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Analysis of genetic relationships in cashew varieties using morphological characters and ISSR markers

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Abstract

In the present paper genetic relationships of twenty five varieties of cashew are described on the basis of morphological characters and ISSR markers. Results obtained for the phenotypic characters based on similarity coefficient were divided into four clusters with 70 per cent similarity. By means of similarity coefficients (S_G), at 70 per cent phenon level the genotypes were broadly grouped into four clusters *i.e.*, cluster I and cluster II both comprising of a single variety Bhaskara and Chintamani-1 respectively, cluster III having six varieties and cluster IV with 17 varieties. The analysis using ISSR markers allowed us to distinguish 25 varieties. A total of 81 distinct DNA fragments ranging from 0.1 to 2.5 kb were amplified by using 10 selected ISSR primers. Genetic similarity analysis was conducted for the presence or absence of bands in the ISSR profile. Cluster analysis clearly showed that 25 varieties of cashew could be grouped into two major clusters based on similarity indices. The first major cluster consists of Priyanka and Madakkathara-1, two moderate yielding varieties. The other major cluster was divided into two sub-minor clusters, one sub-minor cluster having single variety and the other sub-minor cluster was represented by 22 varieties. Among the 25 varieties, Kanaka and Vridhachalam-3 showed the highest similarity indices (92%). The analysis of genetic relationships in cashew using morphological traits and ISSR banding data can be useful for plant improvement, descriptions of new varieties and also for assessment of varietal purity in plant certification programmes.

Keywords: Anacardium occidentale, genetic similarity, ISSR marker, morphological character

Introduction

The 'gold mine in waste land', the cashew *(Anacardium occidentale* L.) from its humble beginning as a crop to check soil erosion has later on emerged as one of the most important dollar earning crops of the country. India is the largest producer, processor and consumer of cashew in the world. It is grown on a large area of 9,53,200 ha in India but the productivity is low with 707 kg ha^{-1} (FAO, 2011). To improve the productivity of the nuts there is a need to select desirable genotypes from the existing gene pool and use superior materials for tree improvement programme. Genetic improvement is limited due to lack of knowledge of genetic diversity of the existing germplasm. Breeding of cashew is mostly based on selection of useful phenotypic and agronomic traits such as nut size, nut weight, colour of apple, size of the fruits, tree canopy, length of panicle and overall yield (Mneney *et al*., 2001). Morphological characters have been used as a powerful tool in the classification of cultivars and as such morphological traits continue to be the first step in the studies of genetic relationships in most breeding programmes (Van Beuningen and Busch, 1997). Therefore, it becomes imperative to quantify the extent of variation among the cashew germplasm by using morphological traits (Chipojola *et al*., 2009). As morphological characters, are tending to be influenced by environmental factors, an evaluation

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with a reliable method like molecular markers would be useful. Among the various PCR-based molecular marker techniques available, random amplified polymorphic DNA (RAPD) (Williams *et al*., 1990) and inter simple sequence repeats (ISSR) (Ziekiewicz *et al*., 1994) markers are useful for diversity analysis. ISSR markers are PCR based like RAPD but are more reproducible than RAPD due to their better stringency (high annealing temperature), require no gene sequence information and targets microsatellite motif. ISSR markers were also used for diversity analysis (Gonzalez *et al*., 2005; Qiao *et al*., 2006; Manimekalai and Nagarajan, 2006; Gajera *et al*., 2011). There are very scanty reports available on genetic variability and mapping study of cashew nut by using molecular markers in combination with the use of qualitative and quantitative characteristics (Archak *et al*., 2003; Samal *et al*., 2004; Aliyu and Awopetu, 2007). The genetic diversity of twenty five varieties of cashew based on morphological characters and ISSR markers are reported and discussed in this paper.

Materials and methods

Plant materials

Fresh leaves from 25 varieties of *Anacardium occidentale* such as BBSR-1, Jagannath, Balabhadra, BPP-4, BPP-6, BPP-8 (H-2/16), Ullal-1, Ullal-3, Ullal-4, Chintamani-1, NRCC-2, Vengurla-1, Vengurla-4, Vengurla-6, Vengurla-7, Madakkathara-1 (BLA-39-4), Madakkathara-2, Dhana (H-1608), Kanaka (H-1598), Priyanka (H-1591), Amrutha (H-1597), K-22-1, Bhaskara, Vridhachalam-3 (M-26/2) and Jhargram-1 were collected from the cashew orchard maintained by the Orissa University of Agriculture and Technology, Bhubaneswar, Orissa, India. Fifteen individual trees from each variety were used for the analysis.

Morphological characteristics

Data on morphological characters such as number of laterals per sq. meter, number of flowering laterals per sq. meter, length of panicle, sex ratio, number of nuts per panicle, nut weight, shelling percentage and yield per plant were recorded for three years. For numerical classificatory analysis, general similarity coefficient (SG) of Gower (1971) was used as a measure of resemblance between different operational taxonomic units and SG values were calculated according to Sneath and Sokal (1973). Based on the matrix of SG values, dendrograms were constructed using the unweighted pair group method using arithmetic average (UPGMA) technique in one of the sequential, agglomerative, hierarchic, non-overlapping (SAHN) clustering methods (Sneath and Sokal 1973).

DNA extraction

Extraction of total genomic DNA was carried out as described by Rout *et al*. (2002) with minor modifications. DNA quantifications were performed by visualizing bands under UV light, after electrophoresis on 1.0 per cent (w/v) agarose gel. Resuspended DNA was then diluted in sterile distilled water to 5 ng μl^{-1} concentration for use in amplification reactions.

Primer selection

A preliminary experiment on 25 selected cashew varieties was carried out to select most suitable primers for analysis. From the amplified pimers, 10 primers were selected for their repeatability, scorability and ability to distinguish between varieties. ISSR primers AM-2, UBC-807, UBC-818, UBC-825, UBC-827, UBC-864 and UBC-872 (custom made from Sigma) exhibited maximum efficiency of discrimination in terms of resolving power. These primers were employed for varietal identification.

Amplification conditions and gel electrophoresis

Polymerase chain reaction (PCR) were carried out in a final volume of 25 µ1 containing 20 ng template DNA, 100 µM each deoxynucleotide triphosphate, 20 ng of ISSR primers (M/S Bangalore Genei, India), 1.5 mM $MgCl_2$, 1 X Taq buffer [10 mM Tris-HCl (pH- 9.0), 50 mM KCl, 0.01% gelatin] and 0.5 U Taq DNA polymerase (M/S Bangalore Genei, India). Amplification was achieved in a thermal cycler (Peqlab, Germany) programmed for 4 min denaturation step at 94 °C, followed by 45 cycles of denaturation at 94 °C for 1 min, annealing at suitable temperature for 1 min and initial extension at 72 °C for 2 min. and finally at 72 °C for 10 min. The amplified products were stored at 4 °C and separated by electrophoresis on 1.5 per cent agarose

gel in TBE buffer for 3 hrs at 100 V. Gene Ruler 100 bp ladder plus (Genei) were used as the size standard to determine the size of the DNA fragments. DNA fragments were made visible by staining the gel with Ethidium bromide and then photographed and the image was analysed using a UVTECH Gel documentation system, UK (Gel Doc G700).

Data analysis

Data were recorded as presence (1) or absence (0) of band from the examination of photographic negatives. Bands with similar mobility to those detected in the negative control, if any, were not scored. The binary data (matrix) prepared was used for calculating Jaccard's coefficient of genetic similarity (Sneath and Sokal, 1973) between all possible pairs of accessions. Similarity coefficient values estimated were used to construct a dendrogram (cluster diagram) using UPGMA and principal co-ordinate analysis (PCA) was also carried out following the software package, NTSYS-pc version 2.1 (Rohlf, 2005). The discriminating power of primers was assessed by calculating percentage of polymorphism, the polymorphic information content (PIC) of each marker and the resolving power (Rp). The PIC content of primers (Powell *et al*., 1996) and the resolving power (Prevost and Wilkinson, 1999) for each primer were calculated as follows:

PIC = 1- Σfi^2, where, '*fi*' is the frequency of i^{th} allele.

Rp = ΣIb, where, b = b and informativeness, Ib = 1- (2 x |0.5-p|) and p is the proportion of individuals containing the band I. The co-phenetic correlation coefficient was calculated and Mantel test (Mantel, 1967) was performed to check the goodness of fit of a cluster analysis to the matrix.

Results and discussion

Morphological characterization

The data on phenotypic characters of twenty five cashew varieties is presented in Table 1. Based on morphological characters, the twenty five varieties of cashew were classified using similarity coefficients. Results obtained for a dendrogram based on similarity coefficients were divided into four clusters with 65 per cent similarity (Fig. 1) *i.e.*, cluster I comprising of a single variety (Bhaskara), cluster II consisting of a single variety, Chintamani-1, cluster III having six varieties and last cluster with seventeen varieties (Fig. 1). At 75 per cent phenon level, third cluster was further divided into two sub-clusters IIIA, IIIB and cluster IV, the largest one

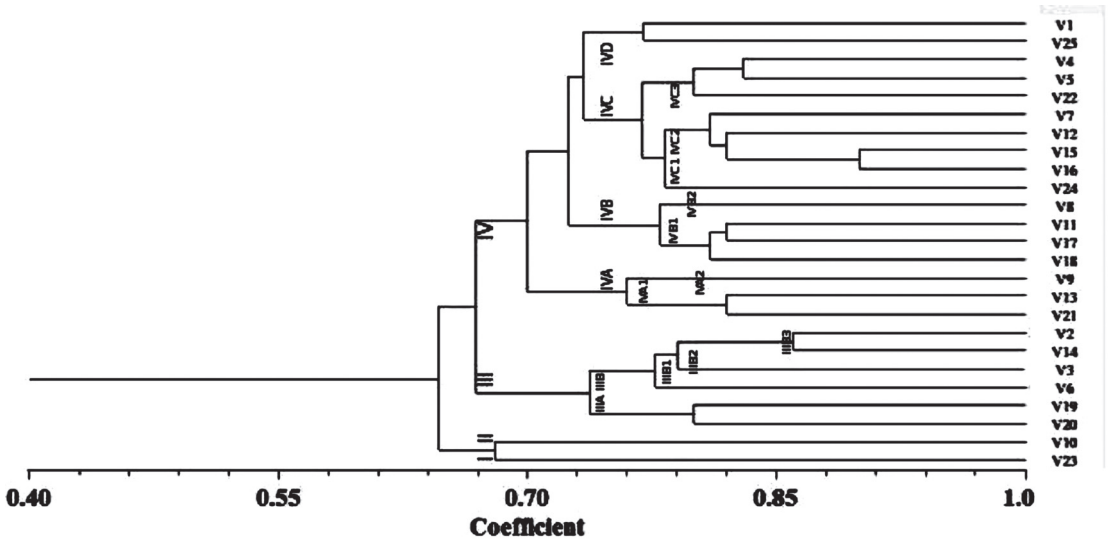

Fig. 1. Dendrogram based on similarity coefficient (S_G) in 25 cashew varieties.

(V1 - BBSR-1, V2 - Jagannath, V3 - Balabhadra, V4 - BPP-4 , V5 - BPP-6, V6 - BPP-8 (H-2/16), V7 - Ullal-1, V8 - Ullal-3, V9 - Ullal-4, V10 - Chintamani-1, V11 - NRCC-2, V12 - Vengurla-1, V13 - Vengurla-4, V14 - Vengurla-7 (H255), V15 - Madakkathara-1 (BLA-39-4), V16 - Madakkathara-2, V17 - Dhana (H-1608), V18 - Kanaka (H-1598), V19 - Priyanka (H-1591), V20 - Vengurla-6 (H -68), V21 - Amrutha, V22 - K 22-1, V23 - Bhaskara, V24 - Vridhachalam-3 (M-26/2), V25 - Jhargram-1)

Table 1. Morphological characteristics of twenty five varieties of cashew (*A. occidentale* L.)

Variety	Parentage	Plant height (m)	Trunk girth (cm)	Canopy spread (m)		Nut yield (kg plant^{-1})	Nut weight (g)	Shelling %	Apple weight (g)
				E-W	N-S				
BBSR-1	WBDC-5 (Vengurla 36/3)	2.60	30.76	2.33	3.23	1.58	4.6	30	50
Jagannath	Bhubaneswar cluster -2 x VTH 711/4	2.42	31.84	3.49	3.65	3.30	8.6	35	62
Balabhadra	Bhubaneswar cluster -1 x H 2/16	2.50	31.13	3.24	3.23	3.69	7.4	33	53
BPP- 4	9/8 Epurupalem	2.23	26.33	1.77	2.00	1.08	5.6	25	30
BPP- 6	T No. 56	2.00	26.33	1.60	1.40	0.72	5.8	26	34
BPP- 8	T. No. 1 x T No. 39	2.25	29.37	2.67	2.91	3.93	7.2	29	55
Ullal-1	8/46 Taliparmba	1.87	27.17	2.03	2.13	0.59	6.6	31	35
Ullal-3	5/37 Manchery	2.63	28.50	2.37	2.37	0.74	7.5	30	37
Ullal-4	2/77 TuniAndhra	2.37	27.17	1.60	2.00	0.96	7.2	30	40
Chintamani-1	8/46 Thaliparmba	2.87	31.83	2.87	3.67	1.23	6.4	30	24
NRCC-2	2/9 Dicherla	2.50	27.50	2.73	2.93	1.87	9.0	32	40
Vengurla-1	Ansur-1	2.13	29.50	2.97	3.37	0.98	6.2	30	30
Vengurla-4	Midnapore Red x Vetore 56	2.03	26.80	1.90	1.80	1.34	8.0	30	42
Vengurla - 7 (H255)	Vengurla -3 x M 10/4 (VRI - 1)	2.23	32.00	3.53	3.67	2.28	10.0	31	60
Madakkathara -1	T No. 39 of Bapatala	2.20	27.83	2.26	2.57	0.66	6.0	27	25
Madakkathara -2	Neduvellur Material	2.13	28.00	2.33	2.17	0.80	7.0	26	30
Dhana (H-1608)	ALGD - 1 x K-30-1	2.37	29.13	2.48	2.62	2.22	8.0	30	48
Kanaka (H-1598)	BLA 139-1 x H-3-13	2.17	28.17	2.03	2.23	1.13	6.0	31	40
Priyanka (H-1591)	BLA 139-1 x K-30-1	1.99	28.30	2.75	2.97	1.32	9.8	28	62
Vengurla -6 (H-68)	Vetore56 x Ansur-1	1.93	29.84	1.86	1.60	1.43	7.4	30	60
Amrutha (H-1597)	BLA 139-1 x H-3-13	1.93	26.07	1.30	1.80	0.78	8.0	31	40
K-22-1	Kottarakkara-22 (Layer -23)	1.82	27.67	1.72	1.72	1.23	6.0	27	65
Bhaskara	Selection	2.97	32.84	3.36	3.87	3.36	7.7	27	50
Vridhachalam-3	M 26/2 Edayanchavadi material	1.97	28.17	2.23	2.17	1.98	7.0	29	40
Jhargram-1	T.No. 16 of Bapatala	2.50	29.80	2.90	3.10	1.05	6.0	30	50
C.D.		0.650	3.878	1.104	0.948	0.452	0.532	2.496	5.424
SE(m)		0.228	1.360	0.387	0.332	0.159	0.187	0.875	1.901
C.V.		17.492	8.181	27.77	22.070	17.059	4.511	5.167	7.403

again divided into four subclusters *i.e*., IVA, IVB, IVC and IVD. The sub-cluster IIIA and IIIB were multivariate groups containing two (Vengurla-6, Bhubaneswar-1) and four (Balabhadra, BPP-8, Jagannath, Vengurla-7) genotypes respectively. The varieties NRCC-2, Dhana and Kanaka were 78 per cent similar with Ullal-3 which was characterized by moderate shelling percentage and higher sex ratio. This pattern of clustering on the basis of previous breeding or genetic history, is similar to the report of Swamy *et al.* (2002) and Aliyu and Awopetu (2007) on cashew. In the subcluster IVC, the variety Madakkathara-1 and Madakkathara-2 were closely associated with 90 per cent similarity by moderate number of flowering laterals, length of panicle, sex ratio and number of nuts per panicle. The varieties Madakkathara-1 and Madakkathara-2 were 82 per cent similar with Vengurla-1 and Ullal-1 which was characterized by similar number of laterals, length of panicles and shelling percentage. Further, the last group IVC3 of sub-cluster IVC consists of K-22-1, BPP-4, BPP-6 which was characterized by highest canopy spread, moderate number of shoots per square meter and shelling percentage. The varieties Bhaskara and Jhargram-1 were 78 per cent similar with regard to phenotypical characters like highest number of shoots per square meter, flowering shoots per square metre, inflorescence length, panicle breadth and nut weight. Accessions from different regions were found to cluster together indicating no correlation between molecular groupings and their geographical origin. Similar observation was also made by Dhanaraj *et al*. (2002). This might have been due to free exchange of germplasm that has taken place between different places. Smith and Smith (1989) suggested that the use of morphological traits is not always the best way to evaluate genetic distance since the degree of divergence between genotypes at the phenotypic level is not necessarily correlated with a similar degree of genetic difference.

ISSR characterization

The primer screening resulted in 10 primers that showed good amplification (Table 2). The reproducibility of amplification products was tested on template DNA from three independent extractions of three clones using leaf samples in different seasons. The amplification profiles of total genomic DNA from 25 varieties with 10 primers produced 81 consistent ISSR markers, ranging in size from 0.1 to 2.5 kb, out of which 11 were monomorphic. The number of DNA fragments generated ranged from 5 to 13 in primers UBC-825, UBC-864 and UBC-872, AM-2. The pattern of ISSR profiles produced by primers UBC-827, UBC-872, AM-2 and UBC-818 is shown in Figure 2. Among the 13 fragments amplified by primer AM-2, two unique bands of 2.13 kb and 1.05 kb were present in the variety Jagannath that clearly distinguished it from the other varieties. Similarly, another unique band of 0.9 kb was observed in Jagannath with primer UBC-818. The primer UBC-825 produced five amplified bands, out of which four were polymorphic and one was monomorphic. Two unique bands of 0.75 kb and 0.40 kb appeared in BPP-4 and Ullal-4 with primer UBC-827. Some of the primers (UBC-841 and UBC-864) gave only six fragments in each variety of cashew. The results also indicate that the primer UBC-872 produced one unique band (1.40 kb) in Priyanka which was different in other varieties. One unique band 1.2 kb was present in the variety Jhargram-1 with primer UBC-807. In BPP-6, eight amplified bands appeared including one unique band having 1.5 kb (Table 3).

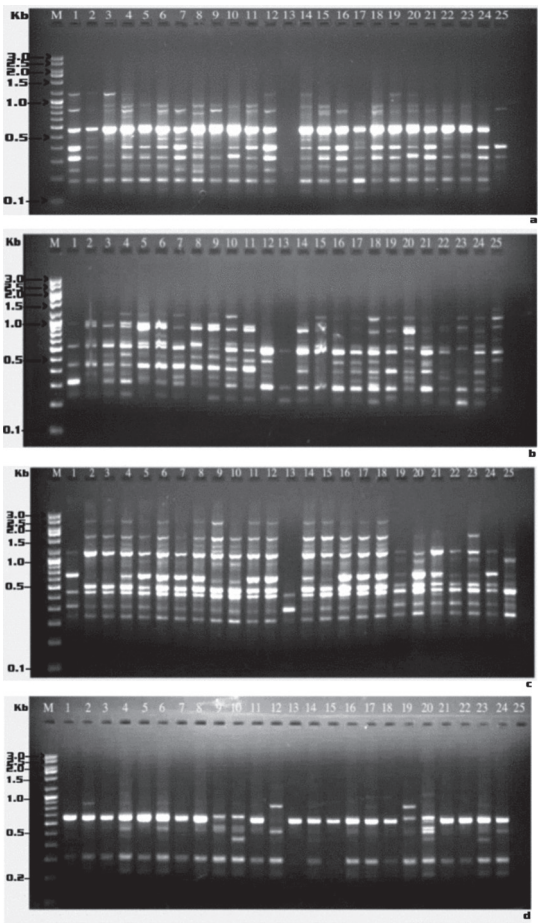

Fig. 2. ISSR patterns of 25 varieties of cashew generated by primer (a) UBC-827 (b) UBC-872 (c) AM-2 (d) UBC-818.
M - kb molecular weight ladder, 1 - BBSR-1, 2 - Jagannath, 3 - Balabhadra, 4 - BPP-4 , 5 - BPP-6, 6 - BPP-8 (H-2/16), 7 - Ullal-1, 8 - Ullal-3, 9 - Ullal-4, 10 - Chintamani-1, 11 - NRCC-2, 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-1

Table 2. Polymorphism detected with 10 ISSR primers in 25 cashew varieties

Primer code	Primer sequence (5'-3')	Total no. of bands	Polymorphism		Band Size (bp)	*PIC	*Rp value
			No. of bands	%			
AM-2	$(AAG)_5GC$	13	10	76.92	350-2500	0.44	18.56
UBC-807	$(AG)_8T$	10	10	100.00	350-1100	0.77	11.68
UBC-818	$(CA)_8G$	8	7	87.50	250-900	0.54	5.76
UBC-825	$(AC)_8T$	5	4	80.00	375-700	0.35	7.20
UBC-827	$(AC)_8G$	11	10	90.91	200-1100	0.57	12.56
UBC-841	$(GA)_8YC$	6	4	66.66	340-775	0.18	6.96
UBC-855	$(AC)_8YT$	10	10	100.00	100-1000	0.68	13.68
UBC-864	$(ATG)_6$	5	4	80.00	500-1000	0.45	6.96
UBC-865	$(CCG)_6$	6	5	83.3	540-975	0.39	7.54
UBC-872	$(GATA)_4$	13	12	92.31	250-1400	0.63	13.68
Total/Mean		81	70			0.51	10.91

*PIC: polymorphic information content; Rp: Resolving power

The maximum resolving power was observed in primer AM-2 (18.56) and the maximum PIC value was obtained by using the ISSR primer UBC-807 (0.77). According to Archak *et al*. (2003) ISSR with its superior genotype index should be the choice for genetic analysis of cashew germplasm.

Table 3. Unique markers identified for different varieties

Primer code	Variety	Unique band size (bp)
AM-2	Jagannath	1050
AM-2	Jagannath	2130
UBC-807	Jhargram-1	1200
UBC-807	BPP-6	1500
UBC-818	Jagannath	900
UBC-827	Ullal-4	750
UBC-827	BPP-4	400
UBC-872	Priyanka	1400

The cluster analysis with ISSR revealed that 25 varieties of cashew can be grouped into two major clusters based on similarity indices. The varieties Priyanka and Madakkathara-1 developed from Cashew Research Station, Madakkathara form the first major cluster with only 60 per cent similarity among them. The maximum numbers of genotypes (23) were represented in Cluster II. Cluster II was again divided into two subclusters. Jagannath (high nut weight variety released from Cashew Research Station, Orissa Agricultural University, Bhubaneshwar), showed an out-group from rest of the varieties of this cluster. The second sub-cluster further divided into two minor sub-clusters BI with 15 varieties and BII with seven varieties (Bhubaneswar-1, BPP-8, Vengurla-7, Kanaka, Vengurla-6, Vridhachalam-3 and Jhargarm-1). Among the 25 varieties, Kanaka and Vridhachalam-3 showed highest similarity indices (92%). Interestingly, it was noted that Vengurla-6 and Vengurla-7 were grouped into a single cluster at 80 per cent similarity level. But Ullal-3 and Ullal-4 not grouped into a single cluster. The varieties Jagannath and Balabhadra, released from Cashew Research Station, Bhubaneswar, grouped in the major cluster. But at 67 per cent similarity level Jagannath formed a distinct cluster. The results suggest that the use of different ISSR primers would enable to assess the genetic diversity of cashew as reported previously including *Citrus spp* (Fang and Roose, 1997), *Vitis vinifera* (Moreno *et al.,* 1998), *Plantago major* (Wolff and Morgan-Richards, 1998), *Olea europea* (Essadki *et al.,* 2006), *Castanea sativa* (Mattioni *et al.,* 2008), *Morus spp* (Kar *et al.,* 2008), *Corylus avellana* (Ferreira *et al.,* 2010) and *Prunus avium*

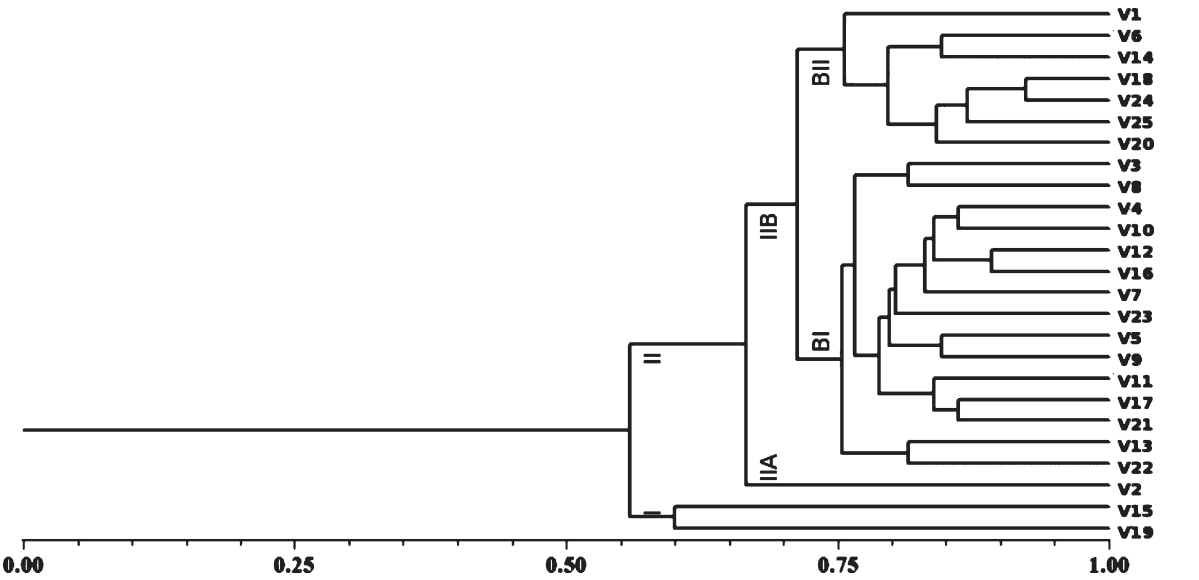

Fig. 3. UPGMA dendrogram showing the genetic relationship within 25 cashew varieties based on ISSR profile

(V1 - BBSR-1, V2 - Jagannath, V3 - Balabhadra, V4 - BPP-4 , V5 - BPP-6, V6 - BPP-8 (H-2/16), V7 - Ullal-1, V8 - Ullal-3, V9 - Ullal-4, V10 - Chintamani-1, V11 - NRCC-2, V12 - Vengurla-1, V13 - Vengurla-4, V14 - Vengurla-7 (H255), V15 - Madakkathara-1 (BLA-39-4), V16 - Madakkathara-2, V17 - Dhana (H-1608), V18 - Kanaka (H-1598), V19 - Priyanka (H-1591), V20 - Vengurla-6 (H -68), V21 - Amrutha, V22 - K 22-1, V23 - Bhaskara, V24 - Vridhachalam-3 (M-26/2), V25 - Jhargram-1)

(Ganopoulos *et al.*, 2011). The present results showed that the variation in ISSRs results from differences in the sequence between SSR loci. The difference in ISSR profile was perhaps due to their adaptability and other significant nut yield potential, shelling types, photosynthesis efficiency and growth characteristics. This methodology is relatively simple to perform, rapid and amenable to automation. The ISSR protocol can be readily used in breeding activities for registration and characterization of even closely related cashew accessions and for cataloguing collections.

Comparison between morphological and molecular data

The comparison between molecular and phenotypic data was determined on the basis of similarity coefficient. Pair wise comparison of accessions indicated relative genetic similarity between accessions ranging from a maximum of 0.86 (between Vengurla-7 and Jagannath) to a minimum of 0.48 (between Amrutha and Balabhadra) in morphological characterisation and from a maximum of 0.92 (Vridhachalam and Kanaka) to a minimum of 0.38 (between Bhaskara and Madakkathara-1) in ISSR. Based on these, the diversity (dissimilarity) ranged from 14 to 52 per cent in morphological characterisation and 8 to 62 per cent with ISSR markers and the diversity skewed around 50 per cent, indicating moderate diversity in the varieties. The results are in confirmative of moderate to high diversity of 6 to 62 per cent (dissimilarity) reported by Thimmappaiah *et al*. (2009) with cashew germplasm.

The low and non-significant value of matrix correlation (r=0.005) suggested that dendrogram based on morphometric and ISSR markers was not conserved. Results obtained with the dendrogram based on similarity coefficient of morphological characters were grouped into four clusters with 65 per cent similarity. Cluster I and II both comprising of a single variety, cluster III having six varieties and cluster IV with seventeen varieties. However, dendrogram obtained by ISSR analysis revealed differences among the clusters. In cluster I, Bhaskara forming a distinct cluster at morphological level showed about more than 70 per cent similarity with 13 other varieties belonging to cluster I at molecular level. Chintamani-1 also formed a single variety group with cluster II showing more than 75 per cent similarity with 15 other varieties belong to cluster I at molecular level. In cluster III, Jagannath which showed 70 per cent similarity at morphological level formed an out-group having about 60 per cent similarity with rest of the varieties at molecular level. Similarly Priyanka and Vengurla-6 which showed about 80 per cent similarity at morphological level were found to be only 60 per cent similar at molecular level. In cluster IV, seventeen varieties showing 70 per cent similarity in morphology also showed 70 per cent similarity at molecular level.

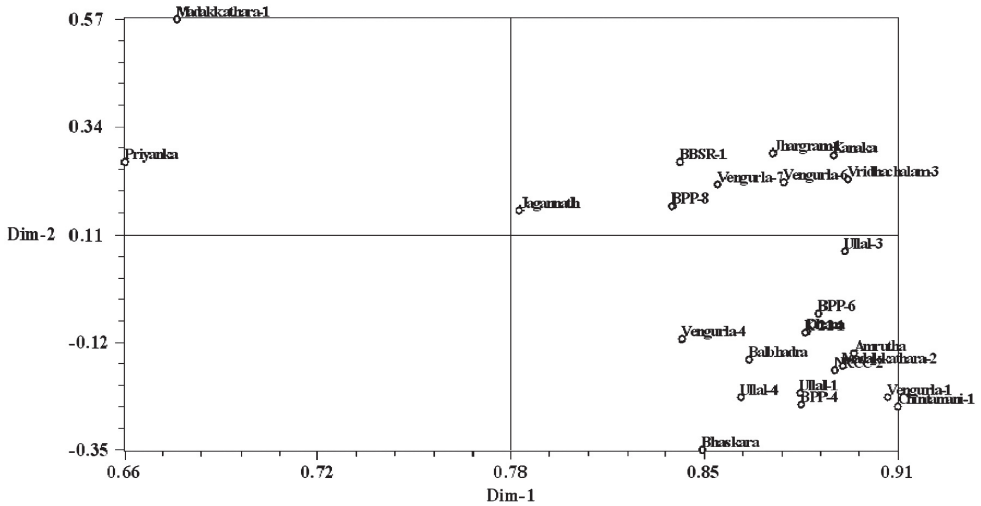

Fig. 4. Two dimensional scaling by principal component analysis (PCA) of 25 cashew varieties using Jaccard's similarity coefficient of ISSR profile

Apart from this, these varieties also had about 70 per cent similarity with Jagannath which belongs to cluster II, Priyanka of cluster III and Madakkathara-1, BPP-8, Vengurla-7 and Bhubaneswar-1 at molecular level. The discrepancies between ISSR data and morphologically based groupings have been reported in *Eriastrum densilfolium* (Brunel and Whitkus, 1997), *Fragaria* spp. (Harrison *et al*., 1997), *Cucurbitaceae spp.* (Sikdar *et al*., 2010) and *Mangifera indica* (Samal *et al*., 2012). Castiglione *et al.*, (1993) managed to define the entire commercial poplar clone tested, including those that could not be distinguished with morphological traits through RAPD and ISSR markers. Phenotypic differences are not necessarily correlated with the number of underlying gene mutations, and differences in phenotypic characters are not necessarily reflections of different genetic events (Bachmann, 1992). In our study, ISSR markers concur with the classical taxonomic groupings with better range of diversity *i.e.*, 8-62 per cent and it can be concluded that morphologically distinct varieties like Bhaskara and Chintamani-1 may exhibit similarity at molecular level, thus showing their genetic relatedness. Likewise varieties which show similar morphological characters may vary widely among themselves at the genotypic level as evident in 'Jagannath' which forms an out-group at molecular level. The phenotypic characters do not seem to be a reliable descriptor to classify the variety, even though they may be useful for making a crude classification of different geographical origin. Consequently, the use of morphological traits is not always the most informative method while evaluating distances and relatedness. Genetic variation can instead be directly measured using DNA analysis as opposed to those estimated from a phenotype. In the present study, there was some correlation between the two types of methods and can be used to evaluate the genetic relationships for plant improvement, in descriptions of new variety and for assessing varietal purity in plant certification and conservation programmes.

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