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Short communication

Assessment of genetic diversity among arecanut accessions by using RAPD markers

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ABSTRACT

The RAPD markers were used to analyze genetic diversity and genetic relationship among 10 arecanut varieties. Out of 30 primers tested, only 11 primers amplified. The highest number of bands was obtained with primer OPH-48 (48) followed by OPH-8 (47) and OPH-46 (46), while minimum number of band was generated with OPH-41 (39). The maximum number of bands per marker per genotype was obtained from OPH-35 (4.8) followed by OPH-8(4.7) and OPH-46 (4.6) and OPH-42 (4.5). The highest PIC value was obtained (0.60) with OPH-35 followed by OPF-10 (0.44), whereas, the lowest PIC value was obtained in (0.17) with OPH-41 primer. The dendrogram showed high variability between the wild and cultivated arecanut varieties.

Key words: Arecanut, genetic diversity, RAPD markers.

Arecanut or betel nut (*Areca catechu* L.) is an important plantation crop and its nuts are chewed regularly by at least 5% of the world population, imported by immigrant users wherever they settle. There are several palms under the genus *Areca* that are native to South and South-East Asia and the Pacific islands. Arecanut is cultivated in an area of about 4,152 ha with the production of 5,800 MT and the second most important agricultural product in Andaman and Nicobar Islands. Areca nut is commercially sold as dried, cured and fresh nut forms. There is a huge genetic diversity of arecanut and its use has a major role in social situations in Andaman and Nicobar Islands of India. Due to maritime climatic and geographical fragmentation, these islands are considered as biological paradise of flora and fauna by naturalists and geneticists and included in the list of biodiversity hot spots (Upadhyay *et al*., 8). The recent development of DNA markers are mainly used to analyse the genetic variability in molecular level of plant populations. Among the various molecular marker techniques developed over the year, RAPD, AFLP and SSR (Manimekalai and Nagarajan, 1; Purushotham *et al*., 4) techniques have been done in nut palms. RAPD techniques have been used in genetic diversity in arecanut (Rivera *et al*., 5) and coconut (Perera *et al*., 3). Very little information is available on the genetic diversity among arecanut populations using molecular markers. Therefore, the present study was attempted.

The young leaf samples of arecanut (Samrudhi, Calicut-35, Calicut-1, Mangala, CARI-Selection1, dwarf arecanut & wild arecanut) were collected from (South Block (SB) & Hill Block (HB) germplasm, maintained at CARI, Andaman were used for the study. The genomic DNA isolation was done by using standard protocol with slight modifications. The DNA was extracted from spindle leaves (pale yellow colour). Five gram of leaf material was frozen in liquid nitrogen and ground to powder in a pestle and mortar. About 250 mg of poly vinyl poly pyrrolidone (PVPP, MW 40,000) was added to the ground powder, mixed well and transferred to a centrifuge tube containing 15 ml of extraction buffer (1 M Tris (pH 8.0), 0.5 M EDTA, 5 M NaCl, 10% sodium dodecyl sulphate and 0.2 M β-mercaptoethanol). The mixture was incubated at 65°C for 1 h with intermittent mixing. To this, same volume of chloroform: isoamyl alcohol (24:1) were added and homogenized on inverting the tubes gently. The mixture was centrifuged at 8,000 rpm for 15 min. Later the supernatant was transferred into equal volume of ice-cold isopropanol. The DNA spool was collected in 2 ml microtube and washed with 70% ethanol. Then put for air-dried and re-suspended in 200 µl Tris EDTA (TE) buffer and incubated in 25 ng/µl of RNase at 37°C for 3 h. Equal volume of chloroform: iso-amyl alcohol was added, mixed well and centrifuged at 8,000 rpm for 15 min. The supernatant was transferred to equal volume of ice-cold isopropanol and DNA. Precipitate was washed with 70% ethanol, air-dried and resuspended in 200 µl TE. To remove contaminant RNA, the sample was treated with 3 µl RNase and then incubated in water bath for 1 h at 37°C and after that equal volume of phenol: chloroform: isoamyl alcohol (24:1) and

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centrifuged at 8,000 rpm for 5 min. at 4°C. DNA was precipitated by chilled absolute alcohol and pelleted by centrifugation at 5,000 rpm for 8 min. The precipitated DNA was spooled, rinsed with 70% ethanol and dissolved in 0.5 ml of TE buffer for further analysis. DNA concentration was measured in a UV spectrophotometer and the intactness was checked in 0.8% agarose gel. Amplification reactions were carried out in 20 µl volume containing 3 µl of template DNA, 10 µl of master mix (Ampliqon), 3 µl of random primer (Operon Technologies, USA) and 4 µl sterile MilliQ water. Amplifications were performed in a thermocycler (G-storm) programmed for an initial denaturation at 94°C for 5 min., 40 cycles of 1 min. denaturation at 94°C, 1 min. annealing at 37°C and 1 min. extension at 72°C, a final extension of 10 min. at 72°C and a holding for 1 h at 10°C. The amplification products in RAPD analysis were separated by electrophoresis in 1.5% agarose gel containing ethidium bromide using 1X TBE buffer (pH 8.0). Separation was carried out by applying constant voltage @ 70 V/cm for 2 h. The sizes of amplified fragments were determined using standard (1 kb and 100 bp DNA ladder mix). Gel was photographed using a gel documentation system (UPV). Genotypes were compared with each other using their amplification profiles and bands of DNA fragments were scored as present (one) and absent (zero). Genetic similarity matrix was generated on the basis of coefficients (Perera *et al.*, 3). Cluster analysis of genotypes was carried out on similarity coefficients using the unweighted pair group method arithmetic average (UPGMA) using NTSYS-PC, version 1.80. The polymorphic information content (PIC) for each RAPD marker was then calculated (Roldan-Ruize *et al*., 6).

In this study, genetic variations of arecanut have been elucidated with help of RAPD markers. Out of the 30 RAPD primers, 11 were selected to detect polymorphism based on their reliability (Table 1) in pooled DNA among 10 arecanut accessions. The size of products ranging from 150 to 1400 bp, and every primer generated polymorphic bands. The PCR analysis of all accessions with 11 polymorphic random markers generated 499 scorable bands with 86% polymorphism (Table 1). Among RAPD markers, OPF-16 produced maximum number of bands (49) and followed by OPF-8 and OPH-35 (48). While, RAPD markers OPH-41 and OPF-9 generated minimum number of bands (39) in the genomic pool. Average number of bands from across in individual genotype was maximum in OPH-35 (9.6) followed by OPH-41 (8.67). The percentage of polymorphism ranged from 50 to 100. Primers OPH-8, OPH-35, OPP-46 and OPF-8 revealed 100% polymorphism, whereas OPF-1

Table 1. Sequence and amplified products exhibited by RAPD primers.

RAPD marker	Marker sequence (5' to 3')	PIC value	MI	Range of amplicon size (bp)	Total No. of bands	Av. No. of bands per genotype	Av. No. of bands across genotype	Amplified bands/ allele	Unamplified band(s)	Amplification (%)	Polymorphic band(s)	Polymorphism (%)
OPH-8	GTCCGTACTG	0.29	2.61	150-1000	47	4.7	6.71	9	1	90	9	100
OPH-35	CGATCGGGAA	0.6	6	380-800	48	4.8	9.6	10	0	100	10	100
OPH-41	ATTTGATCGC	0.17	1.19	300-1500	39	3.9	8.67	9	1	90	7	77.77
OPH-42	ACGCTGATCA	0.28	2.52	270-1000	45	4.5	7.5	10	0	100	9	90
OPP-46	GCAGTACTCC	0.29	2.9	300-1000	46	4.6	7.67	10	0	100	10	100
OPF-1	ACGGATCCTG	0.38	1.9	200-800	47	4.7	5.11	10	0	100	5	50
OPF-8	GGGATATCGG	0.3	2.7	250-1400	48	4.8	3.25	9	0	90	9	100
OPF-9	CCAAGCTTCC	0.35	1.75	250-850	39	3.9	3.14	8	2	80	5	62.5
OPF-10	GGAAGCTTGG	0.44	3.08	350-1050	45	4.5	4.1	8	2	80	7	87.5
OPF-15	CCAGTACTCC	0.29	2.61	450-1500	46	4.6	0.29	10	0	100	9	90
OPF-16	GGAGTACTGG	0.33	2.64	270-1080	49	4.9	0.33	9	1	90	8	88.9
Average								102	0.63	92.72	8	86.06

PIC = Polymorphic information content, MI = Marker index

showed 50% polymorphism. The PIC value reflected from each primer frequency, and it has differs from one locus to another. The PIC value was ranged from 0.17 to 0.60 with an average of 0.33 per primer. The highest PIC was obtained in OPH-35 (0.60), while lowest was found in OPH-4 (0.17).

The dendrogram showed genotypes grouped into two major clusters (Fig. 1). First cluster had only one wild accession and second cluster had two sub-clusters and it was further divided into two CAL 35 HB and DWARF, CAL 1HB and CAL 1SB were present in the second sub-cluster with 85% similarity. The second minor cluster contained five genotypes namely MSB, SSB, SHB, CSSB and MHB with genetic similarity (78%) at molecular level. It has been reported that maximum genetic distance matrix, 47% between cultivars Mohit Nagar Interse and Mohit Nagar and the minimum genetic distance (22%) was between the genotypes Maidan Local and Sree Mangala (5%).

The genetic relationships obtained by RAPD analysis are in accordance with previous results (Purushotham *et al*., 4; Rivera *et al*., 5). The dendrogram shows that huge variability exists between wild arecanut and cultivated varieties. Based on the dendrogram pattern, it may be concluded that the CARI-Sel-1 might be a natural cross between the Mangla and Samruddhi. The major clusters showed the genetic similarity with genotypes among the arecanut species at molecular level. This data provides the information on genetic diversity between the arecanut species. The 11 primers used in RAPD analysis showed polymorphism within arecanut accessions. This study illustrated the possibility of molecular marker application to assess the genetic relationship in germplasm conservation and varietal identification in arecanut.

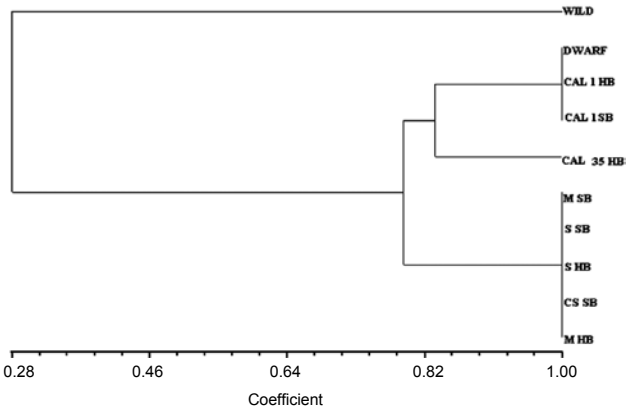

Fig. 1. Dendrogram of diversity analysis for arecanut varieties using RAPD primers.

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