	0.64	1.92
OPC-03	GGGGGTCTTT	3	2	66.67	400-1000	0.45	0.90
OPC-04	CCGCATCTAC	8	7	87.50	200-1000	0.64	4.48
OPC-05	GATGACCGCC	4	4	100.0	400-1500	0.82	3.28
OPC-06	GAACGGACTC	6	5	83.33	200-1000	0.68	3.40
OPC-08	TGGACCGGTG	8	8	100.00	350-1000	0.90	7.20
OPC-10	TGTCTGGGTG	3	2	66.67	200-1000	0.56	1.12
OPC-12	TGTCATCCCC	4	2	50.00	300-1500	0.32	0.64
OPC-18	TGAGTGGGTG	11	10	90.91	200-2500	0.58	5.80
OPK-18	CCTAGTCGAG	10	10	100.00	100-1000	0.88	8.80
OPK-20	GTGTCGCGAG	3	1	33.33	200-1000	0.54	0.54
OPM-01	GTTGGTGGCT	4	2	50.00	200-1500	0.38	0.76
OPM-05	GGGAACGTGT	4	3	75.00	400-1000	0.78	2.34
OPM-08	TCTGTTCCCC	5	4	80.00	100-1500	0.81	3.24
OPN- 06	GAGACGCACA	2	1	50.00	400-1500	0.68	0.68
OPN-08	ACCTCAGCTC	3	2	66.67	600-2000	0.62	1.24
OPN-12	CACAGACACC	4	3	75.00	400- 1000	0.67	2.01
	Total/Mean	103	84	81.55		0.64	2.95
ISSR							
AM-2	$(AAG)_5GC$	13	10	76.92	350-2500	0.44	4.40
UBC-807	$(AG)_8T$	10	10	100.00	350-1100	0.77	7.70
UBC-818	$(CA)_8G$	8	7	87.50	250-900	0.54	3.78
UBC-825	$(AC)_8T$	5	4	80.00	375-700	0.35	1.40
UBC-827	$(AC)_8G$	11	10	90.91	200-1100	0.57	5.70
UBC-864	$(ATG)_6$	5	4	80.00	500-1000	0.45	1.80
UBC-872	$(GATA)_4$	13	12	92.31	250-1400	0.63	7.56
AM-5	$(AAG)_8T$	9	8	88.9	300-1500	0.52	4.16
AM-6	$(GACA)_4$	8	7	87.5	300-1200	0.48	3.36
AM-8	$(AG)_8G$	6	6	100.0	200-1500	0.73	4.38
AM-12	$(ACC)_8T$	8	7	87.5	300-1000	0.68	4.76
AM-13	$(AG)_8CTT$	6	5	83.3	400-2000	0.70	3.50
AM--15	$(CT)_4AGA$	6	6	100.0	250-1000	0.82	4.92
AM-18	$(GT)_8CTC$	8	8	100.0	250-1500	0.81	6.48
	Total/Mean	116	104	89.65		0.60	4.56

unique band of 800 bp was observed in genotypes 'Vengurla-4' and 'Madakkathara' with primer OPC 04. The primer OPA 07 showed distinguished fragments in different genotypes of cashew, out of which nine were polymorphic. On the other hand, a total of 116 bands ranging in size from 200 to 2500 bp were detected among the 25 genotypes employing 14 ISSR primers (Table 3). The ISSR primers (AM 2 & UBC 872) produced the maximum 13 bands followed by UBC 827 (11 bands). The lowest number of bands was observed in UBC 825 (5 bands) and the average number of bands per primer was found to be 8.21. From a total of 116 ISSR bands, 104 bands (89.63 %) were found to be polymorphic whereas, 12 bands were monomorphic. The level of polymorphism generated by ISSR (89.63%) was higher than that revealed by RAPD primer (81.55%). The pattern of ISSR profiles produced by the primer UBC 872 and UBC 827 are shown in Fig. 2B. The comparative analysis of RAPD and ISSR marker used in diversity study of 25 genotypes are presented in Table 3. The polymorphic information content varied from 0.60 to 0.64 with an average of 0.62 and the marker index from 2.95 to 4.56 with an average of 3.75. Combining the markers of RAPD and ISSR, a total of 219 bands were detected, out of which 188 bands (85.84 %) were polymorphic with an average of 5.52 polymorphic markers per primer (Table 3). The maximum polymorphism was observed both in RAPD and ISSR markers with high level of genetic variation existing within the genotypes because of different genetic source of origin. In the present study, the result showed a significant correlation between RAPD and ISSR markers whereas a very less correlation was detected

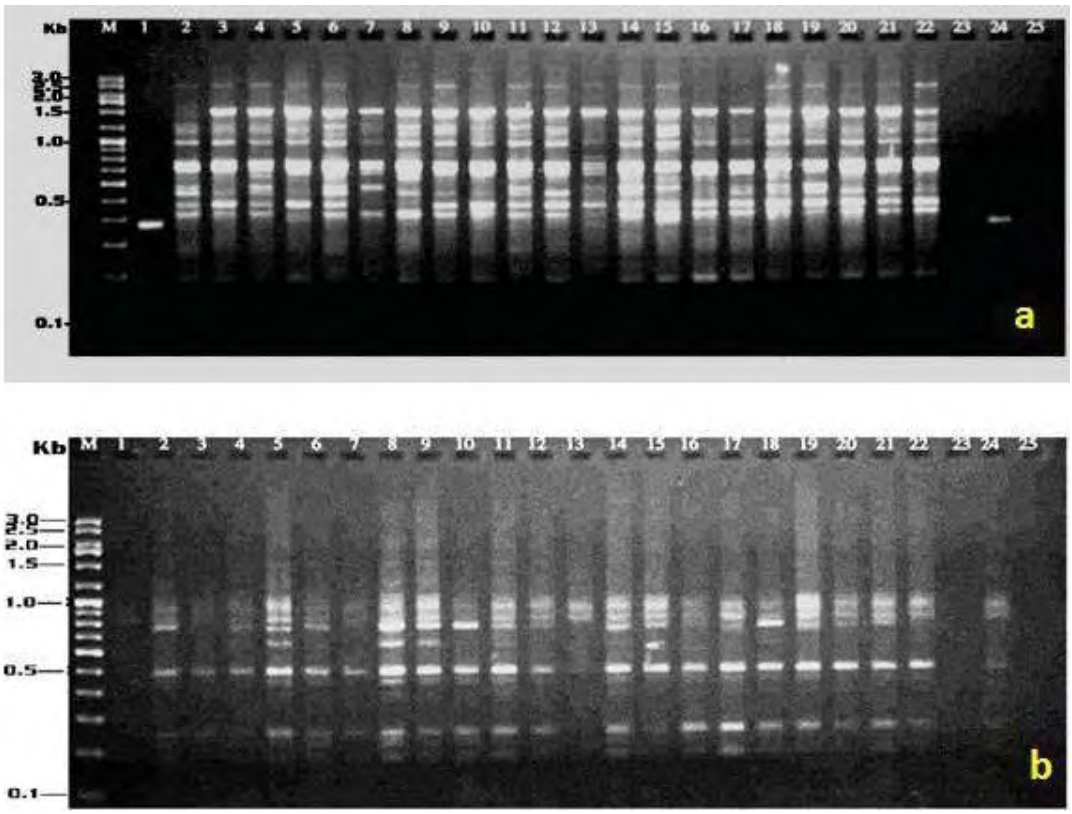

Fig. 2A. RAPD profile of 25 cashew genotypes generated by using primers OPC 18 (a), OPC 04 (b) .
M = kb molecular weight ladder, 1 = BBSR-1, 2 = Jagannath, 3 = Balbhadra, 4 = BPP-4 , 5 = BPP-6, 6 = BPP-8 (H-2/16), 7 = Ullal-1, 8 = Ullal-3, 9 = Ullal-4, 10 = Chintamani, 11 = NRCC-2, 12 = Vengurla-1, 13 = Vengurla-4, 14 = H255, 15 = Madakkathara-1 (BLA-39-4), 16 = Madakkathara-2, 17 = Dhana (H-1608), 18 = Kanaka (H-1598), 19 = Priyanka (H-1591), 20 = H -68, 21 = Amrutha, 22 = K 22-1, 23 = Bhaskara, 24 = Vridhachalam-3 (M-26/2), 25 = Jhargram.

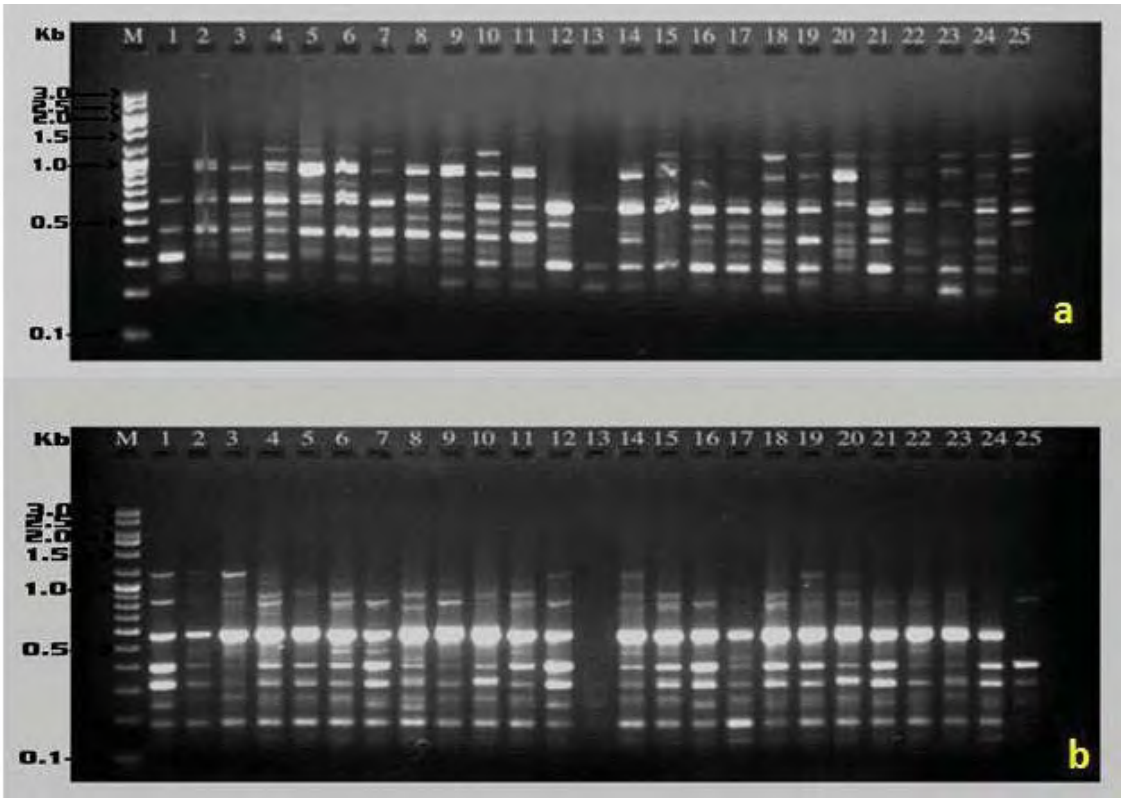

Fig. 2B. ISSR profile of 25 cashew genotypes generated by using primers UBC 872 (a), UBC 827 (b)
M = kb molecular weight ladder, 1 = BBSR-1, 2 = Jagannath, 3 = Balbhadra, 4 = BPP-4 , 5 = BPP-6, 6 = BPP-8 (H-2/16), 7 = Ullal-1, 8 = Ullal-3, 9 = Ullal-4, 10 = Chintamani, 11 = NRCC-2, 12 = Vengurla-1, 13 = Vengurla-4, 14 = H255, 15 = Madakkathara-1 (BLA-39-4), 16 = Madakkathara-2, 17 = Dhana (H-1608), 18 = Kanaka (H-1598), 19 = Priyanka (H-1591), 20 = H -68, 21 = Amrutha, 22 = K 22-1, 23 = Bhaskara, 24 = Vridhachalam-3 (M-26/2), 25 = Jhargram.

Table. 3. Polymorphic data obtained by using 20 RAPD and 14 ISSR primers in 25 genotypes of *Anacardium occidentale*

Name of the Primrs	Number of primer used	Total number of bands	Total number of polymorphic bands	Polymorphism (%)	Band range (bp)	Mean PIC	Marker Index (MI)
RAPD	20	103	84	81.55	200-2000	0.64	2.95
ISSR	14	116	104	89.65	250-2500	0.60	4.56
Mean	34	219	188	85.84		0.62	3.75

between molecular and morphological markers. The genotypes are grouped on the basis molecular markers as grown in different regions. The pair-wise comparison of genetic similarity of all the genotypes ranged from a maximum of 0.87 (between 'Ullal -1' and 'Vengurla-1') to a minimum of 0.48 (between 'Priyanka' and ' Bhaskara') in combining of ISSR and RAPD markers. The maximum genetic variation was due to the genotypes developed through selection. The present result indicate that the genetic diversity of 25 genotypes ranged from 81.55 to 89.55 % which indicate the higher range of polymorphism. The similar results as also reported earlier by Dhanaraj *et al.* (2002) and Thimmappaiah *et al*. (2009). A dendrogram was plotted using similarity coefficient derived from cumulative RAPD and ISSR profile data and is presented in Fig. 3. The dendrogram was generated on the basis of similarity matrix. On the basis of similarity matrix, there were two major clusters with 62% similarity. The maximum numbers of genotypes (19) were represented in major cluster-II whereas, 5 genotypes formed in the major cluster I. The major cluster-II was further divided into two minor clusters *i.e.* III & IV. The minor cluster III having one genotype 'Jagannath' with 0.66 similarity index and minor cluster IV having 18 genotypes. The minor cluster IV further regroup into two sub-minor cluster groups *i.e.* V & VI. The sub-minor cluster V having 14 genotypes and VI having 4 genotypes having the similarity index varies from 0.77 to 0.87. The sub-minor cluster V further divided into two groups *i.e.* VII & VIII. The cluster VII having two genotypes 'Bulbhadra' and ' BPP-4' with 76% similarity with each other. The cluster VIII having 12 genotypes is divided into two groups *i.e.* IX & X. The cluster IX having two genotypes *i.e.*' Ullal-3' and ' NRCC-2' had 75% similarity with each other. The cluster X having 10 genotypes having two groups i.e XI & XII. The cluster XI had one genotype 'Dhana' and cluster XII included 9 genotypes with two subclusters *i.e.* XIII and XIV. The cluster XIII had 6 genotypes with maximum similarity 87% between 'Ullal-1' and 'Vengurla-1' as these genotypes were developed through selection. The cluster XIV with four genotypes had maximum 83% similarity between 'Amrutha' and 'Bhaskara'. The two dimensional scaling of principal components (PCA) exhibited distribution of genotypes into different groups according to the polymorphic banding pattern (Fig. 4). The genotypes from different source of origin / regions were found to form different cluster indicating high correlation between molecular groupings and their morphological attributes. The present findings did not corroborate with the observations made by Dhanaraj *et al.* (2002). This might be due to the selection of genotypes through cross breeding.

This investigation revealed that the association between the results of the two methods used is fairly strong to evaluate the genetic relationships and useful for cashew improvement. This is also helpful in duplicate/genotype identification and genetic conservation.

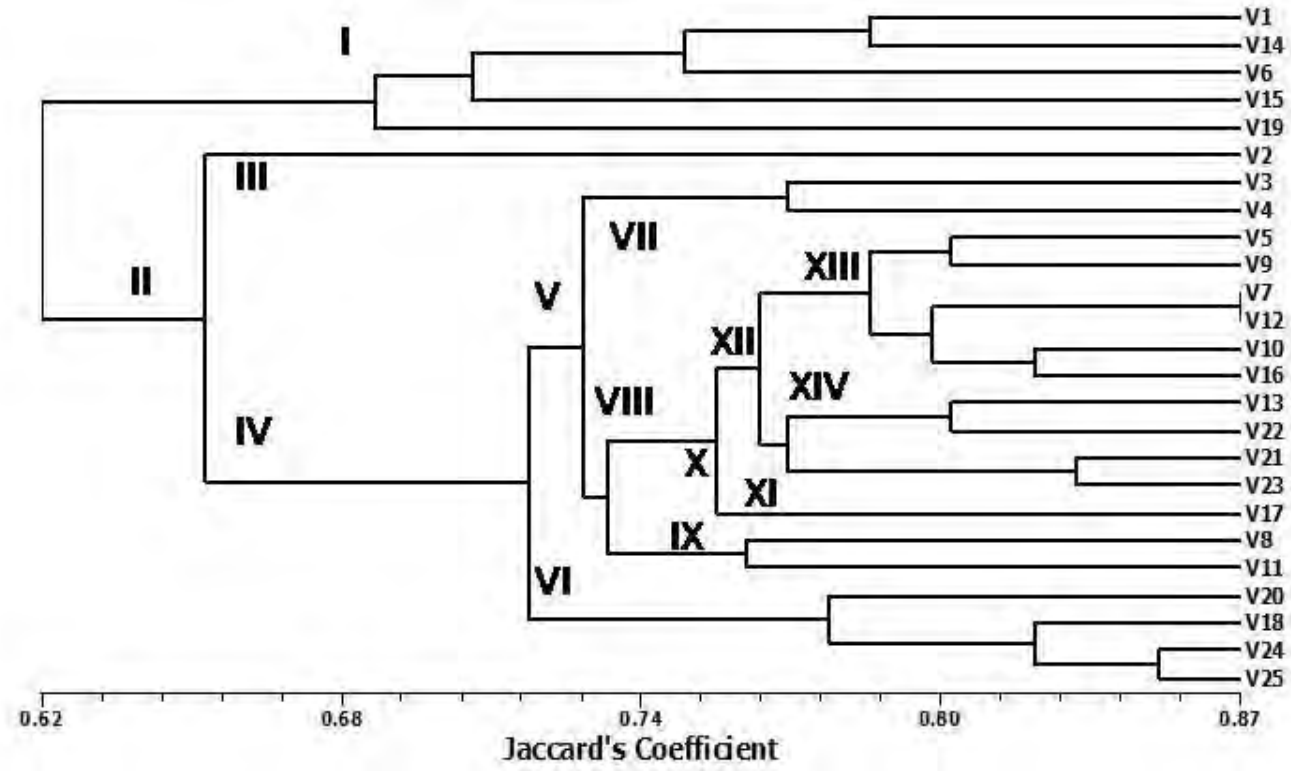

Fig. 3. Dendrogram generated from cumulative RAPD and ISSR profile data depicting relationship among 25 cashew genotypes presented in Table 1

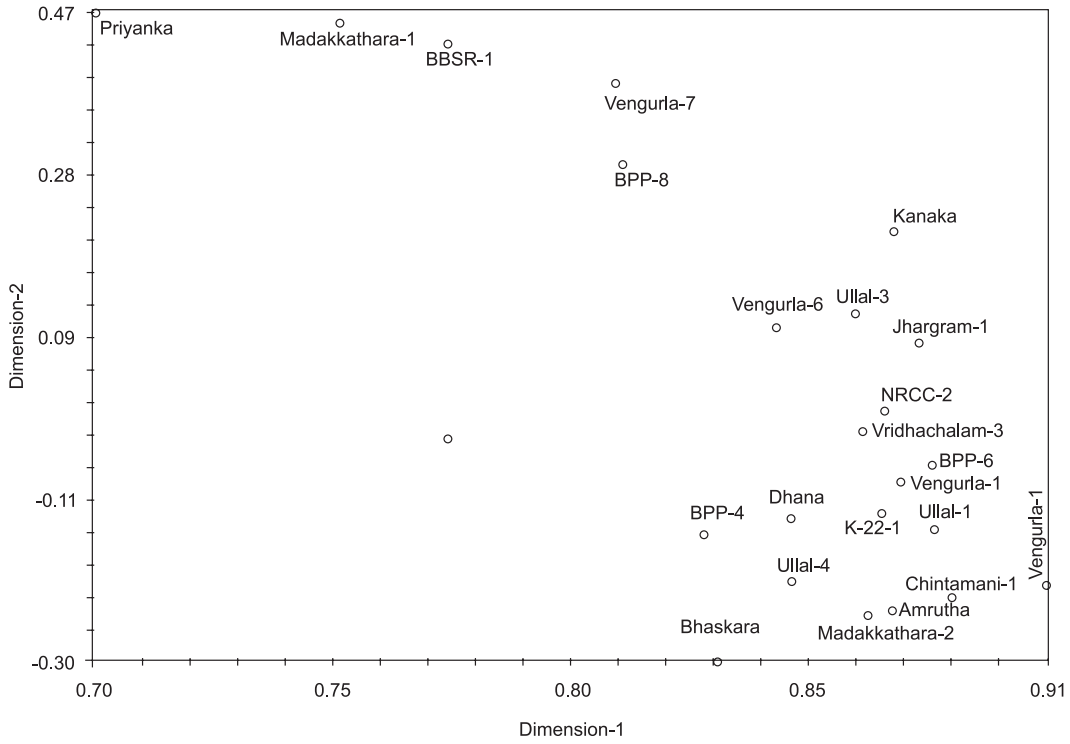

Fig. 4. Two dimensional scaling by principal component analysis (PCA) of 25 cashew genotypes using Jaccards similarity coefficient generated from cumulative RAPD and ISSR profile data. Number denoting the plotted data points represent the respective genotypes.

Acknowledgements

The authors are thankful to Department of Agricultural Biotechnology, College of Agriculture, OUAT, Bhubaneswar to provide laboratory facility. Author (R.D.) wishes to thank ICAR for financial assistance.

References

Aliyu, O.M. and J.A. Awopetu, 2007. Assessment of genetic diversity in three populations of cashew using protein-isozyme electrophoresis analysis. *Genetic Resour. Crop Evol.*, 54: 1489-1497.

Archak, S., A.B. Gaikwad, D. Gautam, E.V.V.B. Rao, K.R.M. Swamy and J.L. Karihaloo, 2003. DNA fingerprinting of Indian cashew (*A.occidentales*) varieties using RAPD and ISSR techniques. *Euphytica*, 230: 397-404.

Ascenso, J.C. 1986a. Potential of the cashew crop-I. *Agric. Intl*., 38: 324-327.

Ascenso, J.C. 1986b. Potential of the cashew crop-II. *Agric. Intl*., 38: 368-371.

Bajpai, A., N. Srivastava, S. Rajan and R. Chandra, 2008. Genetic diversity and discrimination of mango accessions using RAPD and ISSR markers. *Ind. J. Hort.*, 65: 377-382.

Cavalcanti, J.J.V. and M.J. Wilkinson, 2007. The first genetic map of cashew (*A. occidentale* L.). *Euphytica*, 157: 131-143.

Dhanaraj, A.L., E.V.V.B. Rao, K.R.M. Swamy, M.G. Bhat, D.T. Prasad. and S.N. Sondur, 2002. Using RAPD's to assess the diversity in Indian cashew (*Anacardium occidentale* L.) germplasm. *Jour. Hort. Sci. Biotechn.*, 77(1): 41-47.

Doyle, J.J. and J.L. Doyle, 1990. Isolation of plant DNA from fresh tissue. *Focus*, 12: 13-15.

Masawe, P.A.L. 1994. *Aspects of Breeding and Selecting Improved Cashew Genotypes (Anacardium occidentale L.)* (Thesis), University of Read, United Kingdom.

Mneney, E.E., S.H. Mantell and M. Bennett, 2001. Use of random amplified polymorphic DNA (RAPD) markers to reveal genetic diversity within and between population of cashew (*Anacardium occidentale* L.). *Jour. Hort. Sci. Biotechn*., 76: 375-383.

Powell, W, M. Morgante, C. Andre, M. Hanafey, J. Voges, S. Tingey, and A. Rafalski, 1996. A comparison of RAPD, RFLP, AFLP and SSR (microsatellite) markers for germplasm analysis. *Mol. Breed*., 2: 230-236.

Rao, E.V.V.B. and K.R.M. Swamy, 1994. Genetic resources of cashew. In: *Advances in Horticulture.* Vol. 9: *Plantation and Spices Crops.* Part-I. K.L. Chadha and P. Rethinam (Eds). Malhotra Publishing House, New Delhi, pp. 79-97.

Rohlf, F.J, 2005. *NTSYS-pc Numerical Taxonomy and Multivariate Analysis System*. Version 2.02, Exeter Publishing Ltd., Setauket, New York, USA.

Samal, S., G.R. Rout, S. Nayak, R.M. Nanda, P.C. Lenka, and P. Das, 2003. Primer screening and optimization for RAPD analysis of Cashew. *Biol. Plant*., 46(2): 301-304.

Samal, S., P.C. Lenk, R.M. Nanda, S. Nayak, G.R. Rout and P. Das, 2004. Genetic relatedness in cashew (*Anacardium occidentale* L.) germplasm collections as determined by randomly amplified polymorphic DNA. *Genetic Resour. Crop Evol*., 51: 161-166.

Sneath, P.H.A. and R.R. Sokal, 1973. *Numerical Taxonomy*. Freemen, San Francisco, CA.

Thimmappaiah, W.G., Santhosh, D. Shobha and G.S. Melwyn, 2009. Assessment of genetic diversity in cashew germplasm using RAPD and ISSR markers. *Sci. Hort.*, 120: 411-417.

Received: June, 2014; Revised: October, 2014; Accepted: October, 2014