Genetic Diversity Revealed Among Rattan Genotypes from Andaman and Nicobar Islands Based on RAPD and ISSR Markers

Abstract

Southeast Asia hosts a great diversity of different rattan genotypes. There are 5 genera and 60 different species of rattan in India. The natural reserves of this species have come under the threat of genetic erosion due to overexploitation in Andaman and Nicobar Islands. This investigation was focused at characterizing 12 rattan genotypes of the genera Calamus, Korthalsia and Daemonorops which yield rattans of commercial importance, based on RAPD and ISSR fingerprints. PCR amplifications with 8 RAPD primers gave an average of 7.37 selected markers/primers, with a maximum of 10 (OPA-4) and minimum of 6 (OPE-02 & OPE-06). Percentage of polymorphic bands ranged from 50 – 70% in RAPD primers. Among 59 selected bands 36 (61.0) were polymorphic. The amplification with seven ISSR primers generated 53 bands and 30(56.60 %) were polymorphic. The highest polymorphism was observed in OPA -7 (75%) in RAPD and IS16 (71.45%) in ISSR. RAPD primers recorded more polymorphism as compare to ISSR primers. The overall average polymorphism of 12 accessions using 15 primers was 58.92 %. Unique fingerprints for 10 Calamus, 1 Korthalsia and 1 Daemonorops genotypes were detected. The outcomes presented in this paper demonstrated the utility of RAPD and ISSR markers in elucidating patterns of genetic variation among genotypes of the three main rattan genera of Andaman and Nicobar Islands and in identifying individual genotypes, which may serve as potential sources of unique genetic material for genetic improvement and conservation.

Keywords: Calamus; UPGMA; cluster analysis; Finger printing; Phylogeny; Andaman and Nicobar

Research Article

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Abbreviations: RAPD-Random Amplified Polymorphic DNA, ISSR-Inter Simple Sequence Repeat, RFLP-Restriction Fragment Length Polymorphism, AFLP-Amplified Fragment Length Polymorphism, SSR-Single Sequence Repeats, SSCP-Single Strand Conformation Polymorphism, GIS- Geographical Information System

Introduction

The oriental spiny, climbing species of palms are referred to as 'rattans' and often are regarded as 'green gold' for their unique characteristics including strength, durability and flexibility. Rattans supply a basic raw material for the cane industry and are well known for their medicinal and traditional uses in basketry and bridge making. There is extensive global demand for both raw and processed canes worldwide, more than 700 million people reportedly trade or use rattan [1]. The long-term survival and evolution of every species depends on the available diversity in its present habitat. Therefore, an understanding of the current diversity status of forest genetic resources is a prime step for developing efficient forest conservation programme and breeding strategies to cope up with the current scenario [2]. The genetic diversity is a fundamental component of biodiversity and is closely related to geographic distribution of genotypes that constitutes subspecies, races or ecotypes. The DNA based diversity detected by molecular markers is usually defined as ''genetic variation'' to differentiate it from the phenotypic variation evolved due to the adaptive potential of populations. The advent of molecular marker techniques, bioinformatics and the use of geographical information system (GIS) could help to develop better methods to survey, sample and assess the genetic diversity [3]. Molecular markers such as restriction fragment length polymorphisms (RFLPs), random amplified polymorphic DNAs (RAPDs), Inter simple sequence repeat (ISSR), amplified fragment length polymorphism (AFLP), single sequence repeats (SSR) and single strand conformation polymorphism (SSCP) are widely applied to reveal genetic diversity, establish varietal or species identification and to understand phylogenetic relationships and genetic mapping.

Rattans (Canes) are spiny climbing palms belonging to the subfamily calamoideae of the family Arecaceae (Palmae). They comprise around 600 species belonging to 13 genera [4] which are concentrated solely in the old world tropics. They are one of the main non-wood forest produce, as a raw material for the furniture industry, supporting livelihood of many forest dwelling communities. It is estimated that more than half a million people are directly employed in harvesting and processing of rattans in the rural areas of South East Asia. INBAR (2012) [5] reported that the international trade in bamboo and rattan amounted to USD 1.9 billion. In India, rattans comprise about 60 species under four genera, viz. Calamus, Daemonorops, Korthalsia and

Plectocomia distributed in three major phyto-geographical areas viz. Peninsular India, Eastern Himalayas and Andaman and Nicobar Islands [6]. Among the genera as well as species level these rattans have larger phenotypic variation. In India alone rattan industries account for 2, 00,000 employees [7]. Rattan contributes 25-35% of the total household income of tribal communities in North Eastern India and Andaman and Nicobar Islands. Rattan furniture is much valued in many countries, and its export from producer countries have steadily increased, over the years, into a multibillion dollar business. Increase in demand of the raw material has resulted in over-exploitation of the natural resource. This, along with the change of land-use pattern, has led to erosion of the biodiveristy of rattans.

The dioecious nature of the Andaman and Nicobar species and indiscriminate harvesting often obstruct seed propagation, and regeneration through suckers and rhizomes is usually very slow. As a result, many species from these reserves have come under threat. The threat to the genetic diversity present in wild populations and the need for preservation of these genetic resources make it imperative for an assessment of the genetic diversity of the rattans of these Islands. Hence, conservation, protection and utilization of the genetic resources of rattans require urgent action. Conservation measures require an understanding of the genetic system and spatial patterns of genetic variation. Several methods are currently available to assess genetic variation in plant species. None of these methods gives a complete picture of the complex structure of genetic variation in the wild plant species. Hence, it is suggested that multiple methods should be adopted simultaneously to investigate the pattern of genetic variation in rattan species [8]. Both genetic markers and quantitative traits are expected to provide valuable information for the genetic characterization and management of rattan species. Isozyme genetic markers have already been identified for several important rattan species in Malaysia and Thailand [9]. However, very little data is available on the genetic diversity of rattans in India. Hence, as a preliminary step, a study on the genetic diversity and conservation of existing species of rattans in Andaman and Nicobar Islands was initiated. In this study, RAPD and ISSR markers were used to determine genetic relationships among twelve rattan genotypes, to evaluate the level of diversity present among the genotypes belonging to three different genera Calamus, Korthalsia and Daemonorops, which yield rattans of commercial importance. The specific objective of this study was to characterize rattan genotypes with RAPD and ISSR markers for future biodiversity conservation strategies and for genetic improvement.

Material and Methods

Plant material and DNA isolation

Rhizomes and seedlings of twelve different rattan genotypes, ten belonging to the genus Calamus and one each of Daemonorops and Korthalsia, were collected from different locations of Andaman and Nicobar Islands, India (Table 1). Identification to genus and species was done following Basu [10]. Total genomic DNA was isolated from the newly sprouted leaves (1gm) and ground using liquid nitrogen in pre chilled mortar and pestle to a fine powder using CTAB method [11]. It was then transferred to pre-warmed extraction buffer and incubated at 65°C for 1 h. An equal amount of chloroform: isoamyl alcohol (24:1) was added, mixed well by gentle inversion and centrifuged. The supernatant was transferred to a fresh tube and DNA was precipitated by adding ¾ volume of isopropanol. After centrifugation, the pellet was washed in 70% ethanol, dried and dissolved in 1X TE buffer. RNA was removed by RNase treatment. Integrity and quantity of extracted DNA were estimated spectrophotometrically and visually verified on 1% agarose gel.

Table 1: Geographical locations of various accessions cane species in A&N Islands.

Sl. No	Accession Name	Place and District	Altitude (MSL)	Latitude	Longitude
1	*Calamus andamanicus*	Garacharma, South Andaman	28m	N 11°60′19.1″	E 92°58′ 43.1″
2	*Calamus baratangensis*	Baratang, South Andaman	25m	N 11° 64′95.3″	E 92° 73′ 15.5″
3	*Calamus basui*	8 Km, Little Andaman	69m	N 12° 56′80.2″	E 92° 81′ 29.1″
4	*Calamus longisetum*	Mount Harriet, South Andaman	145m	N 11°65′61.4″	E 92°73′ 62.4″
5	*Calamus pahestris*	Mount Harriet, South Andaman	125m	N 11°65′61.4″	E 92°73′ 62.4″
6	*Calamus viminalis*	Chidiyatappu, South Andaman	74m	N 11° 59 ′83.5″	E 92°71 ′43.5″
7	*Daemonovps kurzianns*	Mount Harriet, South Andaman	125m	N 11°65′61.4″	E 92°73′ 62.4″
8	*Korthalsia laciniosa*	Mount Harriet, South Andaman	125m	N 11°65′61.4″	E 92° 73′ 62.4″
9	*Calamus dilaceratus*	Campbell Bay, Nicobar	73m	N 7°55.8″	E 93° 90′ 88.9″
10	*Calamus nicobaricus*	Campbell Bay, Nicobar	88m	N 7°03 ′86.8″	E 93° 86′ 23.7″
11	*Calamus pseudorivalis*	Campbell Bay, Nicobar	56m	N 7° 59.5″	E 93° 90′ 82.9″
12	*Calamus semierectus*	Campbell Bay, Nicobar	77m	N 7°03 ′89.8″	E 93° 82′44.7″

DNA amplification conditions and gel electrophoresis

Eight primers were selected based on more number of polymorphic bands out of 20 RAPD primers. DNA amplification was carried out in 10µl reaction volume containing 25ng genomic DNA, 1x PCR- Colored Mix (*Shrimpex* Genomics *India,* Pvt Ltd. Chennai) and 20ng of primer (Shrimpex, Chennai)**,** in an Applied Bio systems eppendorf nexus gradient thermal cycler. It was programmed to fulfill 40 cycles (for RAPD analysis) or 35 cycles (for ISSR analysis) after an initial denaturaton cycles for 2min denaturation at 94°C.Each cycle consisted of a denaturation step 1 min at 94 °C , an annealing step for1 min at 32 °C (for RAPD analysis) or 35 °C (for ISSR analysis) and an extension step at 72 °C for2 min, followed by extension cycle 5 min at 72 °C .The amplified products of each were size fractioned by electrophoresis on a 1.5% agarose gel with 0.1% ethidium bromide in 1X TAE buffer and visualized on UV transilluminator and photographed. Experiment with each primer was done three times and those primers which gave reproducible fingerprints were considered for data analysis.

RAPD and ISSR data analysis

The banding patterns obtained from RAPD and ISSR were scored as present (1) or absent (0), each of which was treated as independent characters regardless of its intensity. Pair-wise similarity matrices were generated by Jaccard's coefficient of similarity [12] byusing theSIMQUAL format of NTSYS-pc [13]. The similarity matrix wassubjected to cluster analysis by unweighted pair group method for arithmetic mean (UPGMA) and a dendrogram was generated using the programme.

Results and Discussion

Information on population genetic structure and the diversity of populations of a threatened species are essential for developing a conservation and utilization strategy. Genetic variability is the basis for creating new types and adaptation and a species without enough genetic diversity is thought to be unable to cope with changing climate [14].The amplification products produced from RAPD and ISSR primers are listed in Table.2 in terms of the percentage of PCR products appeared in the genotypes studied. Total of 484 DNA fragments were amplified in 8 primers. There were 36 polymorphic bands, out of 59 amplified bands and the average percentage of polymorphism between the 12 genotypes of 8 primers was 61 and average number of polymorphic bands per primer was 4.5. OPA-7 primer gave maximum polymorphic band of 75%. Total of 412 DNA fragments were amplified in 7 primers. There were 30 polymorphic bands, out of 53 amplified bands and the average percentage of polymorphism between the 12 genotypes of 7 primers was 56.60 and average numbers of polymorphic bands per primer was 4.28. IS2 primer gave maximum polymorphic band of 72.45% (Table 3 & Figure 1).

Table 2: RAPD and ISSR primers, total numbers of bands amplified, numbers of polymorphic bands, proportion of polymorphic bands and PIC value.

Primer Name	Primer Sequences 5' to 3'	Total Numbers of Bands	Polymorphic Bands	Percentage of Polymorphic Bands (%)	PIC
RAPD					
OPAJ-14	CACCCGGATG	7	4	57.14	0.672
OPA-4	AATCGGGCTG	10	7	70.0	0.590
OPA-7	GAAACGGGTG	8	6	75.0	0.365
OPA-11	CAATCGCCGT	7	4	57.14	0.254
OPE-8	ACGCAACC	7	4	57.14	0.198
OPX-20	CCCAGCTAGA	8	4	50.00	0.137
OPE-02	GGT GCG GGAA	6	4	66.66	0.224
OPE-06	GGGAATTCGG	6	3	50.00	0.209
Total		59	36	483.08	2.649
Mean		7.375	4.5	60.385	0.331
ISSR					
IS-12	GTGTGTGTGTGTGTTG	8	5	62.5	0.281
IS-16	GGATGGGATGGAT	7	5	71.42	0.332
IS-3	AGCACGAGCAGCAGCGT	8	5	62.5	0.294
IS-15	GTGTGTGTGTGTGTAT	8	4	50	0.172
IS-2	AGCACGAGCAGCAGCGG	8	5	62.5	0.229
IS-4	GGAGAGGAGAGGAGA	6	3	50	0.188
IS-13	GTGTGTGTGTGTGTCA	8	3	37.5	0.157
Total		53	30	396.42	1.653
Mean		7.57	4.285	56.63	0.236

Table 3: A comparative list of showing different markers details (RAPD, ISSR and RAPD+ISSR) obtained for 12 genotypes of Cane species.

Primers	RAPD	ISSR	RADP + ISSR
Number of primers used	8	7	15
Total number of polymorphic bands	36	30	66
Total number of monomorphic bands	23	23	46
Total number of bands	59	53	112
Total number of bands amplified	484	412	896
Percentage polymorphism (%)	61.0	56.60	58.92
Average number of bands/primer	7.37	7.57	7.46
Average number of polymorphic bands/primer	4.5	4.28	4.4

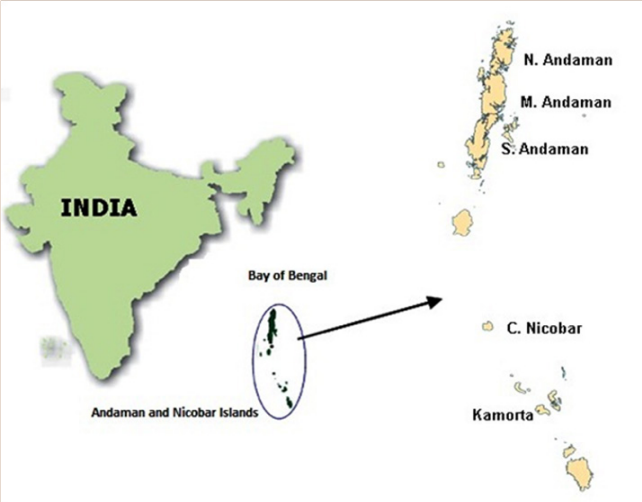

Figure 1: Map showing the Andaman and Nicobar Islands physical settings.

Cluster analysis of RAPD based on Jaccard's similarity coefficient using UPGMA identified two main groups (Figure 2). Group I consists of seven accessions with two different genera of 1, 11, 10, 2, 4, 7and 3. The first subgroup was formed five accessions and second subgroup was formed by three accessions of 2 and 3.Group II was formed by 5, 6, 8, 12 and11. The 87% similarity between the species of *Calamus baratangensis* and *Calamus basui*. *Calamus species* is 70% similarity with *Daemonorp skurzianuus* and 71.5% similarity with *Korthalsia laciniosa. Daemonorps kurzianuus* was 60% similarity with *Korthalsia laciniosa.*

The clustering pattern of 12 genotypesof ISSR markersbased on UPGMA analysis with Jaccard's similarity coefficient using from 0.60 to0.7.ISSR dendrogram obtained two main Groups (Figures 3&4). Group I consist of seven accessions with two different genus species 1, 2, 4, 6, 3, 10 and 12. The group I was subdivided into two subgroups and the first subgroup was formed five accessions and second subgroup was formed by three accessions. Group II was formed by 5, 7, 9, 11 and 8. Similarity between the species of *Calamus dilaceratha* and *Calamusp seudorivalis* was 79%. *Calamus species* was 68% similarity with *Daemonorps kurzianuus* and 66% similarity with *Korthalsia laciniosa. Daemonorps kurzianuus* was 60 % similarity with *Korthalsia laciniosa.* Pai [15] observed that a high amount of genetic variation and divergence amongpopulations and moderate variation within populations of *A. calamus* in South Eastern Ohio, USA use ISSR markers. Similar results were obtained in ISSR studies of populations of *Ceriopstagal* in Thailand and China [16].

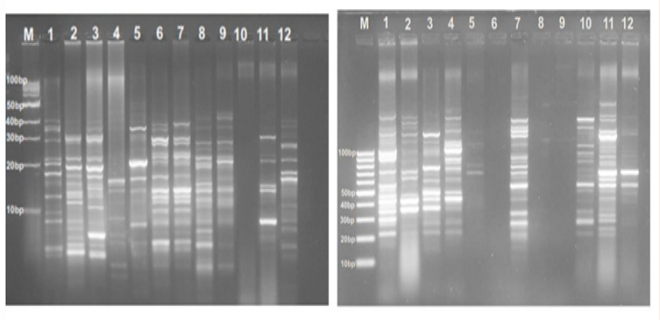

Figure 2: Gel image of RAPD (OPA-4primer) in left and ISSR (IS-2 primer) in right.

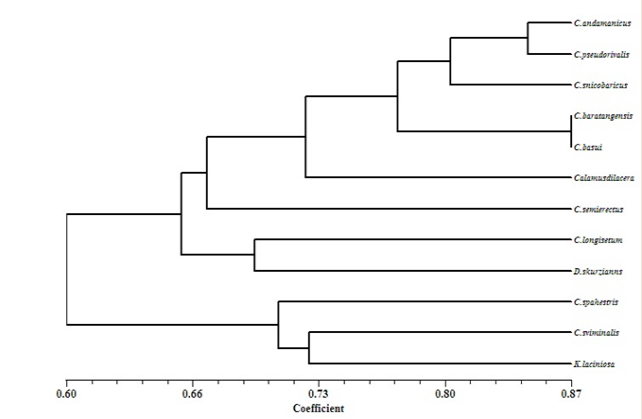

Figure 3: Dendrogram of RAPD markers.

Analysis of RAPD + ISSR based on Jaccard's similarity coefficient using UPGMA identified two main groups. The Jaccard's similarity coefficient of UPGMA analysis of twelve accessions from 0.62 to 0.80. Group I consist of seven accessions of same genus species 1, 2, 4, 6, 3 10 and 12. Group II included five accessions with different genera.The group I was subdivided into two subgroups

at the genetic distance of 0.68. The first subgroup was formed five accessions and second subgroup was formed by three accessions.

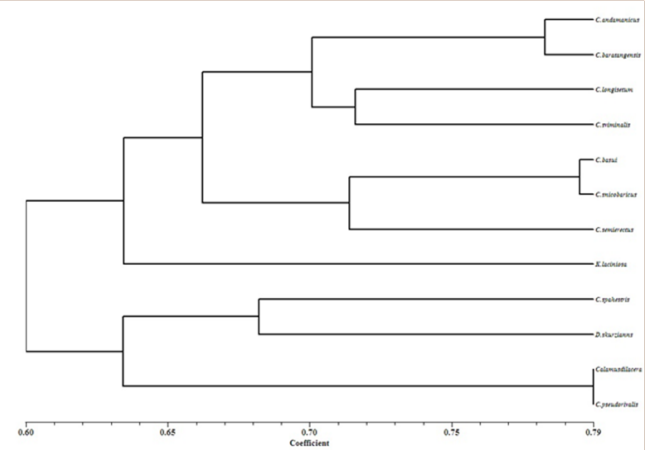

Figure 4: Dendrogram of ISSR markers.

Group II was formed by 5, 7, 9, 11 and 8.The 87 % similarity between the genotype of *Calamus baratangensis* and *Calamus basui. Daemonorps kurzianuus* and *Korthalsia laciniosa* were 64 5 similarity with each other. *Daemonorps kurzianuus* was 68 % similarity with genus *calamus. Korthalsia laciniosa* was 64% similarity with genus *Calamus.* RAPD and ISSR markers are two fingerprinting approaches used widely to identify and determine relationships at the cultivar levels and species [17]. There were previous reports regarding the use of ISSR and RAPD for comparative analysis for plants [18]. The present study revealed that each method is useful and enlightening for evaluating genetic diversity.

Conclusion

Based on the present study, percentage of polymorphism RAPD makers was more efficient than the ISSR markers. But not more variation between the RAPD and ISSR markers.RAPD primers showed 61.0% of polymorphism whereas ISSR primers showed only 56.60% of polymorphism. Similarly Sreekumar & Renuka [19] studied that higher genetic diversity in *Calamus thwaitesii* using RAPD markers showed that genetic diversity was distributed (70.79%) within populations and only (29.21%) among populations; Priyanka Giri et al. [20] reported that genetic diversity of six populations of *Acorus calamus* L. using randomly amplified polymorphic DNA (RAPD) markers; Ja-Hyun Lee et al. [21] reported that Genetic Diversity in Genus *Acorus* using seven, 43.33% of monomrphic and average 7.57 bands per primer. Maximum was observed in IS 16 RAPD Markers. Prabalee Sarmah et al. [22] revealed that a considerable degree of polymorphism (98.1%) was detected among the Calamus, Plectocomia and Daemonorops genotypes. In the present study revealed that ISSR marker showed 56.66 % of polymorphism similarly Abdul et al. [23] reported that lower Genetic diversity of *A. calamus* populations in South and North East India using inter simple sequence repeat (ISSR) markers showed 53(51.5%) were polymorphic,50 (48.5%) were monomorphic and average 7.35 bands per primer was observed. Maximum number of polymorphic bands was observed in UBCS. Similarly Avani et al. [24] revealed that 33.7% of polymorphism in RAPD markers and 63.7% of polymorphism in ISSR markers in endangered Indian sweet flag (*Acorus calamus* L.). Low genetic diversity may decrease the potential of plant populations to survive in a changing environment. There is a crucial need to take active measures to protect these rattans against further loss of genetic diversity. From this study it can be concluded that the utility of RAPD and ISSR markers in elucidating patterns of genetic variation among genotypes of the three main rattan genera of Andaman and Nicobar Islands and in identifying individual genotypes, which may serve as potential sources of unique genetic material for genetic improvement and conservation.

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Conflict of Interest

There is no financial or any conflict of interest exists.

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