Analysis of genetic relationships between populations of cashew (*Anacardium occidentale* L.) by using morphological characterisation and RAPD markers

S. Samal[1], G.R. Rout[2], P.C. Lenka[1]

[1]*Orissa University of Agriculture and Technology, Bhubaneswar, Orissa, India*
[2]*Regional Plant Resource Centre, Nayapalli, Bhubaneswar, Orissa, India*

ABSTRACT

In the present paper genetic relationships of twenty varieties of cashew are described on the basis of morphological characters and RAPD (Randomly Amplified Polymorphic DNA) markers. Results obtained for the phenotypic characters based on similarity coefficient were divided into four clusters with 70% similarity. By means of similarity coefficients (S_G), cluster I was found to consist of twelve varieties. Cluster II consisted of a single variety, NRCC-1, cluster III consisted of six varieties and cluster IV had only one variety, Vridhachalam-2. The analysis started by using RAPD markers that allowed us to distinguish 20 varieties. A total of 80 distinct DNA fragments ranging from 0.2 to 3.0 kb were amplified by using 11 selected random 10-mer primers. Genetic similarity analysis was conducted for the presence or absence of bands in the RAPD profile. Cluster analysis clearly showed that 20 varieties of cashew grouped into two major clusters based on similarity indices. The first major cluster comprised one minor cluster. The other major cluster was divided into two sub-minor clusters, one sub-minor cluster having three varieties and the other sub-minor cluster was represented by 15 varieties. Among the 20 varieties, Ullal-3 and Dhana (H-1608) showed the highest similarity indices (87%). It was noted that Vengurla-2 and Vengurla-3 were not grouped into a single cluster but Vengurla-4 has 82% similarity to Vengurla-3. The variety Vengurla-2 has very close similarity (85%) with variety Vridhachalam-3 (M-26/2). The analysis of genetic relationships in cashew using morphological traits and RAPD banding data can be useful for plant improvement, descriptions of new varieties and also for assessment of variety purity in plant certification programmes.

Keywords: *Anacardium occidentale*; genetic similarity; morphological character; RAPD marker

Cashew (*Anacardium occidentale* L.) is an important tropical tree belonging to the family Anacardiaceae and native to tropical America, from Mexico and West Indies to Brazil and Peru. The cashew tree is also pantropical, especially in coastal areas. Major producers of cashew nuts are India, Tanzania, Mozambique and Kenya. However, its production is constrained by low and variable nut yields, nut quality and susceptibility to pests and fungal diseases, particularly *Helopeltis* and powdery mildew, even though efforts have been made to improve this crop through breeding and other agronomic measures since 1950 (Northwood and Tsakiris 1967). Cashew yields are reported to range from 0–48 kg/tree, with an average yield of 800–1000 kg/ha. Genetic improvement is limited by the lack of knowledge of genetic diversity of the indigenous germplasm of both India and other countries. Moreover, breeding of cashew is mostly based on traditional methods of selection of useful traits which in turn are phenotypes such as nut size, nut weight, sex ratio, length of panicle and yield performance (Mneney et al. 2001). Although classical phenotype features are still extremely useful, the efficiency of selection may be reduced by developmental stages or by environmental effects on measured traits. Broadening the genetic base by introducing new alleles present in exotic germplasm (Faenza et al. 1982) and a systematic exploitation of heterosis (Masawe 1994) have been suggested as means to overcome some of the problems. 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). Apart from this, cultivar identification is mainly based on morphological traits. This approach has, however, limited usefulness since the variation in morphological traits is influenced by environmental factors. Therefore polymorphic markers are needed for identification of varieties, for estimation of genetic similarity among and between the populations or varieties. RAPD analysis primarily detects differences in the nuclear genome because there are large numbers of unique nucleotide sequences available for primer binding in the nuclear genome relative to organellar genomes. An RAPD assay is the cheapest method for identification of the genotypes within a short period and also requires only a limited amount of DNA. The development of randomly amplified polymorphic DNA markers, generated by the polymerase chain reaction (PCR) using arbitrary primers, has provided a new tool for the detection of DNA polymorphism (Williams et al. 1990). RAPD analysis has also been used to study the genetic relationship in a number of tree species (Ziegenhagen et al. 1993, Kra-

bel et al. 1998, Casas et al. 1999, Ravishankar et al. 2000, Rout et al. 2003). In this paper we investigate genetic similarity of 20 varieties of cashew by using morphological characteristics and RAPD markers.

MATERIAL AND METHODS

Plant materials. Leaves of twenty varieties of *Anacardium occidentale* Vars. BPP-2, BPP-3, BPP-4, BPP-8 (H-2/16), Vridhachalam-2 (M-44/3), Vridhachalam-3 (M-26/2), Ullal-2, Ullal-3, Vengurla-2, Vengurla-3, Vengurla-4, Vengurla-7, H-320, Madakkathara-1 (BLA-39-4), Dhana (H-1608), Kanaka (H-1598), Priyanka (H-1591), Jhargram-1, NRCC-1 and NRCC-2 were collected from each variety individually in an ice box from a Cashew Orchard maintained by the Orissa University of Agriculture and Technology, Bhubaneswar, Orissa, India. About ten individuals of each variety were selected for determination morphological characteristics and DNA isolation.

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 (S_G) of Gower (1971) was used as a measure of resemblance between different operational taxonomic units and S_G values were calculated according to Sneath and Sokal (1973). Based on the matrix of S_G values, dendrograms were constructed using the UPGMA (unweighted pair group method using arithmetic average) technique in one of the SAHN (sequential, agglomerative, hierarchic, non-overlapping) clustering methods (Sneath and Sokal 1973).

DNA extraction. Total DNA was extracted from semi-mature leaves (2.5 g) by a modified method of Rout et al. (2002). DNA quantifications were performed by visualising bands under UV light, after electrophoresis on 1.0% (w/v) agarose gel. Resuspended DNA was then diluted in sterile distilled water to 5 ng/µl concentration for use in amplification reactions.

Primer screening. Forty 10-mer primers, corresponding to kits A, B, D and N from Operon Technologies (Alameda, California), were initially screened using five varieties to determine the suitability of each primer for the study. Primers were selected for further analysis based on their ability to detect distinct, clearly resolved and polymorphic amplified products within the varieties of cashew. To ensure reproducibility, the primers generating no, weak, or complex patterns were discarded.

PCR amplifications. A set of forty random decamer oligonucleotides purchased from Operon Technologies, Inc. (Alameda, California, USA) was used as single primers for the amplification of RAPD fragments. Polymerase Chain Reactions (PCR) were carried out in a final volume of 25 µl containing 20 ng template DNA, 100µM each deoxynucleotide triphosphate, 20 ng of decanucleotide primers (M/S Operon Technology, Inc., Alameda, CA 94501, USA), 1.5mM $MgCl_2$, 1× Taq buffer [10mM Tris-HCl (pH 9.0), 50mM KCl, 0.01% gelatine] and 0.5 U Taq DNA polymerase (M/S Bangalore Genei, India). Amplification was achieved in a PTC 100 thermal cycler (MJ Research, USA) 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 extension at 72°C for 2 min, finally at 72°C for 10 min. Amplification products were separated alongside a molecular weight marker (1 kb 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 visualised under UV light. Gel photographs were scanned through Gel Doc System (Gel Doc. 2000, BioRad, USA) and the amplification product sizes were evaluated using the software Quantity One (BioRad, USA).

Data were recorded as presence (1) or absence (0) of band from the examination of photographic negatives. Each amplification fragment was named by the source of the primer (Operon, Advanced Biotechnologies), kit letter or number, primer number and its approximate size in base pairs. Bands with similar mobility to those detected in the negative control, if any, were not scored. Similarity index was estimated using the Dice coefficient of similarity (Nei and Li 1979). Cluster analyses were carried out on similarity estimates using UPGMA (unweighted pair-group method using arithmetic average) in NTSYS-PC, version 1.80 programme (Rohlf 1995).

RESULTS AND DISCUSSION

Morphological characters

Eight morphological characters of twenty varieties of cashew are presented in Table 1. Based on morphological characters, the twenty varieties of cashew were classified using similarity coefficients. Results obtained for a dendrogram based on similarity coefficients were divided into four clusters with 70% similarity (Figure 1). By means of similarity coefficients (S_G), the phenon level, the genotypes were broadly grouped into four clusters, i.e. cluster I comprising 12 varieties, cluster II consisting of a single variety (NRCC-1), cluster III having six varieties and cluster IV with one variety only (Vridhachalam-2). At 75% phenon level, cluster I, the largest cluster, was further divided into three sub-clusters (IA, IB, IC) and cluster III into two groups, IIIA and IIIB. Sub-clusters IA, IB and IC were multivariate groups containing four (Vridhachalam-3, Ullal-2, Vengurla-7, BPP-8), five (Vengurla-3, Dhana, Vengurla-4, H-320, NRCC-2) and three (Vengurla-2, Kanaka, Madakkathara-1) genotypes, respectively. When the phenon level was drawn at 80%, IB and IC were further divided into two groups, IB_1, IB_2 and IC_1, IC_2, respectively. Cluster IIIA was further divided into three groups, namely $IIIA_1$ (Ullal-3), $IIIA_2$ (Priyanka), $IIIA_3$ (BPP-2, BPP-3 and BPP-4). Cluster analysis of twenty varieties of cashew is represented in Figure 1. In sub-cluster IA, the varieties Vridhachalam-3 (M-26/2) and

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

Variety	No. of laterals/m^2	No. of flowering laterals/m^2	Length of panicle (cm)	Sex ratio	No. of nuts/panicle	Net weight (g)	Shelling (%)	Yield (kg/plant)
BPP-2	19.31	16.75	21.10	0.61	2.95	5.20	27.45	3.42
BPP-3	13.56	11.62	21.45	0.45	3.05	5.11	28.67	3.68
BPP-4	17.69	12.62	17.12	0.38	2.5	5.17	29.78	2.85
BPP-8	23.33	22.25	18.80	0.14	4.85	7.98	29.65	11.60
Vridhachalam-2	26.12	24.88	12.62	1.12	3.35	5.33	31.20	2.65
Vridhachalam-3 (M-26/2)	20.50	19.94	19.38	0.18	3.97	5.93	29.70	6.67
Ullal-2	23.19	19.62	19.85	0.20	3.98	6.38	28.60	4.57
Ullal-3	16.56	15.12	19.98	0.41	5.00	7.28	30.15	3.58
Vengurla-2	22.56	22.12	20.57	0.55	6.40	4.56	32.00	7.50
Vengurla-3	23.94	23.31	22.25	0.79	4.60	8.43	26.50	8.82
Vengurla-4	24.25	23.42	18.60	0.40	4.68	7.70	27.92	7.40
Vengurla-7	23.38	20.62	19.47	0.29	3.30	8.78	30.50	4.43
H-320	19.81	18.44	18.10	0.43	3.72	7.77	27.10	6.75
Madakkathara-1	20.75	19.31	14.77	0.55	5.32	5.39	28.62	7.40
Dhana (H-1608)	25.12	22.81	20.45	0.39	4.22	7.98	26.50	9.95
Kanaka (H-1598)	22.06	20.19	18.85	0.86	4.97	5.66	31.12	8.50
Priyanka	17.83	15.69	17.17	0.77	2.65	6.75	28.00	6.50
Jhargram-1	21.00	19.38	20.25	0.10	1.00	6.23	28.85	1.23
NRCC-1	20.06	16.75	24.24	0.16	1.55	7.33	30.45	2.12
NRCC-2	22.88	20.19	20.12	0.75	3.40	8.27	28.57	6.47
SE (mean)	0.806	0.795	0.553	0.038	0.265	0.128	0.435	0.306
CD 5%	2.385	2.353	1.636	0.112	0.784	0.378	1.287	0.905

Four individuals/variety, three replications

Ullal-2 were closely associated at 90% similarity by a moderate number of flowering laterals, length of panicle, sex ratio and number of nuts/panicle. The varieties Vridhachalam-3 and Ullal-2 were 80% similar to varieties BPP-8 and Vengurla-7. In sub-cluster IB_2, the varieties Vengurla-3 and Dhana (H-1608) were 86% and 83% similar to sub-cluster IB_1 having the varieties H-320 and NRCC-2. The varieties Vengurla-3 and Dhana (H-1608) were 82% similar to Vengurla-4. Sub-cluster IC_1 comprised the variety Madakkathara-1 (BLA-39-A), which was characterised by high sex ratio, low nut weight and moderate yield per kg. Further, the varieties BPP-2, BPP-3 and BPP-4 showed 78% similarity to Priyanka (H-1591), 75% similarity to Ullal-3 and 70% similarity to the variety Jhargram-1. The variety Vridhachalam-2 (M-44/3) formed the highest major cluster with 58% similarity to the other 19 varieties.

Molecular characters

The primer screening resulted in 11 primers that showed good amplification (Table 2) and 18 random primers that did not give any amplification products. Other 11 primers showed amplification but the intensity of the fragments was very low. 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 20 varieties with 11 random primers produced 80 consistent RAPD markers, ranging in size from 0.2 to 3.0 kb, out of which 16 were monomorphic. The number of produced DNA fragments ranged from 4 to 12 in primers OPN-12 and OPA-2. The pattern of RAPD profiles produced by primers OPA-03, OPA-02, OPN-05 and OPA-09 is shown in Figure 2. Among the 12 fragments amplified by primer OPA-12, two unique bands of 2.13 kb and 1.05 kb were present in the variety BPP-2 that clearly distinguished it from the other varieties. Similarly, another unique band of 0.612 kb was observed in NRCC-1 with primer OPA-2. The primer OPA-03 produced six amplified bands, out of which two were polymorphic and four were monomorphic. Two unique bands of 1.47 kb and 0.40 kb appeared in NRCC-2 and Ullal-3 with primer OPA-7. Some of the primers (OPA-08, OPA-09 and OPN-12) gave only four fragments in each variety of cashew. The results also indicate that the primer OPN-13 produced one unique band (1.75 kb) in BPP-2 which was different in other varieties. Three unique bands (1.77, 1.44 and 1.04 kb) were present in the variety Jhargram-1 with primer OPN-14. In BPP-3, eight amplified bands appeared including one unique band having 1.5 kb

with primer OPN-3. The primer OPN-5 showed distinguished fragments in different varieties of cashew, out of which nine were polymorphic bands. These similarity coefficients were used to generate a tree from cluster analysis by UPGMA method (Figure 3). The cluster analysis indicates that 20 varieties of cashew can be grouped into two major clusters based on similarity indices. The one major cluster comprises one minor cluster having one variety only, BPP-2. The other major cluster is divided into two sub-minor clusters, one sub-minor cluster having three varieties, BPP-4, BPP-8 (H-2/16) and NRCC-2, and the other sub-minor cluster is represented by 15 varieties. The variety BPP-2 showed about 70% similarity to the variety BPP-3. Each variety and all varieties could be identified by 11 random 10-mer primers. Among the 20 varieties, Ullal-3 and Dhana (H-1608) showed the highest similarity indices (87%). The results indicated that Vengurla-2 and Vengurla-3 were not grouped into a single cluster but Vengurla-4 showed 82% similarity to Vengurla-3. The variety Vengurla-2 has very close similarity (85%) to the variety Vridhachalam-3 (M-26/2). On the whole, the genetic relationships based on RAPDs markers concur with classical taxonomic groupings. These results are in close correspondence with the other studies in woody tree species (Dehghan-Shoar et al. 1997, Casas et al. 1999, Ravishankar et al. 2000).

Comparison of molecular and phenotypic data

A comparison of molecular and phenotypic data was made on the basis of similarity coefficients. Results obtained from a dendrogram based on similarity coefficients of morphological characters were grouped into four clusters with 70% similarity. Cluster I comprised twelve varieties, cluster II consisted of a single variety (NRCC-1), cluster III had six varieties and cluster IV one variety (Vridhachalam-2). However, a dendrogram obtained by RAPD analysis revealed differences between the clusters. In cluster I, twelve varieties showed 70% similarity in morphology and they also showed 70% similarity at

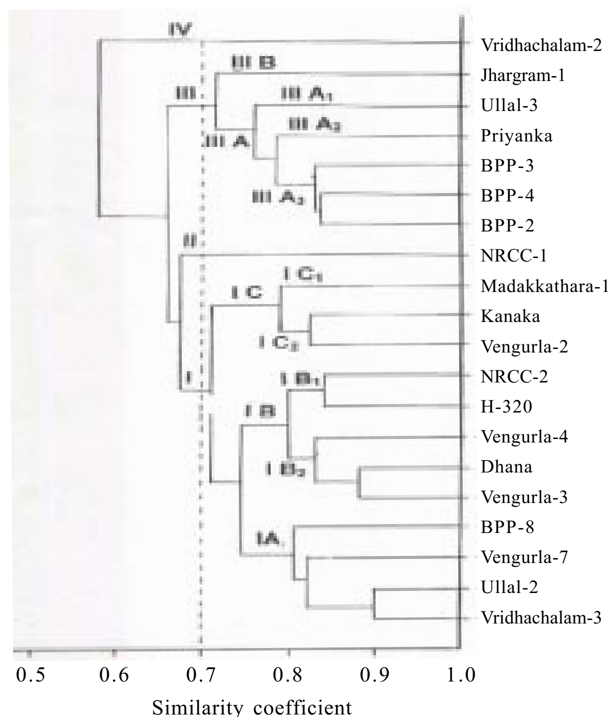

Figure 1. Dendrogram based on similarity coefficients of 20 varieties of cashew using morphological characters

C1 = BPP-2, C2 = BPP-3, C3 = BPP-4, C4 = BPP-8 (H-2/16), C5 = Vridhachalam-2 (M-44/3), C6 = Vridhachalam-3 (M-26/2), C7 = Ullal-2, C8 = Ullal-3, C9 = Vengurla-2, C10 = Vengurla-3, C11 = Vengurla-4, C12 = Vengurla-7, C13 = H-320, C14 = Madakkathara-1 (BLA-39-4), C15 = Dhana (H-1608), C16 = Kanaka (H-1598), C17 = Priyanka (H-1591), C18 = Jhargram-1, C19 = NRCC-1, C20 = NRCC-2

Table 2. Total number of amplified fragments and number of polymorphic bands generated by PCR using selected random decamers in 20 varieties of cashew

Name of primer	Sequence of primer	Total No. of amplification products	No. of polymorphic products	Size range (kb)
OPA 02	5'-TGCCGAGCTG-3'	12	10	0.3–2.1
OPA 03	5'-AGTCAGCCAC-3'	6	2	0.5–1.6
OPA 07	5'-GAAACGGGTG-3'	8	8	0.3–1.4
OPA 08	5'-GTGACGTAGG-3'	4	3	0.4–1.1
OPA 09	5'-GGGTAACGCC-3'	4	3	1.0–2.0
OPN 12	5'-CACAGACACC-3'	4	1	0.8–1.9
OPN 13	5'-AGCGTCACTC-3'	5	3	0.7–1.9
OPN 14	5'-TCGTGCGGGT-3'	8	8	0.4–2.0
OPN 03	5'-GGTACTCCCC-3'	8	8	0.4–3.0
OPN 05	5'-ACTGAACGCC-3'	10	9	0.4–2.7
OPN 06	5'-GAGACGCACA-3'	11	9	0.2–1.7

a molecular level. Apart from this, these varieties also displayed about 70% similarity to NRCC-1, which belongs to cluster II, Priyanka, Ullal-3 and Jhargram-1 of cluster III and Vridhachalam-2 of cluster IV at a molecular level. Likewise NRCC-1 forming a distinct cluster (cluster II) at a morphological level showed about more than 70% similarity to other 13 varieties belonging to cluster I and III at a molecular level.

In cluster III, BPP-3, which showed 70% similarity at a morphological level, formed an out group displaying about 60% similarity to the remaining varieties at a molecular level. Similarly, BPP-2 and BPP-4, which showed about 85% similarity at a morphological level, were found to be only 60% similar at a molecular level. Similarly like NRCC-1, Vridhachalam-2 also formed a distinct cluster (cluster IV) at a morphological level but it also had more than 70% similarity to 14 varieties belonging to cluster I and III. At a morphological level (70% similarity), the varieties NRCC-1 and Vridhachalam-2 formed distinct clusters but they showed about 80% similarity at a molecular level. It can be concluded from these observations that morphologically distinct varieties such as NRCC-1 and Vridhachalam-2 can exhibit similarity at a molecular level, thus showing their genetic relatedness. Likewise, the varieties that have similar morphological characters can be largely different from each other at the genotypic level as evident in BPP-3, which forms an out group at a molecular level. 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 (Hamrick and Godt 1989). According to these authors, molecular markers provide a better coverage of the genome, resulting in a better estimate of relationships. The discrepancies between RAPD data and morphologically based groupings were reported in *Eriastrum densifolium* (Brunell and Whitkus 1997), in *Fragaria* spp. (Harrison et al. 1997), in *Hordeum vulgare* (Papa et al. 1998) and in *Rhenum* spp. (Persson et al. 2000). Keskitalo et al. (1998) reported that the morphological and RAPD data on *Tanacetum vulgare* are grouped into genotypes according to geographic origin. Castiglione et al. (1993) managed to define all the commercial poplar clones tested, including those that could not be distinguished by morphological traits through RAPD markers. In our study, RAPD markers concur with the classical taxonomic groupings with genetic relationships. 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 opinion, this phenotypic character does not seem to be a reliable descriptor to classify the variety, even though it may sometimes 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. Instead, genetic variations can be measured directly using DNA analysis as opposed to those estimated from a phenotype.

In the present study, however, the correlation between the two types of methods is fairly strong to evaluate the genetic relationships useful for plant improvement, descriptions of new varieties and also for assessment of variety purity in plant certification and conservation programmes.

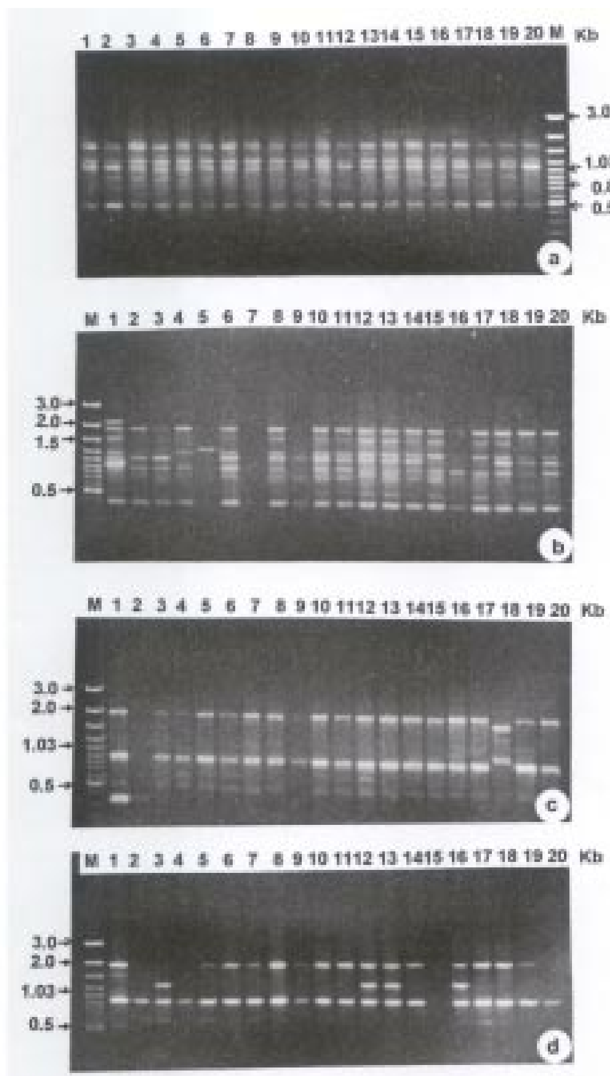

Figure 2. RAPD patterns of 20 varieties of *Anacardium occidentale* generated by primers

(a) OPA-03 (5'-AGTCAGCCAC-3'); (b) OPA-02 (5'-TGCCGAGCTG-3'); (c) OPN-05 (5'-ACTGAACGCC-3'); (d) OPA-09 (5'-GGGTAACGCC-3')
M = kb molecular weight ladder
1 = BPP-2, 2 = BPP-3, 3 = BPP-4, 4 = BPP-8 (H-2/16), 5 = Vridhachalam-2 (M-44/3), 6 = Vridhachalam-3 (M-26/2), 7 = Ullal-2, 8 = Ullal-3, 9 = Vengurla-2, 10 = Vengurla-3, 11 = Vengurla-4, 12 = Vengurla-7, 13 = H-320, 14 = Madakkathara-1 (BLA-39-4), 15 = Dhana (H-1608), 16 = Kanaka (H-1598), 17 = Priyanka (H-1591), 18 = Jhargram-1, 19 = NRCC-1, 20 = NRCC-2

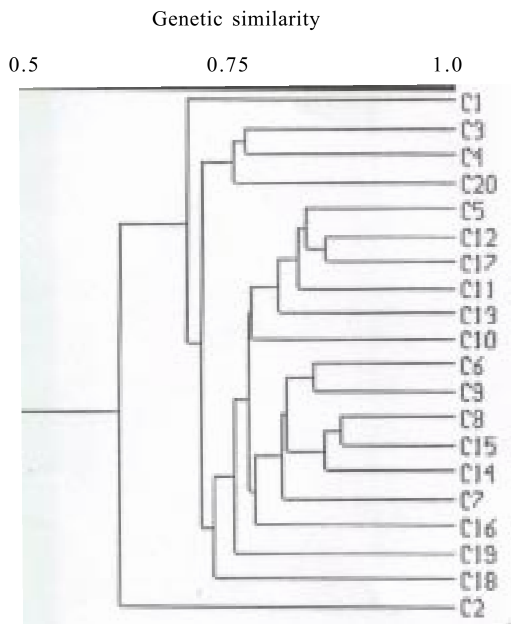

Figure 3. UPGMA dendrogram showing the genetic relationships within 20 varieties of cashew

C1 = BPP-2, C2 = BPP-3, C3 = BPP-4, C4 = BPP-8 (H-2/16), C5 = Vridhachalam-2 (M-44/3), C6 = Vridhachalam-3 (M-26/2), C7 = Ullal-2, C8 = Ullal-3, C9 = Vengurla-2, C10 = Vengurla-3, C11 = Vengurla-4, C12 = Vengurla-7, C13 = H-320, C14 = Madakkathara-1 (BLA-39-4), C15 = Dhana (H-1608), C16 = Kanaka (H-1598), C17 = Priyanka (H-1591), C18 = Jhargram-1, C19 = NRCC-1, C20 = NRCC-2

Acknowledgements

The authors wish to acknowledge the assistance of Department of Forest and Environment, Government of Orissa for undertaking this study. The authors are also thankful to Dr. S.P.S. Khanuja, Director, CIMAP, Lucknow, for his kind help.

REFERENCES

Bachmann K. (1992): Phenotypic similarity and genetic relationship among populations of *Microseris bigelovi* (Asteraceae: Lactuceae). Bot. Acta, *105*: 337–342.

Brunell M.S., Whitkus J. (1997): RAPD marker variation in *Eriastrum densifolium* (Polemoniaceae): implications for subspecific delimitation and conservation. Syst. Bot., *22*: 543–553.

Casas A.M., Lgartua E., Balaguer G., Moreno M.A. (1999): Genetic diversity of *Prunus* rootstocks analyzed by RAPD markers. Euphytica, *110*: 139–149.

Castiglione S., Wang G., Damiani G., Bandi C., Bisoffi S., Sala F. (1993): RAPD fingerprints for identification and for taxonomic studies of elite poplar (*Populus* spp.) clones. Theor. Appl. Genet., *87*: 54–59.

Dehghan-Shoar M., Hampton J.G., Gardiner S.E. (1997): Genetic analysis among and within populations forming ecotypes and cultivars of lucerne, *Medicago sativa* (Leguminosae), using RAPD fragments. Plant Syst. Evol., *208*: 107–119.

Faenza V., Conticini L., Partel L. (1982): Le collezioni nazional e internazionali di Anacardio in Tanzania. Riv. Agric. Subtrop. Trop., *76*: 191–219.

Gower G.C. (1971): A general coefficient of similarity and some of its properties. Biometrics, *27*: 857–871.

Hamrick J.L., Godt M.J. (1989): Allozyme diversity in plant species. In: Brown A.D.H., Clegg M.T., Kahler A.L., Weir B.S. (eds.): Plant population genetics, breeding and genetic resources. Sinauer Assoc., Sunderland, Mass: 43–63.

Harrison R.F., Luby J.J., Furnier G.R., Hancork H. (1997): Morphological and molecular variation among populations of octaploid *Fragaria virginiana* and *F. chloronum* (Rosaceae) from North America. Am. J. Bot., *84*: 612–620.

Keskitalo M., Linden A., Valkonen J.P.T. (1998): Genetic and morphological diversity of Finnish tansy (*Tanacetum vulgare* Asteraceae). Theor. Appl. Genet., *96*: 1141–1150.

Krabel D., Vornam B., Herzog S. (1998): PCR-based random amplification of genomic DNA from single pollen grains of tree. Angew. Bot., *72*: 10–13.

Masawe P.A.L. (1994): Aspects of breeding and selecting improved cashew genotypes (*Anacardium occidentale* L.). [Thesis.] Univ. Read., UK.

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

Nei M., Li W.H. (1979): Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc. Nat. Acad. Sci., USA, *76*: 5269–5273.

Northwood P.J. Tsakiris A. (1967): Cashew nut production in Southern Tanzania – Early yields from a cashew spacing experiment. East Afr. Agric. Forest. J., *33*: 81–82.

Papa R., Attene G., Barcaccia G., Ohgata A., Konishi T. (1998): Genetic diversity in landrace populations of *Hordeum vulgare* L. from Sardinia, Italy, as revealed by RAPDs, isozymes and morphological traits. Plant Breeding, *117*: 523–530.

Persson H.A., Rumpunen K., Mollerstedt L.K. (2000): Identification of culinary rhuarb (*Rheum* spp.) cultivars using morphological characterization and RAPD markers. J. Hort. Sci. Biotechnol., *75*: 684–689.

Ravishankar K.V., Anand L., Dinesh M.R. (2000): Assessment of genetic relatedness among mango cultivars of India using RAPD markers. J. Hort. Sci. Biotechnol., *75*: 198–201.

Rohlf F.J. (1995): NTSYS-PC Numerical Taxonomy and Multivariate Analysis System. Version 1.80, Exeter Software, Setauket, New York.

Rout G.R, Bhatacharya D., Nanda R.M., Nayak S., Das P. (2003): Evaluation of genetic relationships in *Dalbergia* species using RAPD markers. Biodiv. Conserv., *12*: 197–206.

Rout G.R., Samal S., Nayak S., Nanda R.M., Lenka P.C., Das P. (2002): An alternative method of plant DNA extraction of cashew (*Anacardium occidentale* L.) for randomly amplified polymorphic DNA (RAPD) analysis. Gartenbauwissenschaft, *67*: 114–118.

Smith J.S.C., Smith O.S. (1989): The description and assessment of distances between inbred lines of maize. II. The utility of morphological, biochemical and genetic descriptors and a scheme for the testing of distinctiveness between inbred lines. Maydica, *34*: 151–161.

Sneath P.H.A., Sokal R.R. (1973): Numerical taxonomy. The principles and practice of numerical classification. Freeman W.H. & Comp., San Francisco, New York.

Williams J.G.K., Kubelik A., Livak K.J., Rafiski J.A., Tingey S.V. (1990): DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucl. Acids Res., *18*: 6531–6535.

Ziegenhagen B., Guillemaut P., Scholz F. (1993): A procedure for minipreparations of genomic DNA from needles of silver fir (*Abies alba* Mill.). Plant Mol. Biol. Rep., *11*: 117–121.

Received on September 5, 2002

ABSTRAKT

Analýza genetické příbuznosti populací ledvinovníku západního (*Anacardium occidentale* L.) s použitím morfologické charakteristiky a markerů RAPD

Je popsána genetická příbuznost 20 odrůd ledvinovníku západního s použitím morfologických znaků a markerů RAPD (náhodně amplifikované polymorfní DNA). Výsledky, které jsme získali pro jednotlivé fenotypové znaky na základě stanovení koeficientu podobnosti, jsme rozdělili do čtyř klastrů se 70% podobností. Pomocí koeficientů podobnosti (S_G) jsme zjistili, že klastr I obsahuje 12 odrůd, klastr II jednu odrůdu (NRCC-1), klastr III tvoří šest odrůd a klastr IV má pouze jednu odrůdu (Vridhachalam-2). Analýza s použitím RAPD markerů umožnila rozlišit 20 odrůd. Celkem bylo amplifikováno 80 rozdílných fragmentů DNA v rozsahu od 0,2 do 3,0 kb s použitím 11 vybraných náhodných dekamerních primerů. Analýzu genetické podobnosti jsme prováděli na přítomnost či nepřítomnost zón v profilu RAPD. Klastrová analýza jednoznačně prokázala, že 20 odrůd ledvinovníku západního lze rozdělit na základě indexů podobnosti do dvou větších klastrů. V prvním větším klastru lze rozlišit jeden menší klastr. Druhý větší klastr se dělí na dva menší subklastry, jeden menší subklastr se třemi odrůdami a další menší subklastr, který představuje 15 odrůd. Z 20 odrůd měly nejvyšší indexy podobnosti (87%) odrůdy Ullal-3 a Dhana (H-1608). Odrůdy Vengurla-2 a Vengurla-3 nebyly zařazeny do jednoho klastru, neboť u odrůdy Vengurla-4 byla nalezena 82% podobnost s odrůdou Vengurla-3. Odrůda Vengurla-2 vykázala velmi blízkou podobnost (85%) s odrůdou Vridhachalam-3 (M-26/2). Analýzu genetické příbuznosti ledvinovníku západního s použitím morfologických znaků a údajů o zónách RAPD lze využít při šlechtění rostlin, k popisu nové odrůdy nebo pro hodnocení odrůdové čistoty v programech certifikace rostlin.

Klíčová slova: *Anacardium occidentale*; genetická podobnost; morfologický znak; marker RAPD

Corresponding author:

Dr. Gyana Ranjan Rout, Plant Biotechnology Division, Regional Plant Resource Centre, Nayapalli, Bhubaneswar, 751 015 Orissa, India
tel.: + 91 674 553 845, fax: + 91 674 550 274, e-mail: grrout@hotmail.com