Plant Science Research 36 (1&2) : 68-76, 2014

Plant Science Research

ISSN 0972-8546

Genetic diversity and phylogenetic relationships in *Pterocarpus* species and its closely related genus *Tipuana* (Fabaceae) as revealed by RAPD and ISSR markers

P. Bal[1], P. C. Panda[1Ψ] and U. B. Mohapatra[2]

[1] Taxonomy & Conservation Division, Regional Plant Resource Centre, Bhubaneswar - 751 015, Odisha

[2] Department of Science & Technology, Government of Odisha, Bhubaneswar - 751 001, Odisha

ARTICLE INFO

Article history:
Received : 18 November 2014
Received in revised form : 21 December 2014
Accepted : 27 December 2014

Keywords:
Molecular phylogeny
RAPD
ISSR
Pterocarpus

ABSTRACT

The genetic relationships among four species of *Pterocarpus* (*P. dalbergioides, P. indicus, P. marsupium* and *P. santalinus*) with sixteen accessions and one accession of *Tipuana tipu* (Fabaceae) were assessed using RAPD and ISSR markers. Twelve RAPD and fourteen ISSR primers were used for analysis of genetic diversity among the species and accessions studied and high degrees of polymorphism was observed with most of the primers used. All the species and accessions had an average similarity of 52% among themselves. Highest similarity (95%) was observed between two accession of *Pterocarpus dalbergioides* and lowest (28%) between *Tipuana tipu* and *Pterocarpus marsupium*. The lone accession of *Tipuana tipu* got separated from the *Pterocarpus* group in the dendrograms constructed using both RAPD and ISSR primers which justify their generic distinctness. Among the four species of *Pterocarpus*, *P. indicus* was distantly related to rest three species and *P. marsupium* and *P. santalinus* were genetically very close to each other exhibiting a similarity of 88.80%.

1. Introduction

Pterocarpus is a pantropical genus of trees belonging to the subfamily Faboideae of the family Leguminosae (Fabaceae). It was recently assigned to the informal monophyletic *Pterocarpus* clade within the Dalbergieae (Lavin *et al*., 2001; Cardoso *et al*., 2012). Most species of *Pterocarpus* yield valuable timber traded as "Padauk"; other common names are "Red Sanders", "Bija Sal" etc. The timbers are valued for their toughness, stability in use, and decorativeness, most having a reddish wood. Most *Pterocarpus* woods contain either water- or alcohol-soluble substances and can be used as dyes. *Pterocarpus santalinus* (Red Sanders) is an endangered species distributed in Eastern Ghat region only and wild populations of *P. marsupium* have been drastically reduced bringing the species under "vulnerable" category (Ved *et al*., 2008). *Tipuana* is another genus of the *Pterocarpus* clade of Dalbergioid legumes which is closely related to the genus *Pterocarpus*. Due to rarity of most of the species of *Pterocarpus* because of deforestation and over-exploitation, it is necessary to develop appropriate conservation and restoration strategies, and in order to achieve this goal information on the genetic diversity and structure of populations will be most useful.

The genetic diversity of many *Pterocarpus* species have been analysed using molecular markers like Random Amplified Polymorphic DNA (RAPD) (Chisha-Kasumu *et al*., 2009; Amri & Mamboya, 2012; Usha *et al*., 2013), Amplified Fragment Length Polymorphism (AFLP) (Rivera-Ocasio *et al*., 2001, 2005), microsatellite (Muller *et al*., 2009; Li *et al*., 2010) and isozyme (Liengsiri *et al*., 1998). Rivera-Ocasio *et al*. (2002, 2005) studied the genetic structure and diversity of island and continental populations

Ψ *Corresponding author; Email:* pcpanda2001@yahoo.co.in

of *Pterocarpus officinalis* in the Caribbean region using AFLP. The inter and intra-population genetic variability in *Pterocarpus angolensis* was evaluated with the application of RAPD markers (Amri & Mamboya, 2012). The nursery raised and forest grown plants of Red Sanders (*Pterocarpus santalinus*) were studied for their genetic variability using RAPD (Usha *et al*., 2013). Li *et al*. (2010) characterized 18 microsatellites for *Pterocarpus indicus* which showed high level of polymorphism and higher resolution for identifying individuals. Liengsiri *et al*. (1998) investigated the mating system in 11 natural populations of *Pterocarpus macrocarpus* in Thailand based on the mixed mating model using 16 isozyme markers. The molecular diversity of *Pterocarpus officinalis*, distributed in Caribbean islands, South and Central America was analysed by Muller *et al*. (2009) to quantify the genetic variation within island, to assess the pattern of differentiation and infer levels of gene flow; with the overall goal of defining a strategy of conservation.

With a view to understand the molecular phylogeny, genetic diversity of 16 accessions of four species of *Pterocarpus* (*P. dalbergioides, P. indicus, P. marsupium* and *P. santalinus*) and one accession of *Tipuana tipu* were assessed using RAPD and ISSR markers.

2. Materials and methods

2.1. Plant materials

Leaf samples of 16 individuals/ accessions belonging to 4 species of *Pterocarpus* and 1 accession of *Tipuana tipu* of the tribe Dalbergioideae were collected from different forest areas of Odisha, Andhra Pradesh, Indian Botanic Garden, Howrah, West Bengal and also from the arboretum of Regional Plant Resource Centre (RPRC), Bhubaneswar. The accession number, locality of collection and abbreviation used for each of the taxon is shown in Table 1. The tender and healthy leaves were used for DNA extraction for molecular analyses.

2.2. Genomic DNA extraction

Genomic DNA was extracted from the leaf tissues using the modified CTAB (cetyl-trimethyl-ammonium-bromide) protocol (Doyle and Doyle, 1990). Two grams of leaf tissues from tender parts were ground with grinding buffer composed of 100 mM sodium acetate (pH 4.8), 500 mM NaCl, 50 mM EDTA (pH 8.0), 50 mM Tris (pH 8.0), 2% Polyvinyl pyrollidone (PVP) and 2% CTAB. Purification of DNA was done twice with extraction of phenol: chloroform: Isoamyl alcohol (25:24:1). RNAse @ 40 µl from 1 mg/ ml was applied in the supernatant to get rid of RNA. The quality and quantity of DNA were checked through 0.8% agarose electrophoresis with standard DNA before PCR amplification.

2.3. RAPD and ISSR analyses

Thirty RAPD and 30 ISSR primers (Operon Technologies, Alameda, USA) were used for PCR analysis based upon their performance and reproducibility. Among them, 26 primers showed distinct polymorphism. The details of primers used, bands amplified and percent of polymorphism detected are presented in Table 2, 3. PCR mixture of 25 µl contained 25 ng of genomic DNA template, 0.6 µq of Taq DNA polymerase (Bangalore Genei, Bangalore, India), 0.3 µM of decamer primers, 2.5 µl of 10 x PCR assay buffer (50 mM KCI, 10 mM Tris-Cl), 1.5 m $MgCl_2$) and 0.25 µl of pooled dNTPs. The PCR condition used for RAPD was: Initial denaturing step at 94°C for 5 minutes followed by 42 cycles of 94°C for 1 minute, 37°C for 1 minute and 72°C for 2 minute, the last cycle, primer extension at 72°C for 7 minutes. For ISSR amplification, the PCR condition was: Initial denaturing step at 94°C for 5 minutes followed by 42 cycles of 94°C for 1 minute, 45° - 55°C for 1 minute and 72°C for 2 minute, the last cycle, primer extension at 72°C for 7 minutes. The amplified products as developed by the primers were separated by agarose (1.5%) gel electrophoresis and documented in gel documentation system (Bio Rad XR, Biorad, USA). O'Gene Ruler™ 100 bp DNA Ladder plus (ladder range 3000 bp to 100 bp from Fermentas Life Sciences, USA) was used as molecular weight marker. Bands were scored for its presence/ absence (1/0) for each primer genotypes combination. Software NTSYS-pc, version 2.1 (Rohlf, 2000) was used for estimation of genetic relatedness among the genotypes using Jaccard's similarity coefficient and clustering was done with UPGMA (unweighted pair group method using arithmetic averages).

3. Results

3.1. RAPD analysis

Four species of *Pterocarpus* and one species of *Tipuana* produced distinct reproducible amplifications with 12 RAPD primers out of 30 primers screened. The DNA profiles obtained from RAPD analysis are represented in Table 2. A total of 56 amplified loci were generated including 49 polymorphic bands. Seven bands were monomorphic in nature and 3 were unique ones. The resolving power of primers ranged from 2.82 (OPA07) to 7.41 (OPA04) whereas the primer index varied from 0.44 to 2.56 in case of primers OPA10 and OPN03 respectively. The RAPD banding pattern is represented in Fig 1. The primers OPA04, OPC02 and OPN03 produced highest number of amplified bands (7.6

Table 1

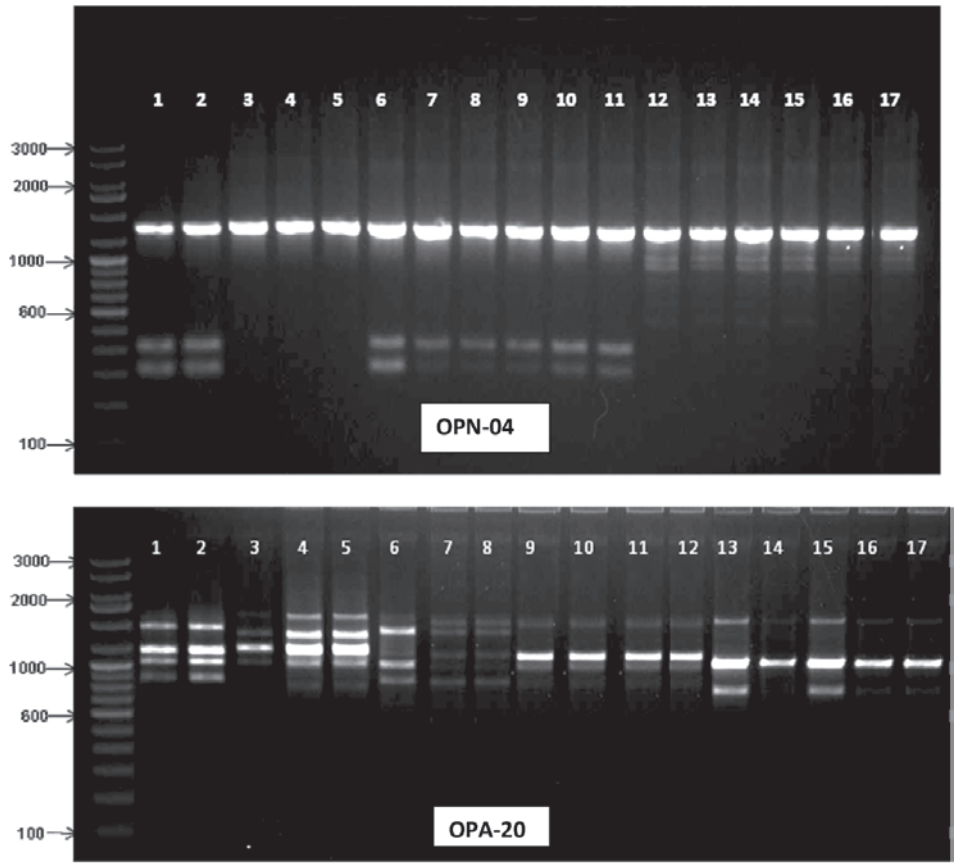
Details of plant samples used for study of genetic diversity and phylogeny

Sl. No.	Samples collection sites	Species	Code used in text, tables and figures
1	RPRC, Bhubaneswar, Odisha	*Pterocarpus marsupium*	PM1
2	Baliguda, Phulbani, Odisha	*Pterocarpus marsupium*	PM2
3	RPRC, Bhubaneswar, Odisha	*Pterocarpus indicus*	PI1
4	RPRC, Bhubaneswar, Odisha	*Pterocarpus indicus*	PI2
5	Indian Botanic Garden, Howrah, West Bengal	*Pterocarpus indicus*	PI3
6	Indian Botanic Garden, Howrah, West Bengal	*Pterocarpus indicus*	PI4
7	State Silviculturist Office campus, Ghatikia, Bhubaneswar	*Pterocarpus santalinus*	PS1
8	State Silviculturist Office campus, Ghatikia, Bhubaneswar	*Pterocarpus santalinus*	PS2
9	State Silviculturist Office campus, Ghatikia, Bhubaneswar	*Pterocarpus santalinus*	PS3
10	Seshachalam Hills, Andhra Pradesh	*Pterocarpus santalinus*	PS4
11	Seshachalam Hills, Andhra Pradesh	*Pterocarpus santalinus*	PS5
12	Seshachalam Hills, Andhra Pradesh	*Pterocarpus santalinus*	PS6
13	Eastern Regional Office, MOEF, Bhubaneswar, Odisha	*Pterocarpus dalbergioides*	PD1
14	Eastern Regional Office, MOEF, Bhubaneswar, Odisha	*Pterocarpus dalbergioides*	PD2
15	Eastern Regional Office, MOEF, Bhubaneswar, Odisha	*Pterocarpus dalbergioides*	PD3
16	Eastern Regional Office, MOEF, Bhubaneswar, Odisha	*Pterocarpus dalbergioides*	PD4
17	Indian Botanic Garden, Howrah, West Bengal	*Tipuana tipu*	T. tipu

Table 2

RAPD primer sequences and band details in different species of *Pterocarpus* and *Tipuana tipu*

Primer Name	Sequence	Range of amplicons	Total no. of bands	No. of polymo-rphic bands	No. of mono-morphic bands	No. of unique bands	Percentage of poly-morphic bands	Resolving Power	Primer Index
OPA 04	5' AATCGGGCTG 3'	200-2700	7	6	1	0	85.71	7.41	2.44
OPA 05	5' AGGGGTCTTG 3'	400-2500	5	5	0	0	100	5.53	2.23
OPA 07	5' GAAACGGGTG 3'	600-1500	2	1	1	0	50	2.82	0.48
OPA 10	5' GTGATCGCAG 3'	850-1500	4	4	0	2	100	4	0.44
OPA 20	5' GTTGCGATCC 3'	800-1500	5	4	1	0	80	5.18	1.47
OPC 02	5' GTGAGGCGTC 3'	650-2000	6	6	0	0	100	6.24	1.98
OPN 02	5' ACCAGGGGCA 3'	900-1845	4	4	0	0	100	3.88	1.8
OPN 03	5'GGTACTCCCC 3'	1000-2500	6	6	0	0	100	4.82	2.56
OPN 04	5' GACCGACCCA 3'	300-1280	3	2	1	0	66.66	3.88	1
OPN 05	5' ACTGAACGCC 3'	700-2000	4	3	1	0	75	5.53	1.36
OPN 06	5' GAGACGCACA 3'	600-2200	5	3	2	0	60	6.12	1.33
OPN 08	5' ACCTCAGCTC 3'	500-1250	5	5	0	1	100	3.06	1.09



Lane No. 1 &2: *P. marsupium*; Lane No. 3 to 6: *P. indicus*; Lane 7 to 12: *P. santalinus*; Lane No. 13 to 16: *P. dalbergioides*; Lane No. 17: *Tipuana tipu*

Fig. 1. RAPD banding pattern of different species and accessions of *Pterocarpus* and *Tipuana tipu*

and 6 respectively), whereas OPA07 produced least number of amplified loci (2). Six primers OPA05, OPA10, OPC02, OPN02, OPN03 and OPN08 showed 100% polymorphism and with OPA07 primer the level of polymorphism was 50%. This was the least polymorphism among all primers used. The average number of bands and polymorphic bands per primer was 4.67 and 4.08 respectively. Primers OPA10 and OPN08 produced 2 and 1 unique bands respectively.

Jaccard's similarity coefficient analysis revealed that all the taxa were related to each other with an average similarity of 53%. Highest similarity (100%) was observed between *Pterocarpus santalinus* (PS3) and *Pterocarpus santalinus* (PS4) and *Pterocarpus dalbergioides* (PD1) and *Pterocarpus dalbergioides* (PD3). Lowest (26%) similarity was observed between *Tipuana tipu* (T.tipu) and *Pterocarpus marsupium* (PM1 and PM2).

A dendrogram was constructed (Fig 2) to derive relationship among the four species of the genus *Pterocarpus* and one species of *Tipuana*. *Tipuana tipu* got separated from all other 16 accessions of four species of *Pterocarpus* sharing a node at 37% similarity. Among the 16 accessions of *Pterocarpus*, four accessions of *Pterocarpus dalbergioides* along with one sample of *Ptercarpus santalinus* formed a cluster and separated from accessions of *Pterocarpus marsupium*-*Pterocarpus santalinus*-*Pterocarpus indicus* at about 42% level of similarity. The later group had a further subdivision one containing the four species of *Pterocarpus indicus* and the other with representative sample of *Pterocarpus marsupium* and *Pterocarpus santalinus*. Both the clusters had a similarity of 47% between them. The two accessions of *Pterocarpus marsupium* and 5 accessions of *Pterocarpus santalinus* shared a common node at 72% similarity level as could be determined from Jaccard's similarity coefficient analysis. The two genotypes of *Pterocarpus marsupium* had close genetic resemblance between them with a similarity of about 98%. Of the five individuals of *Pterocarpus santalinus* studied there was a further subdivision into two clusters of 2 and 3 at about 89% similarity level. The individuals of each cluster of *Pterocarpus santalinus* exhibited close genetic resemblance among themselves.

3.2. ISSR analysis

The results obtained from the molecular fingerprinting by ISSR primers in 17 accessions of four species of *Pterocarpus* and one species of *Tipuana tipu* are represented in Table 3. Out of the 30 ISSR primers tested, 14 primers produced good and reproducible amplified product. A total of 79 bands were amplified which include 71 polymorphic, 8 monomorphic and 7 unique bands. The size of amplicons ranged from 200bp to 3000bp. The resolving power of primers ranged from 2.82 [(GA)8G] to 8.35 $(GGA)_4$ and the primer index varied in the ranges of 0.72 [C(GA)9T] to 3.36 $(GGA)_4$. The ISSR banding pattern is shown in Fig 3. Primers (CT)8A and (GGA)4 produced highest number of amplified loci (8) whereas $(GACA)_4$ produced least number

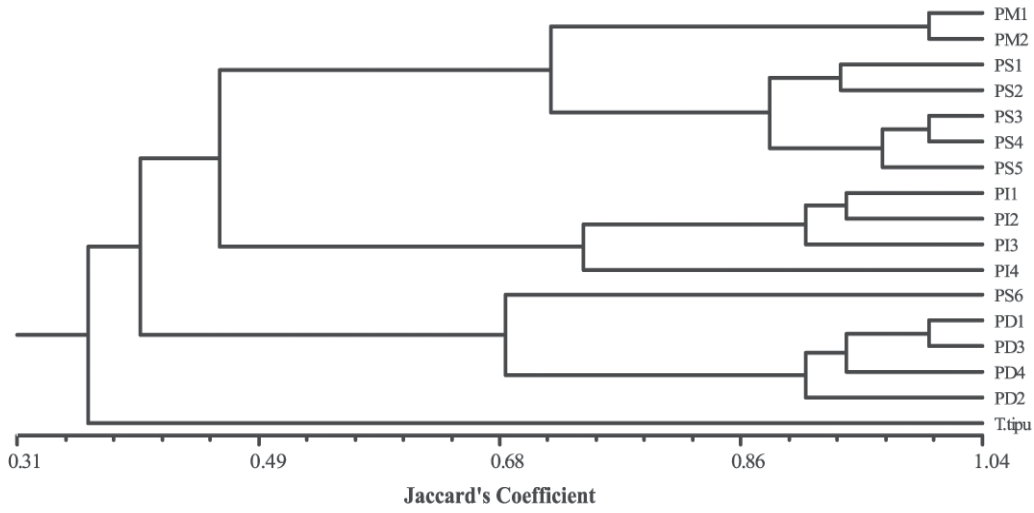
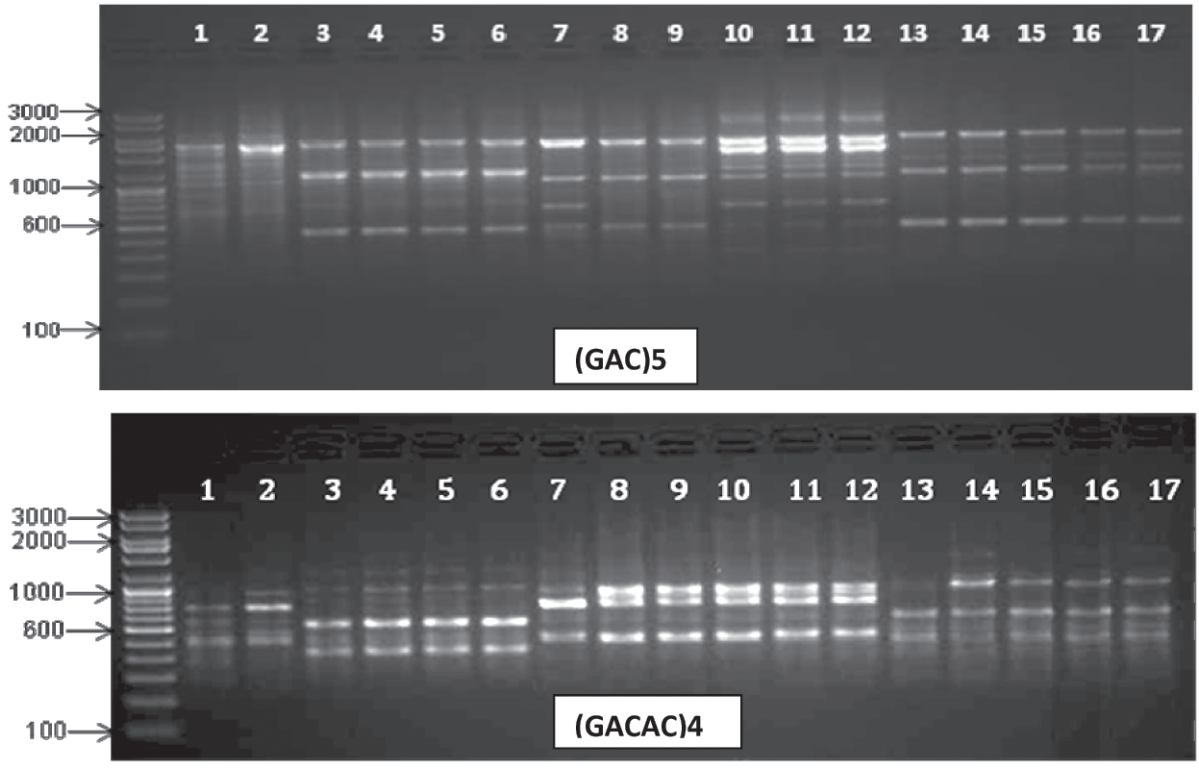


Fig. 2. Dendrogram showing genetic relationship among different species and accessions of *Pterocarpus* and *Tipuana tipu* as revealed from RAPD analysis



(Lane No. 1 &2- *P. marsupium*; Lane No. 3 to 6. *P. indicus*; Lane 7 to 12- *P. santalinus*; Lane No. 13 to 16- *P. dalbergioides*; Lane No. 17- *Tipuana tipu)*

Fig. 3. ISSR banding pattern of different species and accessions of *Pterocarpus* and *Tipuana tipu*

(3) of bands. Seven primers yielded 100% polymorphic bands but the polymorphism observed with (AG)8C primer was only 60%. The average number of amplified bands and polymorphic bands per primer was 5.64 and 5.07 respectively. (AG)8C amplified maximum number of monomorphic loci (2), and the primer (GACA)8G and (GACAC)4 produced 3 and 2 unique bands respectively.

All the taxa were related to each other with an average similarity of 52% as per the Jaccard's similarity coefficient analysis. Highest similarity (0.96) was observed between two accessions of *Pterocarpus dalbergioides* (PD1 and PD2) and lowest (0.29) between *Tipuana tipu* and an accession of *Pterocarpus marsupium* (PM2) (Table 3). Highest numbers of bands (49) were amplified in case of *Pterocarpus*

Table 3

ISSR primer sequences and band details in different species of *Pterocarpus* and *Tipuana tipu*

Primer Name	Sequence	Range of amplicons	Total no. of bands	No. of polymo-rphic bands	No. of mono-morphic bands	No. of unique bands	Percentage of poly-morphic bands	Resolving Power	Primer Index
(AG)8	5'GAGAGAGAGAGAGAG3'	600-1845	4	4	0	0	100	3.06	1.52
(AGG)6	5'AGGAGGAGGAGGAGGAGG3'	600-2000	6	5	1	0	83.33	6.47	2.12
(CT)8A	5'CTCTCTCTCTCTCTCTA3'	300-1900	8	8	0	1	100	7.41	3.24
(CT)8T	5'CTCTCTCTCTCTCTCTT3'	750-1500	4	4	0	0	100	4.82	1.69
(GA)8G	5'GAGAGAGAGAGAGAGAG3'	700-2000	4	4	0	0	100	2.82	1.48
(GA)9T	5'GAGAGAGAGAGAGAGAGAT3'	900-2300	4	3	1	0	75	6.71	0.72
(GAC)5	5'GACGACGACGACGAC3'	600-3000	7	6	1	0	85.71	7.06	1.79
(GACA)4	5'GACAGACAGACAGACA3'	600-1845	3	3	0	0	100	3.65	1.30
(GACA)8G	5'GACAGACAGACAGACAGACAGACAGACAGACAG3'	600-2500	7	7	0	3	100	5.18	1.69
(GACAC)4	5'GACACGACACGACACGACAC3'	450-1845	7	6	1	2	85.71	7.06	0.79
(GGA)4	5'GGAGGAGGAGGA3'	700-3000	8	7	1	0	87.5	8.35	3.36
(GTGC)4	5'GTGCGTGCGTGCGTGC3'	500-2300	7	7	0	0	100	6.71	3.10
(AG)10	5'AGAGAGAGAGAGAGAGAGAG3'	480-1000	5	4	1	1	80	7.06	0.89
(AG)8C	5'AGAGAGAGAGAGAGAGC3'	200-650	5	3	2	0	60	7.76	1.26

santalinus (PS4*)* and lowest (32) in *Pterocarpus marsupium* (PM1).

The dendrogram (Fig 4), in the first place, separated the lone accession of *Tipuana tipu* at about 36% similarity level. The large cluster was further subdivided into two sub clusters; one with the 4 accessions of *Pterocarpus indicus* and the other with the 12 accessions of *Pterocarpus marsupium, Pterocarpus santalinus* and *Pterocarpus*

3.3. RAPD and ISSR combined markers

The cladogram (Fig 5) constructed taking both RAPD and ISSR data showed separation of *Tipuana tipu* at the first division sharing a common node at 36.4% similarity level. The large cluster containing 16 accessions of four *Pterocarpus* species was further segregated into two sub clusters; one containing the 4 accessions of *Pterocarpus indicus* and the other with the rest of the species. These

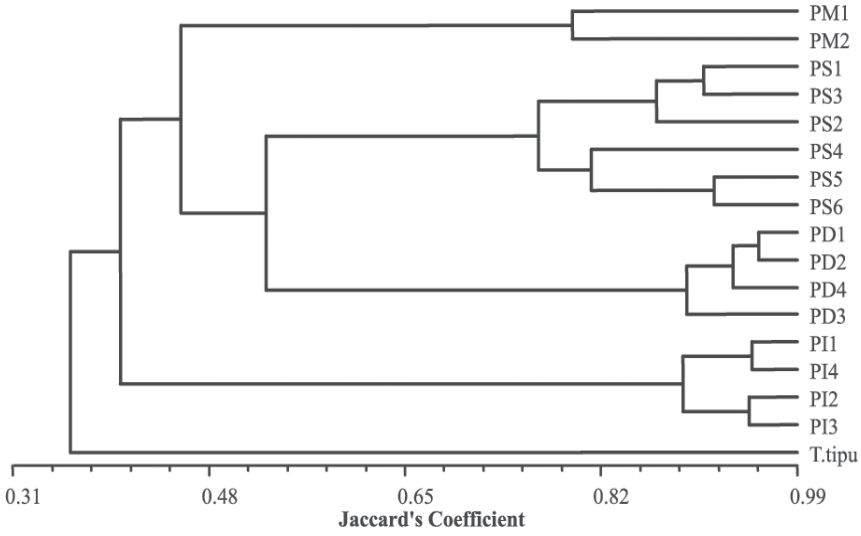


Fig 4. Dendrogram showing genetic relationship among different species and accessions of *Pterocarpus* and *Tipuana tipu* as revealed from ISSR analysis

dalbergioides. Both the clades shared a common node at 40% level of similarity.

Further division of the *Pterocarpus marsupium-Perocarpus santalinus-Pterocarpus dalbergioides* clade in the dendrogram placed *Pterocarpus marsupium* in a different group with a genetic resemblance of approximately 45%. Subsequently all the 6 accessions *Pterocarus santalinus* and 4 accessions of *Pterocarpus dalbergioides* formed distinct clusters having 52.4% genetic commonness. Among the 6 accessions of *Pterocarpus santalinus,* there was two sub-groups of 3 each with a similarity of 76.2%. Three accessions of *Pterocarpus santalinus* (PS1, PS2 and PS3) collected from the experimental plantation of the State Silviculturist Office campus, Ghatikia, Bhubaneswar and the other 3 collected from Seshachalam hills of Andhra Pradesh (PS4, PS5 and PS6) maintained their genetic distinctness. Varying levels of genetic relatedness was observed among the 4 accessions of *Pterocarpus dalbergioides* ranging from 89% to 95.8%. All genotypes of a particular species formed clear clusters justifying their status as distinct biological species. The two accessions of *Pterocarpus marsupium* also had approximately 79% similarity.

above two clades had genetic similarity to the extent of 42.2%. With further grouping of taxa, the four accessions of *Pterocarpus dalbergioides* got separated from the rest sharing a common node at 46.8% level of genetic similarity. The larger group had 6 accessions of *Pterocarpus santalinus* and two accessions of *Pterocarpus marsupium* exhibiting as much as 52% similarity between them. Individuals of each species showed close genetic relatedness among them and levels of similarity ranged from 84%-95%. The two accessions of *Pterocarpus marsupium* had as high as 88.8% genetic closeness. Similarly, the two *Pterocarpus dalbergioides* accessions (PD1 & PD3) were found to have 95% similarity. Among the *Pterocarpus* species analyzed for genetic diversity, *Pterocarpus dalbergioides* accessions had much close similarities among them followed by *Pterocarpus indicus* accessions.

4. Discussion

Bentham (1865) placed *Pterocarpus* and *Tipuana* along with other genera like *Machaerium* and *Cyclolobium* in the sub-tribe Pterocarpeae of the tribe Dalbergieae of the family Leguminosae. However, these taxa were classified under a separate tribe Pterocarpeae keeping the members of

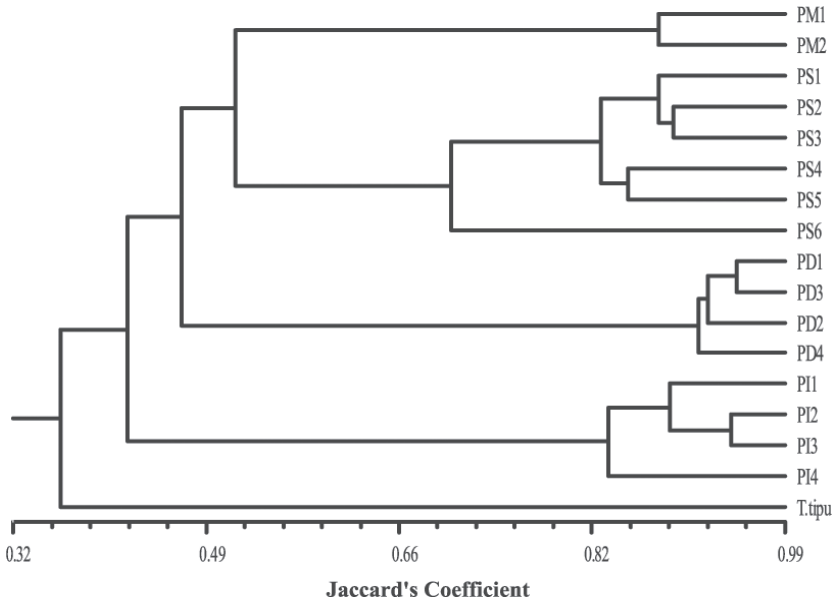


Fig. 5. Phylogeny of different species and accessions of *Pterocarpus* and *Tipuana tipu* as inferred from combined RAPD and ISSR marker analyses.

Dalbergia in a different tribe Dalbergieae (Hutchinson, 1964). However, as per later taxonomic treatments (Rojo, 1972; Polhill, 1981; Lavin *et al*., 2001), both the genera along with the genus *Dalbergia* are placed under the legume tribe Dalbergieae. *Pterocarpus* and *Tipuana* can be morphologically distinguished from each other by their fruit characters. While the fruits of *Pterocarpus* species are attenuated around the seed-chamber normally forming a wing, the fruits of *Tipuana tipu* are winged from style or stipe. During the present study, it was observed that the lone representative of *Tipuana tipu* maintained a distinct identity by getting separated from species and accessions of *Pterocarpus* in the cladogram constructed using RAPD and ISSR data and the combination of the two markers. Both the genera shared only 36.4-37% genetic similarity which justifies their status as two biological taxa distinct from each other as inferred by taxonomists based on morphological data. Saslis-Lagoudakis *et al*. (2011) using several DNA markers established genetic relationship among species of *Pterocarpus* and allied genera and placed *Tipuana* in a distant place in the phylogentic tree. Similar observations were also made by Klitgard *et al*. (2013). The present finding is in conformity with the results obtained in the above studies.

Of all the species of *Pterocarpus*, *P. santalinus* and *P. marsupium* were found to have close genetic similarity (52%) between them. Saslis-Lagoudakis et al. (2011) and Klitgard *et al*. (2013) used a number of DNA markers like rbcL, matK, trnL and nrlTS2 to derive phylogeny of Asian/ Indo-Malayan species of *Pterocarpus* and also reported close genetic affinity between *P. marsupium* and *P. santalinus*. Morphologically, *P. santalinus* can be differentiated from *P. marsupium* by having flowers in axillary racemes and edge of pods between stalk and stylar point being concave. In *P. marsupium*, the flowers are borne in terminal panicles and edge of pods between stalk and stylar point is concave. The other two species viz. *Pterocarpus indicus* and *P. dalbergioides* did not come together in a cluster and got separated in the dendrogram at different times with varying levels of genetic similarity with *P.marsupium-P.santalinus* clade. However, Saslis-Lagoudakis *et al*. (2011) and Klitgard *et al*. (2013) in a study of molecular phylogeny of Asian *Pterocarpus* detected close genomic affinity between *P. indicus* and *P. dalbergioides*. The findings of the present study confirms the system of classification based on morphology and molecular phylogeny derived from DNA markers except the species relationship between *P. dalbergioides* and *P. indicus*. However, the results are likely to change with application of more molecular markers and analysis of substantially high number of samples.

Acknowledgements

The authors wish to thank the Chief Executive, Regional Plant Resource Centre, Bhubaneswar and Head of the Department of Botany, North Orissa University, Baripada for providing laboratory and field facilities and to the Chief Conservator of Forests, Eastern Regional Office, MOEF, Bhubaneswar for permitting to collect plant materials from their garden.

References

Amri, E. & Mamboya, F. (2012). Genetic diversity in *Pterocarpus angolensis* populations detected by Random Amplified Polymorphic DNA markers. Int. J. Plant Breed. Genet. 6(2): 105-114.

Bentham, G. (1865). Leguminosae. In: Bentham, G. & Hooker, J.D. (eds.), *Genera Plantarum*, vol. 1. London: Reeve & Co., pp. 434–600.

Cardoso, D., Queiroz, L. P. de, Pennington, R. T.., De Lima, H. C., Fonty, E., Wojciechowski, M. F. & Lavin. M. (2012). Revisiting the phylogeny of Papilionoid Legumes: New insights from comprehensively sampled early-branching lineages. Amer. J. Bot. 99 (12): 1991-2013.

Chisha-Kasumu, E., Woodward, S. & Price, A. (2009). RAPD markers demonstrate genetic diversity in *Pterocarpus angolensis* from Zimbabwe and Zambia. Southern For. J. For. Sci. 71: 63-70.

Doyle, J. J. & Doyle, J. L. (1987). A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem. Bull. 19: 11–15.

Hutchinson, J. (1964). *The Genera of Flowering Plants*, Vol. 1, Oxford University Press, pp.516.

Klitgard, B. B., Forest, F., Booth, T. J. & Saslis-Lagoudakis (2013). A detailed investigation of the Pterocarpus clade (Leguminosae:Dalbergieae): Etaballia with radially symmetrical flowers nested within the papilionoid-flowered *Pterocarpus.* South Afr. J. Bot. 89: 128-142.

Lavin, M., Pennington, R.T., Klitgaard, B. B., Sprent, J. I., de Lima, H.C., *et al*. (2001). The dalbergioid legumes (Fabaceae): delimitation of a pantropical monophyletic clade. Amer. J. Bot. 88: 503–533.

Liengsiri, C., Boyle, T. J. B. and Yeh, F. C. (1998). Mating System in *Pterocarpus macrocarpus* Kurz in Thailand. Amer. Genet. Assoc. 89:216–221.

Li, C., Bin Sman, M. H., Fong, Y. & Hong, Y. (2010). Isolation and characterization 1 of 18 polymorphic microsatellite loci for *Pterocarpus indicus* (Leguminosae). Mol. Ecol. Resour. 10:1098-1105.

Muller, F., Voccia, M., Ba, A. & Bouvet, J. M. (2009). Genetic diversity and gene flow in a Caribbean tree *Pterocarpus officinalis* Jacq.: a study based on chloroplast and nuclear microsatellites. Genetica 135: 185-198.

Polhill, R. M. (1981). Dalbergieae. In: *Advances in Legume Systematics*, Part 1, (Polhill, R. M. and Raven, P.H. eds.). Royal Botanic Gardens, Kew, Richmond, Surrey, pp. 233-242.

Rivera-Ocasio, E., Aide, T. M. and McMillan, W.O. (2002). Patterns of genetic diversity and biogeographical history of the tropical wetland tree, *Pterocarpus officinalis* (Jacq.), in the Caribbean basin. Mol. Ecol. 11: 675–683.

Rivera-Ocasio, E., Aide, T. M. & McMillan, W. O. (2005). The influence of spatial scale on the genetic structure of a widespread tropical wetland tree, *Pterocarpus officinalis* (Fabaceae). Conser. Genet. 7: 251-266.

Rohlf, F. J. (2000). NTSYS-pc: numerical taxonomy and multivariate analysis system, version 2.1. Exeter Software: Setauket, New York.

Rojo, J. P. (1972). *Pterocarpus* (Leguminosae - Papilionaceae) revised for the world. Lehre, Germany: Verlag Von J. Cramer.

Saslis-Lagoudakis, C.H., Klitgaard, B. B., Forest, F., Francis, L., Savolainen, V., *et al*. (2011). The use of phylogeny to interpret cross-cultural patterns in plant use and guide medicinal plant discovery: An example from *Pterocarpus* (Leguminosae). PLoS ONE 6(7): e22275 (doi:10.1371/journal.pone.0022275)

Usha, R., Jhansi Rani, S., Geetha, Prasuna, T. (2013). Genetic relationship between quality and non-quality wood of *Pterocarpus santalinus* L. (Red Sanders) –an endemic tree species by using molecular markers. J. Chem. Pharma. Sci. 6 (3): 189-194.

Ved, D. K., Kinhal, G. A., Ravikumar, K., Sumathi, R., Ravishankar, R., Mahapatra, A. K. and Panda, P. C. (2008). *Conservation Assessment and management Prioritization for Medicinal Plants of Orissa*. RPRC, Bhubaneswar & FRLHT, Bangalore, pp. 1-124.