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SHORT RESEARCH ARTICLE

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Microsatellite markers-based assessment of genetic diversity and population structure in arecanut (*Areca catechu* L.)

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Abstract

Genetic relationships existing amongst 60 arecanut accessions were assessed by employing nine polymorphic microsatellite primers. A total of 42 alleles were detected, with an average of 4.66 alleles per locus. The PIC values ranged from 0.50 to 0.78, with an average of 0.69. Expected heterozygosity (H_e) was highest for the exotic accessions (0.31), moderate for Konkan- I and II accessions, while the least heterozygosity was observed for the accessions from Maidhan tract (Karnataka) and Andaman and Nicobar Islands. Mean fixation index (F_{ST}) of 0.28 indicated a high level of population differentiation. The UPGMA cluster analysis grouped the accessions in to two major clusters- the indigenous Konkan accessions clustered separately in a distinct cluster along with Maidhan accessions. In the second major cluster, indigenous accessions from North East India formed a unique sub-cluster, while accessions from Andaman and Nicobar Islands were grouped with exotic accessions.

Keywords: Accessions, *Areca catechu*, Genetic diversity, Microsatellite/SSR markers.

Arecanut (*Areca catechu* L.) plays an important role in Asian culture, possessing significant economic, religious, cultural and medicinal importance. India is the largest producer of the arecanut in the world. Despite the outstanding agronomic and socio-economic significance of arecanut palm, attempts to improve knowledge about the biodiversity of arecanut palm have been limited mainly to the phenotypic description of cultivars and genetic diversity analysis using RAPD markers (Bharath et al. 2015). However, a deeper insight into the extent of genetic diversity of arecanut populations is becoming an urgent priority to guide the use of this diversity in improvement. DNA based markers can overcome the shortcomings of morphological and biochemical markers. With this perspective, the present investigation was carried out to understand diversity and population structure of this crop by using SSR markers.

Sixty arecanut accessions comprising 43 indigenous and 17 exotic, conserved in the National Arecanut Gene Bank at ICAR-Central Plantation Crops Research Institute, Regional Station, Vittal, Karnataka State, India, were utilized for the study (Table 1). DNA was extracted from young spindle leaf tissues (4 replications per accession) using the protocol of Rajesh et al. (2007). Spectrophotometric measurements (A260/A280 ratio) and agarose gel electrophoresis (0.8%) were utilized to assess the quality and quantity of the differentially extracted DNA. A set of nine SSR primer pairs, developed previously by Hu et al. (2009), were used for diversity analysis (Table 2). PCR reactions were conducted in volumes of 20 µl containing 35 ng genomic DNA, 0.2 µM each of forward and reverse primers (SIGMA), 50 µM of each dNTPs (Bangalore Genei, India), 1X buffer and 0.3 Units of *Taq* DNA polymerase (Bangalore Genei, India). PCR amplifications were performed in a thermal cycler (Eppendorf Mastercycler) with an initial denaturation at 94°C for 5 min followed by 30 cycles of denaturation for 1 min at 94°C, 2 min at the different annealing temperatures standardized for the individual SSR locus (Table 2) and extension for 2 min at 72°C with a final extension for 5 min at 72°C. After amplification, a volume of 3µL of loading dye was added to each amplified product.

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Table 1. Sixty arecanut accessions which are used in the current study

S.No.	Accessions	Country of collection/Geographic provinces/state of collection
I.	**Exotic Accessions**	
1.	Fiji-I	Fiji
2.	Mangala	China
3.	Sri Lanka-I	Sri Lanka
4.	Indonesia-IV	Indonesia
5.	Indonesia-VI	Indonesia
6.	Saigon-I	Vietnam
7.	Saigon-II	Vietnam
8.	Saigon-III	Vietnam
9.	Sri Lanka-II	Sri Lanka
10.	Singapore	Singapore
11.	Br.Sol.Islands-I	British Solomon Islands
12.	Br.Sol.Islands-II	British Solomon Islands
13.	Br.Sol.Islands-III	British Solomon Islands
14.	Fiji-II	Fiji
15.	Saigon-V	Vietnam
16.	Saigon-VI	Vietnam
17.	Saigon-VII	Vietnam
II.	**Indigenous Accessions**	
A.	**Andaman and Nicobar Islands**	
18.	Andaman & Nicobar Islands-I	Andaman and Nicobar Islands
19.	Andaman & Nicobar Islands-II	Andaman and Nicobar Islands
20.	Andaman & Nicobar Islands-III	Andaman and Nicobar Islands
21.	Andaman & Nicobar Islands-IV	Andaman and Nicobar Islands
22.	Andaman & Nicobar Islands-V	Andaman and Nicobar Islands
23.	Andaman & Nicobar Islands-VI	Andaman and Nicobar Islands
B.	**Konkan-I**	
24.	Asud	India (Maharashtra)
25.	Varand	India (Maharashtra)
26.	Murud	India (Maharashtra)
27.	Diveagar-I	India (Maharashtra)
28.	Diveagar-II	India (Maharashtra)
29.	Shriwardhana-B	India (Maharashtra)
30.	Shriwardhana-M	India (Maharashtra)
31.	Karle	India (Maharashtra)
32.	M.Raigad	India (Maharashtra)
C.	**Maidhan**	
33.	Sagar	India (Karnataka)
34.	Hirehalli Tall (H. Tall)	India (Karnataka)
35.	Hirehalli Dwarf (H. Dwarf)	India (Karnataka)
36.	S.K. Local	India (Karnataka)
37.	Wayanad	India (Kerala)
D.	**Konkan-II**	
38.	Vellinggauthan	India (Goa)
39.	Curti	India (Goa)
40.	Ponda-SR	India (Goa)
41.	Khandolla	India (Goa)
42.	Tamsule	India (Goa)
43.	Banstari	India (Goa)
44.	Keri-B	India (Goa)
45.	Talkatta-1	India (Maharashtra)
46.	Talkatta-2	India (Maharashtra)
47.	Vengurla	India (Maharashtra)
48.	Daboli	India (Maharashtra)
49.	Ratnagari	India (Maharashtra)
E.	**North East**	
50.	Badarpur-I	India (Assam)
51.	Badarpur-II	India (Assam)
52.	Badarpur-III	India (Assam)
53.	Hylakandi	India (Assam)
54.	Cachhar	India (Assam)
55.	Dauki Hills-1	India (Meghalaya)
56.	Dauki Hills-II	India (Meghalaya)
57.	Mowlong-I	India (Meghalaya)
58.	Mowlong-II	India (Meghalaya)
59.	Jawai-I	India (Meghalaya)
60.	Jawai-II	India (Meghalaya)

The amplification products were subjected to electrophoresis and resolved in a 3% high-resolution agarose gel using 1X TBE buffer and the gel was run at 80V for 4-5 h in an electrophoresis unit. The ethidium bromide-stained gels were visualized and photographed using a gel documentation and analysis system (Gel Doc XR, Bio-Rad, CA, USA). Amplification reactions were repeated thrice for checking reproducibility.

The alleles were scored individually compared with a 100 bp molecular ladder (Bangalore Genei, India). Each band generated by SSR primers was considered as an independent locus. Clearly resolved, unambiguous bands were scored visually for their presence or absence. The scores were obtained in a matrix with '1' and '0', which indicate the presence and absence of bands respectively in each sample. The percentage polymorphism was calculated for each primer based on the number of polymorphic bands.

Table 2. Diversity statistics for the nine SSR markers studied

S.No.	Locus	Annealing temperature (°C)	Allele size (bp)	No. of alleles	Effective no. of alleles (Ne)	Shannon's information index (I)	PIC
1.	AC01	50	241–256	6	4.41	1.58	0.77
2.	AC06	50	245–251	2	2.10	0.76	0.50
3.	AC07	50	294–304	6	4.19	1.54	0.76
4.	AC08	50	294–304	5	2.40	1.12	0.58
5.	AC14	50	259–275	4	2.47	0.99	0.59
6.	AC23	50	221–246	5	3.51	1.39	0.71
7.	AC28	50	210–245	4	4.49	1.61	0.78
8.	AC29	55	173–196	5	4.13	1.46	0.75
9.	AC30	55	171–217	5	3.82	1.36	0.73
	Mean	-	-	**4.67**	**3.50**	**1.31**	**0.69**

Table 3. Allele polymorphism (AP), observed heterozygosity (Ho), expected heterozygosity (He) and fixation index (f) in arecanut accessions

S.No.	Accessions	AP	Ho	He	f
1.	Konkan-I	2	0.00	0.21	1.00
2.	Konkan-II	2	0.16	0.28	0.43
3.	Maidhan	0	0.0	0.0	0.0
4.	North East	0	0.0	0.0	0.0
5.	Andaman and Nicobar Islands	0	0.0	0.0	0.0
6.	Exotic	2	0.0	0.31	1.00
	Mean	**1.0**	**0.03**	**0.14**	**0.80**

The average Polymorphism Information Content (PIC) was calculated by applying the formula given by Powell et al. (1996). The observed number of alleles, effective number of alleles (Ne), Shannon's Information Index (I) and F-Statistics were worked out for nine microsatellite loci using the software Population Genetic Analysis (POPGENE) version 1.31. The expected and observed heterozygosity across the 60 arecanut accessions were worked out using the software Genetic Data Analysis (GDA).

The binary data score was used to construct a dendrogram. The genetic associations between the accessions were evaluated by calculating the Dice similarity coefficient (Dice 1945) for pair-wise comparisons based on the proportions of shared bands produced by the primers. A similarity matrix was generated using the NTSYS-PC software, version 2.0. The similarity coefficients were used for cluster analysis, and dendrogram was constructed by the Unweighted Pair-Group Method with Arithmetic Average (UPGMA).

A total of 42 alleles were detected with most of the markers revealing four alleles or more. The number of alleles at each locus varied from 2 (AC06) to 6 (AC01 and AC07), with a mean of 4.67 alleles per locus. The effective number of alleles per locus (Ne) ranged from 2.10 (AC06) to 4.49 (AC28), with a mean of 3.50. Shannon's Information Index ranged from 0.76 (AC06) to 1.61 (AC28) with a mean of 1.31. The PIC value, which is a measure of polymorphism for a marker locus, varied from 0.50 (AC06) to 0.78 (AC28) among the nine microsatellite loci, the average being 0.69 (Table 2).

Among the accessions of different regions, expected heterozygosity was highest for the exotic populations (0.31), followed by indigenous collection Konkan-II (0.28) and Konkan-I (0.21) (Table 3). The least heterozygosity (0.0) was observed for the Maidhan, North East, Andaman and Nicobar Island group. The observed heterozygosity (*Ho*) for all the accessions was less than expected (*He*), indicating a tendency towards inbreeding within the population. The fixation index (*f*) ranged from 0.0 to 1.00 with a mean of 0.80 (Table 3).

The dendrogram constructed using UPGMA clustering revealed that the 60 accessions formed the two major clusters. Cluster 1 contained 26 accessions from Maidhan, Konkan I and Konkan II regions. There were two sub-clusters within Cluster 2, comprising of exotic (23 accessions) and indigenous accessions in one sub-cluster and accessions from Northeast India (11 accessions) in the second sub-cluster. The percentage of similarity coefficient varied from 0.31 to 0.96 between the accessions, indicating a moderately high level of genetic diversity among the accessions studied. The groups showed 31% similarity based on Dice's coefficient. Maximum similarity (0.96) was seen between Saigon-6 and Saigon-7 and between Daukihills-1 and Jawai-1. Minimum similarity (0.10) was seen between Sri Lanka-I and Varand, Mowlong II and Asud, British Solomon Island-II and Hirehalli Dwarf, British Solomon Island-III and Sagar, British Solomon Island-III and Hirehalli Tall, Badharpur-II and Sagar. These accessions could be used for hybridization studies for obtaining highly heterotic progenies.

Most of the exotic accessions collected from East Indies group clustered in a single group; this group contained the highest biodiversity indicating the possible center of origin of this crop. The grouping of indigenous accessions from North East and Andaman and Nicobar Islands with exotic accessions could be explained by a common genetic origin of arecanut in South East Asian region, and their subsequent

movement and domestication in the Indian peninsula via Andaman and Nicobar Islands and North East India, possibly via human interventions, as reported earlier by Bavappa et al. (1982).

The result of the study thus provides the first comprehensive information on the genetic variability/ relationship and phylogeny among arecanut accessions from diverse geographical regions using SSR markers. This study will form the basis of further studies on germplasm characterization and in arecanut breeding programmes (*e.g.* selection of possible parents, genotype fingerprinting, varietal identification, phylogenetic analysis and marker assisted selection).

Authors' contribution

Conceptualization of research (KSA, NRN); Designing of the experiments (NRN, MKR); Contribution of experimental materials (KSA, NRN); Execution of field/lab experiments and data collection (BGB, NRN, MKR); Analysis of data and interpretation (BGB, NRN, MKR); Preparation of manuscript (BGB, NRN, MKR, KSA).

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