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Genetic diversity of *Pterocarpus dalbergioides* (Andaman Padauk) accessions of Andaman and Nicobar Islands

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Abstract

Forty three phenotypically superior accessions of *Pterocarpus dalbergioides*, identified from different locations of the Andaman and Nicobar Islands, were subjected to genetic diversity analysis using 26 polymerase chain reaction (PCR) markers [12 Random Amplified Polymorphic DNAs (RAPDs) and 14 Inter Simple Sequence Repeats (ISSRs)]. The percentage of polymorphic bands detected by ISSR and RAPD was 37.04 and 37.62 %, respectively. RAPD primers OPN 04 and OPN 08 gave 53% & 63 % polymorphism respectively. Among ISSR primers, (GACA)8G gave 100% polymorphism. Clustering pattern of accessions of *Pterocarpus dalbergioides* remained more or less similar in RAPD and combined data of RAPD and ISSR. Present study confirmed fair extent of genetic variation within the species even though it is exposed to a wide range of environmental conditions across Andaman and Nicobar Islands, India.

Key words: *Andaman and Nicobar Islands, Pterocarpus dalbergioides Cluster analysis, dendrogram, genetic variability, marker and PCR.*

Introduction

Andaman padauk (*Pterocarpus dalbergioides* Roxb.) belonging to the family Fabaceae is a very important endemic species of Andaman group of Islands. With tall stature of about 80-127 feet and buttress roots, it is designated as the State Tree of Andaman and Nicobar Islands. Its speciality ornamental wood has wide suitability to make antique furniture (Jaisankar *et al*., 2023). The tree is highly valued in the isles for ornamental and decorative wood work and has emerged as the principal timber tree of the Andaman Islands triggered economic revolution in the isles (Rao, 2000). Over the years, the Padauk stand is considered severely affected due to continuous logging operations and poor regeneration status. It was widely accepted that a balance has to be made between utilization and its conservation.

Information about the genetic variations present within and between various plant populations and their structure and level can play a beneficial role in the efficient utilization of plants. Recent advances in molecular biology; PCR provides powerful techniques for characterizing and evaluating genetic diversity and population evolution among species. Molecular markers are nucleotide sequences which can be investigate through the polymorphism present between the nucleotide sequences of different individuals. During the last three decades, the world has witnessed a rapid increase in the knowledge about the plant genome sequences and the physiological and molecular role of various plant genes, which has revolutionized the molecular genetics and its efficiency in plant breeding programmers. DNA markers are used in characterizing genotypes and to measure genetic relationships more precisely than other markers (Soller and Beckmann, 1983). The PCR techniques has offered new markers systems for diagnose of genetic diversity in large number of studies (Saiki *et al*., 1988). Polymerase chain reaction (PCR) led to the development of two simple and a quick techniques called random amplified polymorphic DNAs (RAPDs) and inter simple sequence repeats (ISSRs) (Wu *et al*., 1994). RAPD is inexpensive and rapid method not requiring any information regarding the genome of the plants and has widely used to ascertain the genetic diversity in several plants (Deshwall *et al*., 2005).

The RAPDs and ISSRs are still very commonly used tools for diversity analysis in less investigated forest tree species. Further, both RAPD and ISSR markers are in

routine use in ecological, evolutionary, phylogenic and genetic diversity studies in plant sciences. DNA markers have proven to be valuable in crop breeding especially in the studies of genetic diversity and in cultivar identification and also useful for various applications in plant breeding. The DNA marker level genetic variability analysis of the endemic economically important Andaman Padauk is very meager and hence, this study was initiated with the objective of analyze the genetic diversity among 43 genotypes collected from different locations of Andaman and Nicobar Islands that could be useful in further identification of inter-specific hybrids, genetic improvement of the species marker assisted selection and genetic resources management in *Pterocarpus dalbergioides*.

Materials and Methods

Plant sample collection

Individual plus trees *Pterocarpus dalbergioides* were identified and collected the seeds for seedling production from 43 places of Andaman and Nicobar Islands. Fresh healthy leaf samples from young seedlings (Table 1) were used for the analysis.

Table 1: Collection sites and tree data different genotypes of *Pterocarpus dalbergioides* collected from different part of Andaman and Nicobar Islands.

Genotype No.	Survey site details			Altitude(m)	Tree Height(m)	Tree Girth(cm)
	Area name	Latitude	Longitude			
1.	Tugapur	12°50'19"N	92°50'59"E	4	22	275
2.	Gandhietty	12°18'28"N	92°47'16"E	7	26	235
3.	Nimbudera	12°43'10"N	92°53'02"E	22	22	178
4.	Karmatang1	12°51'26"N	92°55'49"E	7	24	340
5.	Nimbudera2	12°43'37"N	92°52'36"E	80	21	285
6.	Kadamtala1	12°20'00"N	92°47'04"E	49	26	390
7.	Baratang	12°10'71"N	92°77'46"E	7	26	515
8.	Jirkatang	11°50'20"N	92°39'14"E	15	24	320
9.	Karmatang2	12°51'23"N	92°55'27"E	8	24	320
10.	Danapur	12°54'85"N	92°53'54"E	4	23	384
11.	Adajig	12°15'30"N	92°48'05"E	36	34	198
12.	Lorojig	12°12'56"N	92°47'41"E	79	22	225
13.	Bakultala	12°30'18"N	92°51'45"E	7	22	148
14.	Yerratajetty	12°28'46"N	92°50'41"E	9	22	235
15.	Rangath1	12°30'11"N	92°53'45"E	87	21	198
16.	Baultala2	12°29'58"N	92°52'24"E	27	27	150
17.	Billigroung	12°40'06"N	92°52'53"E	12	24	190
18.	Kadamtala2	12°30'39"N	92°46'41"E	30	22	135
19.	Betapur	12°42'54"N	92°55'83"E	32	22	290
20.	Sipighat1	11°36'41"N	92°40'51"E	24	22	210
21.	Sipighat2	11°36'42"N	92°40'57"E	28	21	220
22.	Sipighat3	11°36'43"N	92°40'44"E	14	23	190
23.	Lamiyabay	13°12'19"N	93°01'22"E	32	26	480
24.	Laxmipur	13°18'21"N	92°57'03"E	103	21	320
25.	Saddlepeak	13°10'17"N	92°59'58"E	108	23	485
26.	Budhanalah	12°33'21"N	92°52'17"E	17	23	185
27.	Lapagram	13°14'29"N	93°00'47"E	39	21	210
28.	Kalpong	13°13'59"N	92°57'48"E	17	24	420
29.	Kishorinagar	13°09'56"N	92°52'26"E	12	23	385
30.	Pembrokebay	13°10'56"N	92°49'43"E	24	24	375

31.	Kalaphad28	13°09'55"N	92°49'33"E	22	21	195
32.	Mohanpur	13°12'36"N	92°53'26"E	105	23	280
33.	Prolobjig	12°23'44"N	92°53'19"E	16	24	480
34.	Longisland	12°23'34"N	92°56'06"E	73	23	390
35.	Sastrinagar	12°14'55"N	92°49'35"E	78	20	167
36.	Borniyal	12°28'44"N	92°49'21"E	25	24	420
37.	Kalighat	13°07'35"N	92°57'57"E	78	22	325
38.	Hutbay	10°38'37"N	92°30'42"E	78	22	195
39.	Katchal	7°57'18"N	93°21'53"E	108	23	275
40.	Rutland	11°26'29"N	92°38'25"E	157	23	210
41.	Sikhanallah	12°13'44"N	92°48'07"E	72	23	185
42.	Rangat2	12°30'50"N	92°53'09"E	117	24	325
43.	Baratang2	12°13'49"N	92°40'06"E	52	23	320

RAPD and ISSR analysis

Twelve ten-mer oligonucleotide RAPD primers of Operon series (Operon technologies, Alameda, CA, USA) and fourteen ISSR primers (Clonitec Inc., USA) were surveyed in genomic DNA of *Pterocarpus dalbergioides* samples. The PCR analysis for RAPD and ISSR was performed in thermal cycler (Bio-Rad Laboratories, Inc., CA, USA) in a final volume of 20 µL as described by Singh *et al.* (2010). The PCR reaction mixture contains 1 µL template DNA (30 ng/ µL), 2 µL of dNTP mix containing 100 µM of each of the four deoxynucleotide triphosphate, 2 µL of decanucleotide primer, 1.6 µL MgCl2, 2 µL Taq buffer (10 mM Tris–HCl pH 9.0, 50 mM KCl), 0.5 U Taq DNA polymerase (Bangalore Genei, Bangalore, India) and 10.9 µL millipore water (Heal Force Water Purification System, Sanghai Canrex Analytic Instruments Co. Ltd, Shangai, China). The PCR products from both RAPD and ISSR analysis were resolved on 1% agarose gel prepared in 1X TAE buffer containing 0.5 µg/ ml of the ethidium bromide (10 mg/ml). Amplified PCR product (5 µL) mixed with 6X bromophenol blue (2 µL) and electrophoresis was performed on at 100 V for 2.5 h. Gel photography was done with UVP MultiDoc-IT Digital Imaging System (UVP LCC, California). Amplicon size on agarose gel was established by comparing with reference bands of 100 bp DNA ladders.

Statistical analysis

The bands from PCR analysis of 43 collections were scored as '1' for presence and '0' for absence and created a binary data matrix. The matrix subjected to similarity analysis by Jaccard's coefficient and employed for clustering the germplasm by sequential hierarchical agglomerative non overlapping (SHAN) based un-weighted pair group method with arithmetic averages (UPGMA) method using NTSYSpc, version 2.02. The allele richness and the polymorphism information content (PIC) was calculated using formula: PICi = 2fi (1 - fi), where, PICi is the polymorphic information content of ith marker, fi is the frequency of the marker band present, (1 - fi) is the frequency of marker band absent. Percentage of expressivity, average number of bands by a marker, range of amplicon size and percent polymorphism were calculated using Microsoft EXCEL software. Principal coordinate analysis (PCA) of the similarity matrix was also used to estimate relationships among different genotypes of *Pterocarpus dalbergioides*.

Results and discussion

The PCR analysis of 12 RAPD and 14 ISSR primers with genomic DNA of 43 samples of *Pterocarpus dalbergioide*s shown in table 2 & 3. In total, 4281 amplicons were generated in 43 samples by 12 RAPDs and 2578 amplicons by 14 ISSRs of them polymorphic markers were 1543 and 970, respectively. The polymorphism of ISSR and RAPD markers showed 37.62% and 37.04% respectively (Table 4). However, it had wide variation for individual markers which ranged from 14.32% (OPA 10) to 63.10% (OPN 08) for RAPD and 19.69% (GTGC)4 to 100% (GACA)8G for ISSR markers.

Number of bands generated in 43 samples by an individual RAPD marker was ranged from 187 (OPN-08) to 464 (OPA-07) (Fig. 1) and with ISSR markers, it varied from 55 (GACA)8G to 350 (GGA)4 (Fig. 2). Amplicon size from RAPD and ISSR analysis was ranged from 0.15 to 3.0 kb. Individually, RAPD primer OPN03 generated maximum bands (17.0) while minimum by OPN 08 (1.0). Polymorphic bands from RAPD analysis ranged from 5.0 (OPA-10) to 7.0 (OPA-04). In ISSR analysis, number of amplicons by an individual primer ranged from 8.0 (AG4) to 12.0 (GACA4) of them, polymorphic bands ranged from 3.0 to 7.0.

Level of polymorphism at individual RAPD and ISSR markers ranged from 14.32 to 63.10% and 19.69 to 100%, respectively. Jaisankar *et al*., (2020) reported that the RAPD primers among *Pandanus* species showed 80.55% polymorphism. Similarly Panda *et al*. (2009) has reported RAPD 43.49% polymorphism between the male populations of *P. tectorius* in which the highest polymorphism was showed by the RAPD primer OPA-09 (91.1%). Genetic polymorphism was showed in *Cycas* species using RAPD at 98.1% (Radha *et al.,* 2015). Number of alleles varied greatly with all markers having average PIC value of 0.448–0.358 for both RAPD and ISSR primers. This is the first report on the use of a DNA based polymorphism assay to assess the level of variability in *Pterocarpus dalbergioides*

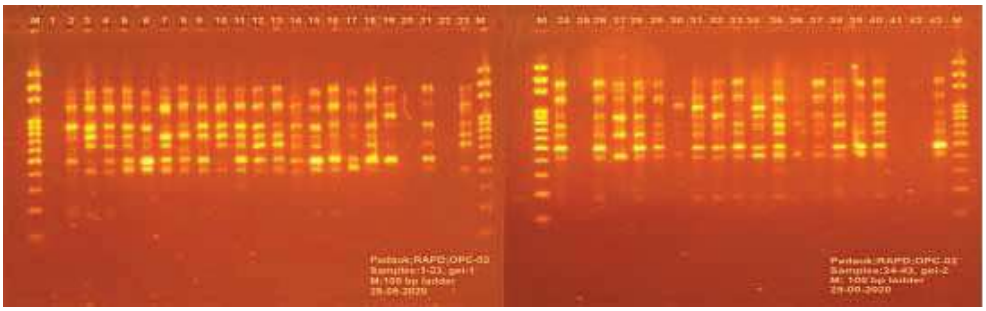

Fig.1. Amplification of padauk accessions with OPC- 02 RAPD primer

Table 2. Description of RAPD markers and their PCR analysis for *Pterocarpus dalbergioides*

Sl. No.	Primer	Sequence	No.of samples amplified	% of expressivity	Av.No. of bands across genotypes	Total No of polymorphic bands	% of polymorphism	Amplicon size(kb)	PIC
1.	OPA 04	AATCGGGCTG	38	88.37	26.64	119	46.85	0.20-1.4	0.477
2.	OPA 05	AGGGGTCTTG	37	86.04	29.00	78	16.81	0.20-1.5	0.493
3.	OPA 07	GAAACGGGTG	38	88.73	25.40	69	27.16	0.15-2.5	0.410
4.	OPA 10	GTGATCGCAG	38	88.37	26.06	56	14.32	0.15-3.0	0.490
5.	OPA 20	GTTGCGATCC	38	88.37	33.54	140	37.94	0.15-3.0	0.438
6.	OPC 02	GTGAGGGCA	36	83.72	31.69	196	47.52	0.15-3.0	0.427
7.	OPN 02	ACCAGGGGCA	36	83.72	24.20	150	41.32	0.15-3.0	0.420
8.	OPN 03	GGTACTCCCC	39	90.69	27.68	195	49.08	0.15-3.0	0.449
9.	OPN 04	GACCGACCCC	37	86.04	24.86	200	53.61	0.25-3.0	0.427
10.	OPN 05	ACTGAACGCC	37	86.04	29.18	114	35.51	0.25-3.0	0.428
11.	OPN 06	GAGACGCACA	38	88.37	22.06	108	32.62	0.15-3.0	0.413
12.	OPN 08	ACCTCAGCTC	37	86.04	23.37	118	63.10	0.25-3.0	0.359

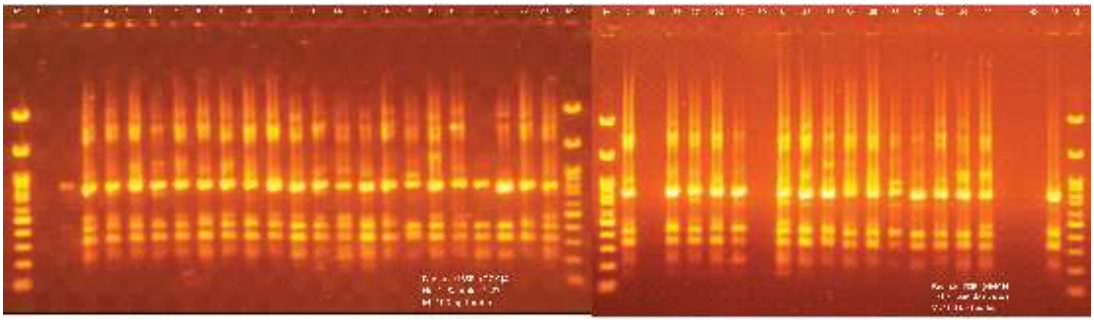

Fig. 2. Amplification of padauk accessions with (GGA)4 ISSR primer

Table 3 : Description of ISSR markers and their PCR analysis for *Pterocarpus dalbergioides*

Sl. No.	Sequence	No. of samples amplified	% of expressivity	Av. No. of bands across genotypes	Total No of polymorphic bands	% of polymorphism	Amplicon size(kb)	PIC
1.	(GA)8G	41	95.34	25.40	75	29.52	0.15-1.15	0.428
2.	(AG)8C	43	100.00	31.10	104	33.44	0.15-1.50	0.365
3.	(AG)10	43	100.00	37.42	67	25.57	0.25-0.85	0.482
4.	(AGG)6	43	100.00	30.40	101	33.22	0.20-2.00	0.446
5.	(GA)9T	43	100.00	28.3	167	73.56	0.25-2.00	0.317
6.	(GAC)5	41	95.34	27.57	86	44.55	0.25-1.15	0.347
7.	(GACA)8G	24	55.81	6.87	55	100.00	0.20-1.00	0.153
8.	(GACA)4	42	97.67	18.75	112	49.77	0.20-1.35	0.350
9.	(GTGC)4	41	95.34	24.75	39	19.69	0.20-1.50	0.394
10.	(GGA)4	38	88.37	29.16	112	32.00	0.15-3.00	0.403
11.	(GACAC)4	41	95.34	19.90	52	26.13	0.20-1.50	0.395
12.	(CT)8A	-	-	-	-	-	-	-
13.	(CT)8T	-	-	-	-	-	-	-
14.	(AG)8	-	-	-	-	-	-	-

Dendrogram analysis for *Pterocarpus dalbergioides* as obtained with RAPD markers

A dendrogram based on UPGMA analysis with RAPD data is shown in fig. 3 representation of the extensive polymorphism observed among three genotypes, as revealed by RAPD primers. Dendrogram based analysis grouped the 43 genotypes into two main clusters. Cluster I comprises with forty accessions and Cluster II comprises 3 accessions with distinct polymorphism with all forty accessions. Further, cluster I divided in two sub-clusters A and B with three and thirty- seven accessions showing more than 65% of similarity. Sub-cluster IB again split into six groups with 65% to 95% similarity. Cluster II also divided into two sub-clusters 2A and 2B with one and two accessions.

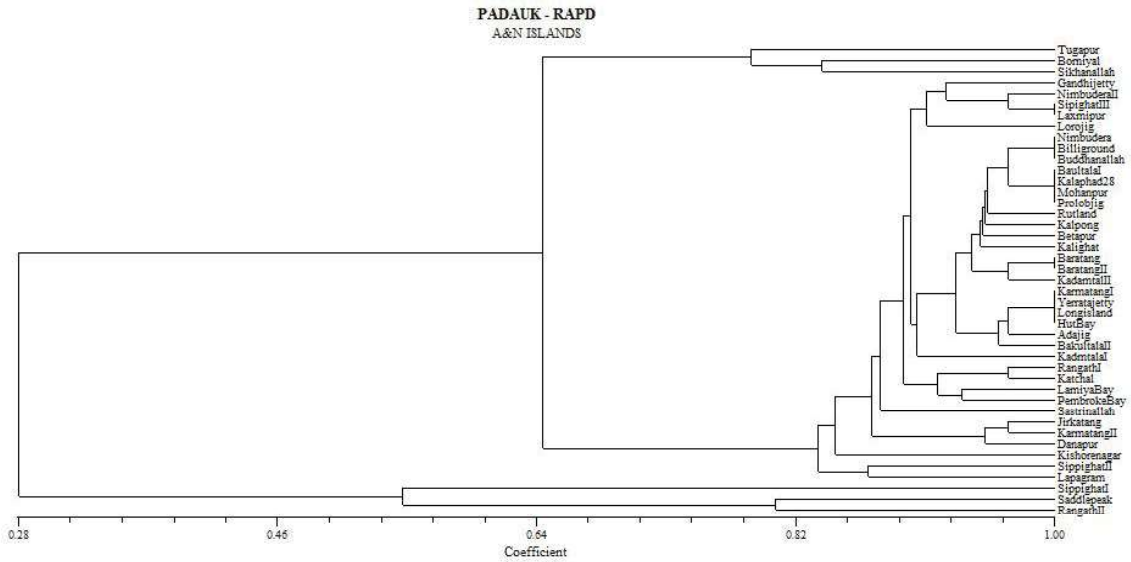

Fig. 3. UPGMA cluster analysis of 43 accessions of *Pterocarpus dalbergioides* using RAPD markers

Dendrogram analysis for *Pterocarpus dalbergioides* as obtained with ISSR markers

A dendrogram based on UPGMA analysis with ISSR data is shown in fig. 4. Jaccard's similarity coefficient ranged from 0.640 to 0.940. The 43 genotypes were grouped into two main clusters. Cluster I comprises with seven accessions and Cluster II comprises 36 accessions. Further, cluster I divided in two sub-clusters A and B with six and one accessions. Sub-cluster again split into two groups with five and one accession respectively with 65% similarity. Cluster II also divided into two sub-clusters 2A and 2B, than the other with 12 and 24 accessions, while in this sub-cluster 2A split in two groups with five and seven accessions showing 75% similarity (coefficient). Sub-cluster 2B spilt into seven more groups showing 75 to 90% similarity (coefficient).

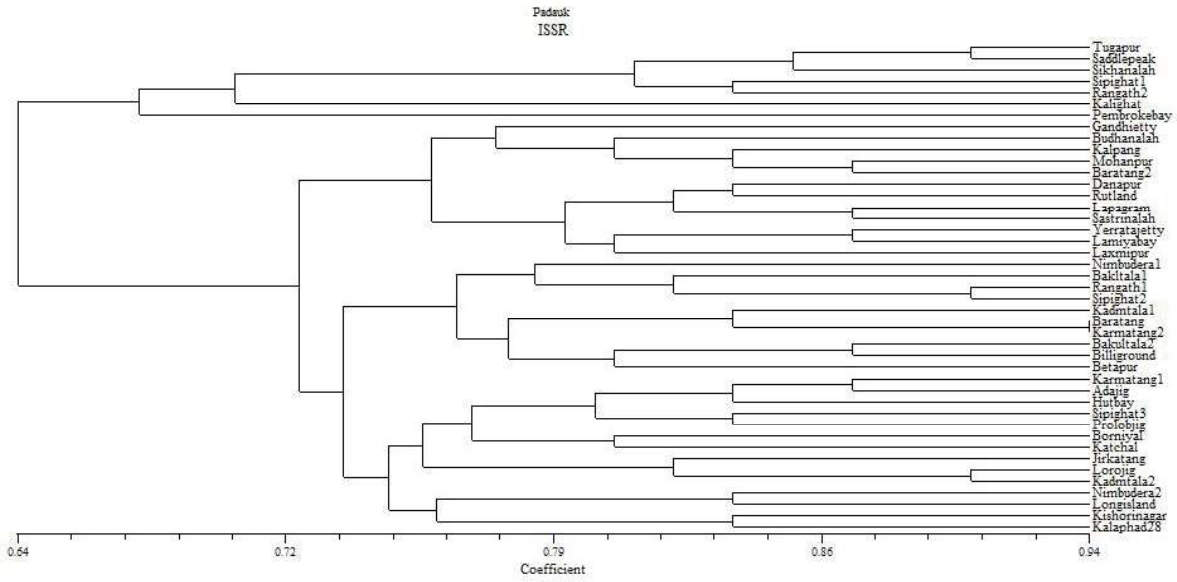

Fig. 4. UPGMA cluster analysis of 43 accessions of *Pterocarpus dalbergioides* using ISSR markers

Comparative analysis of RAPD and ISSR markers

The ISSR and RAPD data were combined for UPGMA cluster analysis fig. 5. The dendrogram and cluster analysis gave a similar clustering pattern to that of ISSR and RAPD analysis, separately, with Jaccard's similarity coefficient ranging from 0.620 to 1.000. Forty-three accessions were grouped in two main clusters with five and 38 accessions respectively. Cluster II again divided into ten sub-clusters with coefficient ranging from 0.870 to 1.000. The RAPD primers showed 37.04% of polymorphism whereas ISSR primers showed 37.62% (Table 4). Similar results were reported by Gupta *et al.,* (2008) with the use of ISSR and RAPD markers for genetic diversity analysis among different *Jatropha curcas* genotypes in India. Singh *et al*., (2010) reported that both RAPD and ISSR exhibit more than 60% similarity with castor and 34% polymorphism across the clones of *J. curcas.* RAPD and ISSR techniques revealed a high level of polymorphism in *P. dalbergioides* genotypes. Li *et al*., (2006) reported that genetic diversity by RAPD and ISSR markers clearly showed that there is no genetic variation within and among *Eichhornia crassipes* populations in Southern China. ISSR marker was more effective for genetic variation than RAPD analysis in *Momordica charantia*. ISSR showed 74.7% polymorphism and RAPD showed 36% polymorphism (Behera *et al*., 2008).

Table 4. Genetic variation of 43 genotypes of *Pterocarpus dalbergioides* compared using RAPDs and ISSRs.

Primers	RAPD	ISSR	RAPD + ISSR
Numbers of primers used	12	14	26
Amplified product range (bp)	150-3000	150-3000	150-3000
Total number of polymorphic bands	1543	970	826
Average numbers of polymorphic bands	128.58	88.18	35.91
Total number of monomorphic bands	2738	1608	389
Average number of monomorphic bands	228.16	146.18	16.91
Total number of bands	4281	2578	1215
Average number of bands	127.63	68.89	52.82
% Polymorphism	37.04	37.62	28.26
PIC = polymorphism information content	0.435	0.448	0.206

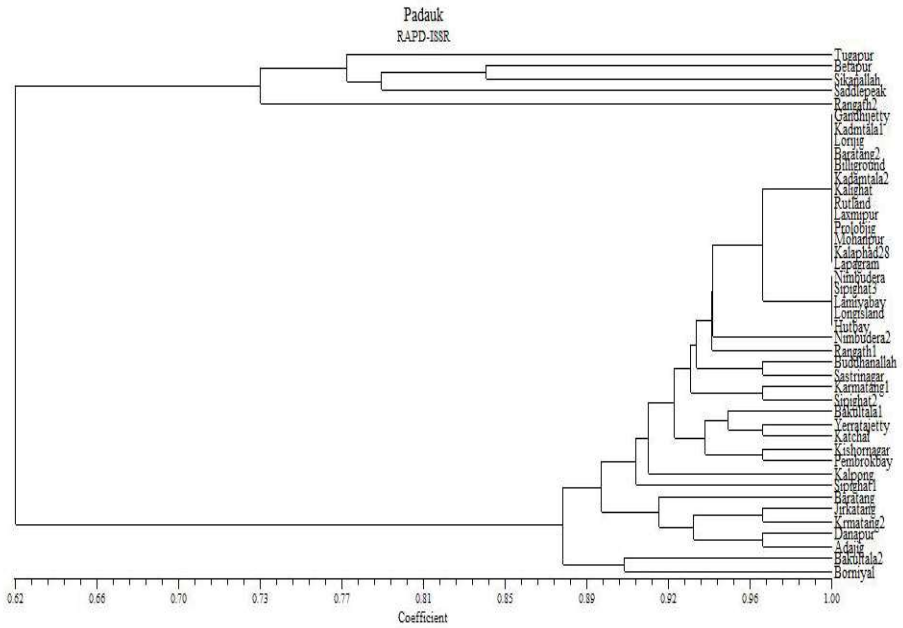

Fig. 5. Dendrogram based on UPGMA cluster analysis of 43 accessions of *Pterocarpus dalbergioides* using RAPD and ISSR markers.

Level of polymorphism in RAPD and ISSR markers in *P. dalbergioides* samples indicates fair extent of variability in the islands. Thus, the results present in this paper, confirms that ISSR and RAPDs have high discriminatory power and can be successfully applied to several genetic diversity among all the 43 genotypes. Hence, *P. dalbergioides* genotypes from Andaman and Nicobar are unique which could be useful for breeding programmes to develop the new superior genotypes as well as those accession needs attention for conservation and utilization for strengthening the coastal ecosystem. Further, studies are needed to take up character tagging for economic traits using molecular tools. The results would be useful in finding out the variation for these traits and to use them in identifying the desirable genotypes.

Acknowledgements

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References

Behera, T.K., Singh, A.K. & Staub, J.E. (2008). Comparative analysis of genetic diversity in Indian bitter gourd (*Momordica charantia* L.) using RAPD and ISSR markers for developing crop improvement strategies. *Scientia Horticulturae*, 115: 209–217.

Deshwall, R.P.S., Singh, R., Malik, K. & Randhawa, G.J. (2005). Assessment of genetic diversity among 29 population of *Azadirachta indica.* A.Juss. using RADP marker. *GeneticResources and Crop Evolution,* 52: 285-292.

Diniz, L.E.C., Sakiyama, N.S., Lashermes, P., Caixeta, E.T., Oliveira, A.C.B., Zambolim, E.M., Loureiro, M.E., Pereira, A.A., & Zambolim, L. (2005). Analysis of AFLP marker associated to the Mex-1 resistance locus in Icatu progenies. *Crop Breed Appl. Biotechnol*. 5:387–393

Doyle, J.J. & Doyle, J.L. (1987). A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin,* 19: 11-15.

Gupta, S., Srivastava, M., Mishra, G.P., Naik, P.K., et al. (2008). Analogy of ISSR and RAPD markers for comparative analysis of genetic diversity among different Jatropha curcas genotypes. *Afric. J. Biotechnol*. 7: 4230-4243.

Jaisankar, I., B. Augustine Jerard, A. Velmurugan & T.P. Swarnam. (2020). Analysis of genetic relationship of *Pandanus tectorius* accessions of Andaman and Nicobar Islands using RAPD and ISSR markers. *Indian J. of Agroforestry , 22 (1) : 24-30.*

Jaisankar, I., S. Srividhya, R. Jayakumara Varadan & Manasseh Ezekiel Moses. (2023). Screening Andaman Padauk (*Pterocarpus dalbergioides*) for drought tolerance. *Indian J. of Agroforestry,* 25(1): 115-119.

Li, W., Wang, B. & Wang, J. (2006). Lack of genetic variation of an invasive clonal plant *Eichhornia crassipes* in China revealed by RAPD and ISSR markers. *Aquatic Botany,* 84: 176–180.

Murray, M.G.,& Thompson, W.F. (1980). Rapid isolation of high molecular weight plant DNA. *Nucl. Acids Res,* 8(9):4321-4325.

Panda, P., Panda, M., Nayak, S.S. & Panda, D.P. (2009). Formulation and evaluation of the tablets prepared with chloroform extract of *Pandanus fascicularis* Lam. leaves. *Research Journal Pharmacognosy and Phytochemistry*, 1: 54- 58.

Radha, P., Urla, R. & Reddy, V.D. (2015). Genetic diversity of Indian *Cycas* species assessed using RAPD markers. *American Journal of Research Communication*, 3(12): 95-105.

Rao, P.S.N. (2000). The Andaman Re- Wood tree *Pterocarpus dalbergioides* Roxb.- An economic timber plant. Short communications.. *Bull. Bot. Surv. India.* **42**(1-4): 149-154.

Saiki, R.K., Gelfand, D.H., Stoeffel, S., Scharf, S.J., Higuchi, R., Horn, G.T., Mullis K.B. & Erlich, H.A. (1988). Primer directed enzymatic amplification of

DNA with a thermostable DNA polymerase. *Science*, 239: 487–491.

Singh, A.S., Gajeraa, B.B., Kumara, N., Punvar, B.S., Ravikirana, R., Subhasha, N. & Jadejab, G.C. (2010). Assessment of genetic diversity in castor (*Ricinus communis* L.) using RAPD and ISSR markers. *Industrial Crops and Products,* 32: 491–498.

Soller, M. & Beckmann, J. S. (1983). Genetic polymorphism in varietal identification and genetic improvement. *Theoretical and Applied Genetics,* 61: 25-33.

Wu, K.R., Jones, R., Dannenberg, L. & Scolnik, P.A. (1994). Detection of micro-satellite polymerase without cloning. *Nucleic Acids Research,* 22: 3257-3258.

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