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SHORT RESEARCH ARTICLE

Identification of polymorphic SSR markers by examining their cross-transferability and investigation of genetic diversity within *Pterocarpus* species

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Abstract

Pterocarpus dalbergioides and *P. marsupium* are deciduous tropical trees in the Fabaceae family, with *P. dalbergioides* native to India's Andaman Islands and *P. marsupium* found in various Asian countries. This research investigated the applicability of specific primers for *P. santalinus* across these species. DNA isolation and PCR amplification were performed on 15 samples from each species. Out of 33 primers, 7 markers were amplified in *P. dalbergioides* and 15 in *P. marsupium*, yielding transferability rates of 21 and 33%, respectively. For *P. dalbergioides,* the effective population size (Ne) was 3.82 ± 1.84, observed heterozygosity (He) 0.42 ± 0.15, and allelic richness (Pa) 5.29 ± 2.01. In *P. marsupium*, Ne was 4.83 ± 0.91, He was 0.56 ± 0.10, and Pa was 7.07 ± 1.27. The average polymorphic information content (PIC) was 0.49 for *P. dalbergioides* and 0.54 for *P. marsupium*. The study underscores the potential of transferred SSR markers to assess genetic diversity in *Pterocarpus* species.

Keywords: Genomic SSRs, cross transferability, polymorphism, tree species, *Pterocarpus.*

The genus *Pterocarpus*, belonging to the Fabaceae family, includes several hardwood tree species found mainly in tropical regions like Africa, the Neotropics, and Indomalaya (Saslis-Lagoudakis et al. 2011). Key Indian species are *Pterocarpus santalinus*, *P. marsupium*, *P. indicus* and *P. dalbergioides*, which are valued for medicinal, ornamental, and furniture uses (Sukhadiya et al. 2019). *P. marsupium*, or "Indian Kino Tree," is significant in various industries and grows in diverse forests across central and southern India (Ahmed et al. 2022) (Figs.1a and b). *Pterocarpus dalbergioides*, known as Andaman padauk, is native to the Andaman Islands and is recognized for its high-quality timber (Fig. 1c). It is culturally significant and is the state tree of the Andaman and Nicobar Islands (Arunkumar and Joshi 2014). Both species, while differing in size and morphology, share a common "Samara" fruit type. They face population declines due to logging, with *P. marsupium* listed as Near Threatened and *P. dalbergioides* as Vulnerable (IUCN, 2020; Mohammad et al. 2022). Genetic studies using Simple Sequence Repeats (SSRs) help assess genetic diversity, crucial for the resilience of these species. SSRs are favored for their high reproducibility and polymorphism rates. Developing species-specific markers can be resource-intensive; hence, cross-species amplification provides a cost-effective alternative (Castillo et al. 2008). Research indicates successful SSR transfer among *Pterocarpus* species (Sneha et al. 2023; Agasthikumar et al. 2022). The present study investigated the cross-transferability of SSR loci from *P. santalinus* to *P. dalbergioides* and *P. marsupium*, contributing

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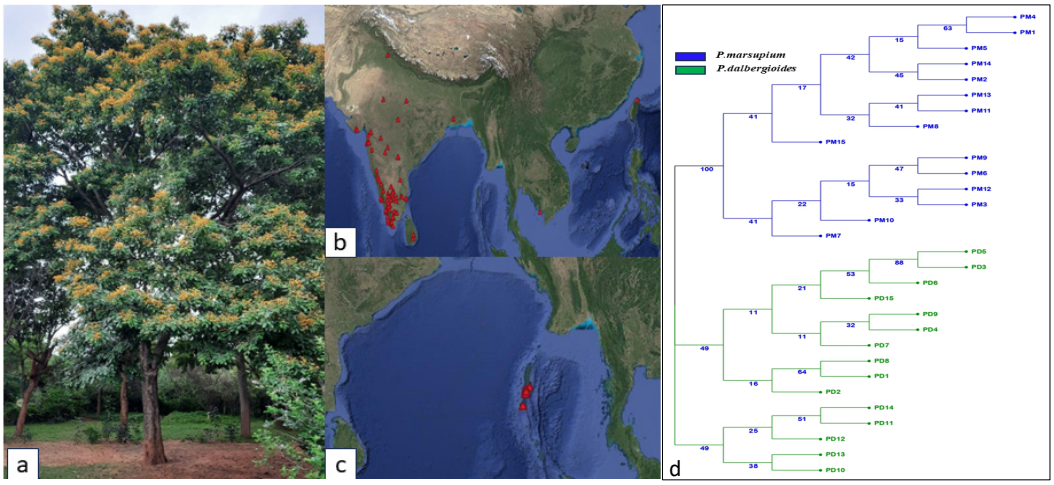

Fig. 1. a) Habitat of *Pterocarpus marsupium,* b) Distribution map-of *Pterocarpus marsupium* c) distribution map of *Pterocarpus dalbergioides,* d) Neighbor-joining tree depicting genetic relationship between *Pterocarpus dalbergioides* and *Pterocarpus marsupium*.

to understanding genetic diversity among these species.

Fifteen leaf samples of *P. dalbergioides* were collected from the Andaman Islands (Latitude 11.750347 and Longitude 92.734963) in collaboration with the local forest department. Additionally, samples of *P. marsupium* were obtained from a research plantation managed by the Karnataka Forest Department in J. B. Kaval, Bangalore (Latitude 13.1102894 and Longitude 77.543312). The specimens were stored in zip-lock bags containing silica gel to facilitate moisture removal for DNA extraction.

Investigation on cross-transferability of DNA markers

The cross-transferability of simple sequence repeats (SSRs) among closely related species within the genus was investigated. The results indicated that SSR markers derived from *P. santalinus* are effectively applicable to *P. marsupium* and *P. dalbergiodes*, highlighting their utility for genetic research and marker development across these taxa. Out of the 33 SSR markers assessed, 7 primer pairs (21%) successfully amplified in *P. dalbergiodes*, whereas 15 primer pairs demonstrated amplification in *P. marsupium*. The remaining markers did not yield amplification in any of the samples (Tables 1 and 2). These findings suggest varying degrees of cross-transferability of the SSR markers between the two species examined. In the species, *P. dalbergioides,* a study utilizing seven amplified molecular markers revealed that two markers, specifically PSSSR-3 and PSSSR-5, exhibited monomorphic characteristics. The observed number of alleles (Na) ranged from 1 to 15, while the effective number of alleles (Ne) demonstrated a range between 1.0 and 14.52. Expected heterozygosity (He) values varied from 0.12 to 0.93, in contrast to observed heterozygosity (Ho), which fluctuated between 0.01 and 0.67. The polymorphic information content (PIC) also displayed variability, with values spanning from 0.12 to 0.93. Furthermore, the average values for Shannon's information index (I) and the fixation index (F) were determined to be 0.96 and 0.54, respectively (Table 3). In the case of *P. marsupium*, out of the 15 markers analyzed, three were found to be monomorphic (specifically, PSSSR-2, PSSSR-6, and PSSSR-16). The number of alleles (Na) ranged from 1 to 13, while the effective number of alleles (Ne) varied between 1.00 and 9.00. The observed heterozygosity

Table 1. Genetic diversity statistics for microsatellite markers on 15 samples of *P. dalbergioides*

Locus	N	Na	Ne	I	Ho	He	F	PIC
PSSSR-2	15	2	1.14	0.25	0.00	0.12	1.00	0.12
PSSSR-3	15	1	1.00	0.00	0.00	0.00	#N/A	-
PSSSR-5	15	1	1.00	0.00	0.00	0.00	#N/A	-
PSSSR-13	15	2	1.80	0.64	0.00	0.44	1.00	0.00
PSSSR-24	13	9	3.98	1.79	0.15	0.75	0.79	0.73
PSSSR-31	14	15	14.52	2.69	0.07	0.93	0.92	0.93
PSSSR-32	15	7	3.33	1.54	0.67	0.70	0.05	0.67
Mean	14.57 ± 0.30	5.29 ± 2.01	3.82 ± 1.84	0.99 ± 0.39	0.13 ± 0.09	0.42 ± 0.15	0.75 ± 0.15	0.49

N = Number of samples, Na = Number of alleles, Ne = Number of effective alleles, I = Shannon's Information Index, Ho = Observed heterozygosity, He = Expected heterozygosity, F = Fixation index

Table 2. Genetic diversity statistics for microsatellite markers on 15 samples of *P. marsupium*

Locus	N	Na	Ne	I	Ho	He	F	PIC
PSSSR-2	14	1	1.00	0.00	0.00	0.00	#N/A	0.00
PSSSR-3	9	2	1.12	0.22	0.11	0.11	-0.06	0.09
PSSSR-8	9	9	8.10	2.14	0.78	0.88	0.12	0.86
PSSSR-6	15	1	1.0	0.00	0.00	0.00	#N/A	0.00
PSSSR-10	15	2	1.47	0.50	0.00	0.32	1.00	0.27
PSSSR-11	15	13	9.00	2.36	0.47	0.89	0.48	0.89
PSSSR-12	9	11	7.36	2.21	0.44	0.86	0.49	0.85
PSSSR-13	15	13	8.82	2.37	0.33	0.89	0.62	0.89
PSSSR-16	15	1	1.00	0.00	0.00	0.00	#N/A	0.0
PSSSR-17	15	6	2.62	1.18	0.93	0.62	-0.51	0.55
PSSSR-21	15	9	3.91	1.78	0.07	0.74	0.91	0.73
PSSSR-24	14	12	8.52	2.31	0.14	0.88	0.84	0.87
PSSSR-28	15	3	1.82	0.77	0.13	0.45	0.70	0.39
PSSSR-29	14	11	8.71	2.29	0.07	0.89	0.92	0.88
PSSSR-31	13	12	8.05	2.31	0.23	0.88	0.74	0.87
Mean	13.4 ± 0.62	7.07 ± 1.27	4.83 ± 0.91	1.36 ± 0.26	0.25 ± 0.08	0.56 ± 0.10	0.52 ± 0.12	0.54 ± 0.3

Table 3. Genetic diversity statistics for *P. marsupium* and *P. dalbergioides*

Species	Na	Ne	I	Pa	He	% transferability	PIC
PD	5.29 ± 2.01	3.82 ± 1.84	0.99 ± 0.39	5.29 ± 2.01	0.42 ± 0.15	21%	0.49
PM	7.07 ± 1.27	4.83 ± 0.91	1.36 ± 0.26	7.07 ± 1.27	0.56 ± 0.10	33%	0.54

PD = P. dalbergioides, PM = P. marsupium, Na = Number of alleles, Ne = Number of effective alleles, I = Shannon's Information Index, Pa = Number of private alleles, He = Expected heterozygosity

(Ho) ranged from 0.07 to 0.93, with a mean value of 0.25. In contrast, the expected heterozygosity (He) ranged from 0.12 to 0.89, yielding a mean of 0.56. Polymorphic information content (PIC) ranged between 0.09 and 0.89. The average values for the Shannon diversity index (I) and fixation index (F) were recorded as 1.36 and 0.42, respectively (Table 3). The mean number of alleles (Na) for *P. dalbergioides* was 5.29 ± 2.01, while the effective number of alleles (Ne) was 3.82 ± 1.84, the expected heterozygosity (He) 0.42 ± 0.15 and Pa of 5.29 ± 2.01. With 21% of marker transferability, a mean polymorphic information content (PIC) of 0.49 was observed. In *P. marsupium* number of alleles (Na) for *P. dalbergioides* was 7.07 ± 1.27, while the effective number of alleles (Ne) was 4.83 ± 0.91, the expected heterozygosity (He) 0.56 ± 0.10 and Pa of 7.07 ± 1.27. With 33% marker transferability, mean polymorphic information content (PIC) of 0.49 and 0.54 was observed in *P. dalbergioides* and *P. marsupium,* respectively (Table 4). The Un-weighted Neighbor Joining (UNJ) tree was constructed for 30 accessions of two species grouped into two major clusters (Fig.1d). Cluster I consisted *P. marsupium* accessions, which can be seen further divided into two major sub-groups. Cluster II consisted of *P. dalbergioides* accessions, which can be seen further divided into two major clusters and four sub-groups. All samples were seen as distinguished from each other.

The *Pterocarpus* genus is known for its strong wood and medicinal properties. However, genetic diversity studies for many species are limited (Johanson et al. 2020). The development of robust molecular markers, particularly SSR markers, is essential for advancing research, given their co-dominant inheritance and genotyping suitability. Previous work led to genome sequencing and the establishment of SSR markers for *P. santalinus* (Sneha et al. 2023). SSR markers have been successfully transferred among closely related species, as demonstrated by their application in *P. dalbergioides* and *P. marsupium*, with transfer rates of 21% and 33%, respectively. The PIC values, indicating the discriminatory power, ranged from 0.12 to 0.93 for *P. dalbergioides* and 0.09 to 0.89 for *P. marsupium*. Notably, the highest PIC value was recorded at 0.93 for marker PSSSR-31. A total of 143 alleles were identified, showcasing polymorphism in both species, with expected heterozygosity values of 0.42 ± 0.15 for *P. dalbergioides* and 0.56 ± 0.10 for *P. marsupium*. UNJ tree analysis clearly differentiated the two species into sister clades, reinforcing the utility of these markers for molecular

studies in *Pterocarpus*. Research across various tree species has demonstrated the transferability of SSR markers. In the *Dalbergia* genus, markers from *D. nigra* and *D. monticola* have been successfully utilized in other species (Buzzati et al. 2016). Other studies have shown similar successes with palm species and legumes, highlighting the markers' efficacy in exploring genetic diversity. The success of SSR transferability depends on the evolutionary relationship between source and target species, influenced by genomic homology and ploidy levels (Dirlewanger et al. 2002). Our findings indicate that *P. marsupium* is more closely related to *P. santalinus* than to *P. dalbergioides*, supported by the notable transferability of SSR markers. Utilization of genetic resources from *P. santalinus*, seven polymorphic SSR markers for *P. dalbergioides* and 15 SSR markers for *P. marsupium*, were identified with transferability rates of 21 and 33%, respectively. Despite the lower transferability, these markers are likely to significantly expand the molecular resources for these timber species and are expected to aid in establishing molecular breeding programs and techniques for assessing genetic diversity in these species.

Authors' contribution

Conceptualization of research (PMK, HRP); Designing of the experiments (PMK, HRP, MVS); Contribution of experimental materials (BNK, KSP); Execution of field/lab experiments and data collection (PMK, BNK, VSK); Analysis of data and interpretation (MVS, AHM, PHV); Preparation of the manuscript (MVS, HRP, PMK).

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