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Molecular Fingerprinting of Indian Medicinal Tree *Saraca asoca* using RAPD Markers

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Saraca asoca **is an important medicinal tree facing a serious problem of reduction from its instinctivetenancy in India.Before formulation of conservation strategies for geographical protection of *S. asoca* genotypes available in India, it is necessary to characterize them. In the current study, the RAPD markers have been utilized effectively for categorization of *S. asoca* collected from 15 diverse sites of India. An initial experiment on the amplification suitability of genomic DNA samples of four *S. asoca* was done with 35 RAPD primers. Among them only twenty sixproved their efficiency in two times repeat amplification.Total 146 bands were amplified and out of these 97 bands were found to be polymorphic. The average numbers of total band was 5.61 while average numbers of polymorphic bands was 3.73. The numbers of bands produced per primer ranged from 3 (OPE-15) to 8 (RUF205). Among all studied markers the highest percentage (100%) of polymorphism was demonstrated by only one marker (OPE-06). The lowest percent of polymorphicm (20%) was demonstrated by marker RUF211. The average percentage of polymorphism was 66.44%. Cluster analysis grouped all the *S. asoca* genotypes under study into two groups. Grouping of genotypes according to their sites of collection demonstrates higher similarity among or between them. The results obtained in the current study may help to formulate conservation strategies for the conservation of *S. asoca* genotypes.**

Keywords: Conservation, Fingerprinting, Genotypes, Molecular markers and Polymorphism.

Molecular fingerprinting technique has been applied enormously for characterization of various medicinal plants, crops as well as tree species (Tripathi *et al*. 2018). In contrast to morphological markers, DNA based markers are not depends on environmental changes. For proper credentialsof closely related botanicals, it is important to use molecular markers. *Saraca asoca* (Roxb.) De Wilde, (Family: Caesalpiniaceae) is an important medicinal tree facing a serious problem of reduction from its instinctivetenancy in India. It is now categorized as 'vulnerable' and considered 'red listed' by the International Union for Conservation of Nature (IUCN) (Senapati *et al*. 2012; Mohan *et al*. 2017). In terms of remedial significance of *S. asoca,* diverse plant parts (seeds, leaves, bark and flowers) have been found better and are in use for the development of different formulations (Hegde *et al*. 2017).

Among all molecular markers Random amplified polymorphic DNA (RAPD) has been found to bewell-situated in concert which does not

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necessitate any former information of DNA targeted for the investigation. The RAPDs have proved their significance for inherent multiplicity estimation in various plant species (Tripathi *et al*. 2013, Khare *et al*. 2013). Before formulation of conservation strategies for geographical protection of *S. asoca* genotypes available in India, it is necessary to characterize them. Molecular characterization of *S. asoca* genotypes have not been performed in at big level in India. So, the current study demonstrates the effective application of RAPD markers for rapid cataloging of fifteen *S. asoca* genotypes collected from diverse parts of India.

MATERIALS AND METHODS

Plant material

Young leaves of the collected *S. asoca* genotypes were taken for genomic DNA extraction. An inclusive detail of collected samples with their collection sites listed (Table 1).

Methods

Genomic DNA was extracted following the steps involved in CTAB method (Doyle and Doyle, 1990). Extracted DNA was purified with RNase treatment. 1X TE buffer was used to dissolve the dried pellet. Dissolved DNA samples were stored at -20 ºC for further use. DNA was quantitatively estimated by UV-spectrophotometer at 260 nm. Decamer (RAPD) primers (Table 2) were used to standardize PCR conditions. DNA templates were amplified with thermal cycler in a total reaction of 20 µl contained template DNA (50 ng), $MgCl_2$ (50 mM), primer (0.4mM), each dNTP (2 mM) and *Taq* Polymerase (1U) following initial denaturation (5 min at 94 ºC) at first step, followed by denaturation (1 min at 94 ºC), annealing (1 min at 37 ºC) and extension (2 min at 72 ºC) in second

Table 1. Details of collection site of Saraca asoca genotypes

S. No.	Place of collection	Genotypes	State	Latitude	Longitude
1	*Kodaikanal-1*	SA1	Tamil Nadu	10°13'56.27"N	77°29'11.04"E
2	*Kodaikanal-2*	SA2	Tamil Nadu	10°13'44.25"N	77°29'18.14"E
3	*Melpallum*	SA3	Tamil Nadu	10°20'15.20"N	77°33'48.49"E
4	*Palani*	SA4	Tamil Nadu	10°20'15.20"N	77°33'48.49"E
5	*Satyamagalum-1*	SA5	Tamil Nadu	11°30'11.56"N	77°14'48.57"E
6	*Satyamagalum-2*	SA6	Tamil Nadu	11°29'13.23"N	77°15'12.32"E
7	*Satyamagalum-3*	SA7	Tamil Nadu	11°29'45.94"N	77°13'29.78"E
8	*Satyamagalum-4*	SA8	Tamil Nadu	11°29'47.28"N	77°13'34.75"E
9	*Munnar road forest area-1*	SA9	Kerala	10°11'47.93"N	77°15'30.20"E
10	*Munnar road forest area-2*	SA10	Kerala	10°11'44.01"N	77°15'36.17"E
11	*Gopal swami hill*	SA11	Karnataka	11°43'12.02"N	76°34'55.86"E
12	*Borivali forest area*	SA12	Maharashtra	19°13'43.93"N	72°53'35.48"E
13	*Vasco*	SA13	Goa	15°23'37.13"N	73°48'47.75"E
14	*Veterinary College, Jabalpur*	SA14	Madhya Pradesh	23°12'54.14"N	79°57'11.22"E
15	*Gwarighat, Jabalpur*	SA15	Madhya Pradesh	23° 6'24.82"N	79°55'20.28"E

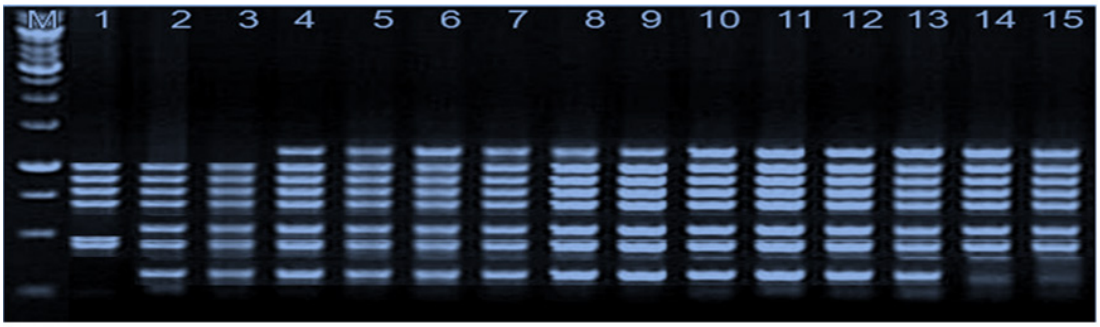

Fig. 1. Electrophoretic banding pattern of RAPD marker OPA-05 amplified with 15 *S. asoca* genotypes

step of 45 cycles. In third step final extension (10 min at 72 ºC) was performed. Amplicons were electrophoresed on 1.5% agarose gel in TAE buffer and images were captured under gel documentation system.

Data analysis

Amplified bands were scored according to their absence '0' or presence '1'. The clustering of genotypes was performed on the basis of Jaccard's similarity coefficient. The dendrogram was constructed by using the UPGMA cluster analysis with help of software NTSYS-pc ver. 2.0 (Rohlf, 2000).

RESULTS AND DISCUSSION

Assessment of inherent dissimilarity is very of the essence for the continuance of plant genetic resources in their native territory (Khare *et al*. 2013). An Initial experiment on the amplification suitability of genomic DNA samples of four *S. asoca* was done with 35 RAPD primers. Among them only twenty six primers (Table 2) proved their efficiency of the amplification ofconsistentsharp bands.These efficient markers were further used for the amplification of DNA of all studied genotypes. Banding pattern of OPA-05 primer is illustrated in fig. 1. During the present study, total 146 bands were amplified and out of these 97 bands were found to be polymorphic and 49 bands were monomorphic. The average numbers of total band was 5.61 while average numbers of polymorphic bands was 3.73. The number of bands produced per primer ranged from 3 (OPE-15) to 8 (RUF205). Among all studied markers the highest percentage (100%) of polymorphism was demonstrated by only one marker (OPE-06). However rest of the markers had less polymorphism comparatively.

Table 2. Sequences of RAPD Primers used in the Study

S.	Primer	5'-3' Sequence	GC content	Total Bands	Polymorphic Bands	Percentage Polymorphism
1.	OPA-5	AGGGGTCTTG	60%	8	3	37.50
2.	OPA-8	GTGACGTAGG	60%	6	4	66.60
3.	OPC-10	TGTCTGGGTG	60%	6	5	83.30
4.	OPC-15	GACGGATCAG	60%	5	4	80.00
5.	OPAP-07	ACCACCCGCT	70%	5	3	60.00
6.	OPAP-13	TGAAGCCCCT	60%	7	5	71.42
7.	OPR-15	GGACAACGAG	60%	6	5	83.30
8.	OPM 05	GGGAACGTGT	60%	7	4	57.10
9.	OPM-06	CTGGGCAACT	60%	7	5	71.42
10.	OPM 13	GGTGGTCAAG	60%	6	5	83.30
11.	OPO-20	ACACACGCTG	60%	6	5	83.30
12.	OPB-18	CCACAGCAGT	60%	5	4	80.00
13.	OPE-06	AAGACCCCTC	60%	4	4	100.0
14.	OPE-15	ACGCACAACC	60%	3	2	66.60
15.	RUF 202	TTGGCGGCCT	70%	4	3	75.00
16.	RUF 205	TGGGTCCCTC	70%	3	2	66.60
17.	RUF 207	CAGGCCCTTC	70%	6	5	83.30
18.	RUF 210	TGCCGAGCTG	70%	5	4	80.00
19.	RUF 211	GGGTAACGCC	70%	5	1	20.00
20.	RUF 215	GCTGCGTGAC	70%	6	2	66.60
21.	RUF 216	CAGCGAACTA	50%	7	2	28.57
22.	RUF 217	CGACTCACAG	60%	5	4	80.00
23.	RUF 218	GGGCCTCTAT	60%	5	4	80.00
24.	RUF 219	CTAGAGGTCC	60%	6	4	66.6.
25.	RUF 220	GGGTGAACCG	70%	6	3	50.00
26.	OPO-03	TCCGATGCTG'	60%	7	5	71.42
			Total	146	97	66.44
			Average	5.61	3.73	—-

The lowest percent of polymorphism (20%) was demonstrated by marker RUF211. The average percentage of polymorphism was 66.44%.

Cluster analysis was performed on the basis of Jaccard's similarity co-efficient generated from RAPD fingerprinting. The clusteranalysis grouped all the *S. asoca* genotypes under study into two groups i.e. group A and group B (Fig. 2). Group A is a minor cluster consisting only two*S. asoca* genotypes namely SA-9 and SA-10. Both of these genotypes were collected from Munnar road forest area, Kerala. Genotypes SA-9 and SA-10 showed higher similarity and group together.

Group B is a major cluster consisting 13 genotypes. Group B was further divided into two sub groups C and D. Sub group C consisted 11 genotypes namely SA-11, SA-4, SA-1, SA-2, SA-3, SA-6, SA-8, SA-7, SA-5, SA-12 and SA-13. Among these eleven genotypes, SA-5 and SA-7 had maximum similarity however SA-13 showed highest genetic divergence from other ten genotypes of sub group C. This genotype was collected from Goa. Eight genotypes collected from different locations of Tamil Nadu state and one genotype from Karnataka, one from Maharashtra and one from Goa are also present in this sub group.The clustering of genotypes in sub group D demonstrated higher similarity among the genotypes collected from Tamil Nadu. However, the genotypes collected from Maharashtra, Goa and Karnataka showed genetic distance from Tamil Nadu genotypes and were clustered distantly. Further, sub group D had only two genotypes coded as SA-14 and SA-15. Both of these genotypes were collected from Jabalpur district of Madhya Pradesh. Geographically, secluded genotypes are tending to build up genetic variability throughout the way of ecological adjustments. The current study is an effort to ascertain the inherent multiplicity backdrop in *S. asoca* with the use of RAPD markers. Moderate levels of polymorphism observed in the current examination divulge that RAPD technique proved its suitabilityfor fingerprinting studies. The results obtained under this study will lay concrete on the wayfor comprehensiveexploration to recognize all the facets of thisdiscrepancy. RAPD technique has been used extensively for thedetection of diversity

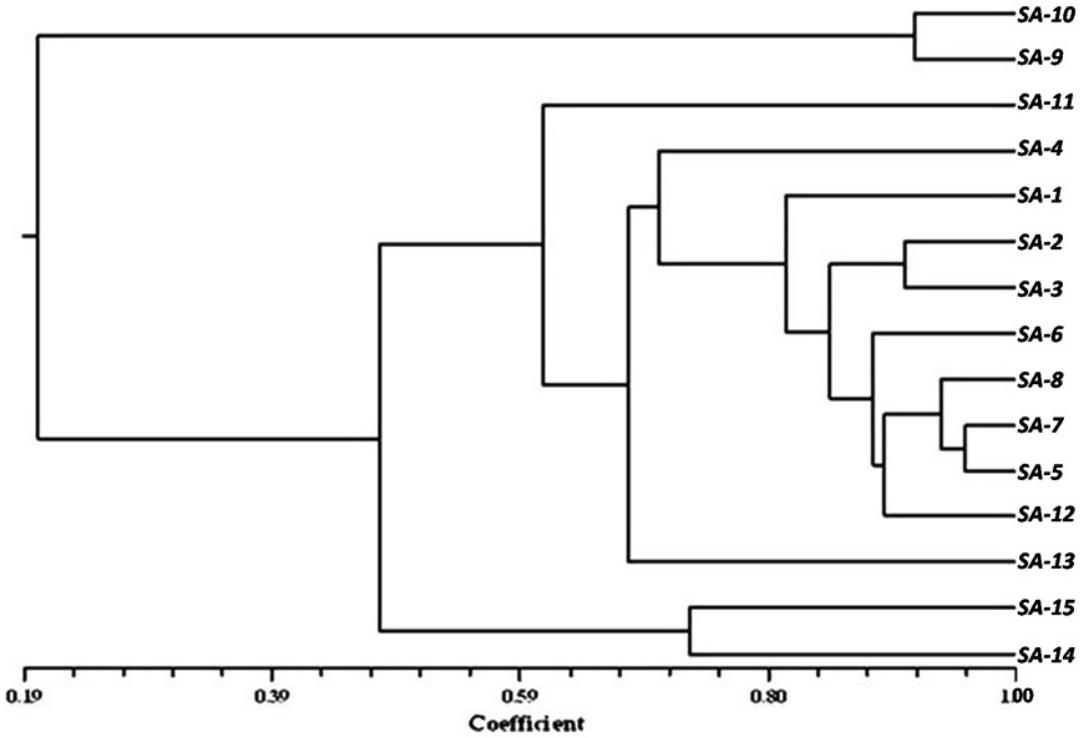

Fig. 2. Dendrogram based on RAPD data representing genetic similarity among 15 *S. asoca* genotypes

among and between medicinal plants (Tripathi *et al*.2012; Pagare *et al*. 2017).

Among all DNA fingerprinting techniques RAPDmarker technique is one of the easiest and cheaperthan other techniques available. Because, RAPD markers are decamer and produce multiple bands targeting multi locus in the genome of objective genotype (Khare *et al*. 2013). The current study demonstrates the suitability of RAPD markers for genetic diversity analysis at DNA level. This particular technique has been employed all the time more formolecular characterization studies in different plant species (Tripathi *et al*. 2013) and it provides helpful figures on assortment all the way through their aptitude to perceive variations at the DNA level.

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Conflict of interest

Authors have declared no conflicts of interests.

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