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Genetic Diversity Estimates of *Santalum album* L. through Microsatellite Markers: Implications on Conservation

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Abstract

Sandalwood (*Santalum album* L.) is the second most expensive wood in the world. There are approximately 16 species of sandalwood (*S. album*, *S. spicatum*, *S. austrocaledonicum*, *S. yasi*, *S. lanceolatum*, *S. ellipticum*, *S. macgregorii*, *S. insulare*) occurring naturally throughout Australia, India, Indonesia, Papua New Guinea and the islands of the South Pacific. In India, *S. album* is found all over the country, with over 90% of the area in Karnataka, Tamil Nadu, Kerala, Andhra Pradesh and Telangana state. It is highly economic tropical tree species because of its scented heartwood and heartwood oil. Several causes have been attributed to the depletion of sandalwood population mainly amongst which theft is causing negative effect on the quality of species by constant removal of superior clones. The aim of this study was to determine the genetic diversity of *S. album*. For this, 177 genotypes of *S. album* from 14 populations of three states (Karnataka, Telangana state and Kerala) in southern India were selected. The genetic diversity and genetic structure were characterized through 25 SSR markers developed by cross amplification of different species of Sandalwood. Under this study, following genetic diversity parameters were estimated at individual level and population level; Number of alleles (Na) 9.107, Effective number of alleles (Ne) 7.56, Observed heterozygosity (Ho) 0.187, Expected heterozygosity (He) 0.861, Shannon information index (I) 2.03, F statistics 0.89, Polymorphic information content (PIC) 0.87 and Gene flow (Nm) 4.98. The estimates of gene flow among the populations of Kodada Telangana (Nm = 15.109); IWST Karnataka (Nm = 13.62) than across other geographical populations (Nm = 9.40). Analysis of molecular variance (AMOVA) revealed that 3% of the total variation was due

to differences among populations and 97% due to differences within the populations. The genetic differentiation among populations (F_{ST}) 0.012 at $p < 0.001$ was significant. Structure clustering at Ks = 3 highlighted three distinct groups with the inferred clusters i. 0.369, ii. 0.304, iii. 0.327. Structure analysis revealed that genetic structure of Kerala and Karnataka and Telangana populations were admixtures and classified in three groups. In addition, all the accessions were clearly divided into three major clusters by UPGMA dendrogram which could be further divided into five sub groups. Principal component analysis (PCA) results showed the combined variation 82.2% of these markers. This study highlights the knowledge of genetic variation in sandalwood across the herd population of sandalwood in India. The highest range of polymorphism was detected with SSR markers developed from *Osyris lanceolata* compared to *Santalum. austrocaledonicum*, *Santalum. insulare and Santalum. spicatum.* This study would help in conservation of the Sandalwood populations with high profile of genetic diversity and selection of clones for genetic improvement program.

Keywords

Sandalwood, Genetic Diversity, Alleles, Microsatellite Markers, PCA

1. Introduction

Santalum album L. is medium sized evergreen hemi-parasitic economically important aromatic tree species. The genus *Santalum* belonging to the family Santalaceae, which consist of 15 species (*S. album*, *S. spicatum*, *S. austrocaledonicum*, *S. yasi*, *S. lanceolatum*, *S. ellipticum*, *S. macgregorii*, *S. insulare*) occurring naturally throughout Australia, India, Indonesia, Papua New Guinea and the islands of the South Pacific. In India, *S. album* is found all over the country, with over 90% of the area in Karnataka, Tamil Nadu, Kerala, Andhra Pradesh and Telangana state. Sandalwood has been categorized as "vulnerable" by International Union for Conservation of natural and natural resources [1] Indigenous to peninsular India, its natural distribution estimation, estimated at about 9600 km^2 is confined predominantly to the two states of Karnataka (500 km^2) and Tamil Nadu (3600 km^2) [2]. In India it found distributed all over the country with over 90% of the area in Karnataka, Tamil Nadu, Andhra Pradesh and Kerala. *Santalum album* is culturally and economically important species for more than fifteen countries [3]. Sandalwood is valued for two important traits, heartwood and essential oil obtained from the heartwood. Both of these are considered to be highly prized and are used in incense, perfumes, medicine and carving [4]. Severe biotic factors including human interference, leading to heavy exploitation and massive clearance, grazing, fire and spike disease have selectively eroded the best trees of sandalwood [5]. Result indicated significant reduction of genetic variability in harvested population. This is causing negative effect on the quality of species by constant removal of superior clones. The aim of this study

was to determine the genetic diversity of *S. album*. For this, 177 genotypes of *S. album* from 14 populations of three states (Karnataka, Telangana state and Kerala) in southern India were selected. The genetic diversity and genetic structure were characterized through 25 SSR markers developed by cross amplification of different species of Sandalwood. This study highlights the knowledge of genetic variation in sandalwood across the highest range of polymorphism was detected with SSR markers developed from *Osyris lanceolata compared to Santalum austrocaledonicum*, *Santalum insulare and Santalum spicatum.*

2. Materials and methods:

a) Sample collection: The gentic diversity of the *Santalum album* was assessed by undertaking survey and sampling along the area of natural populations and plantations from the stretch of 1,162 Km (Telanagana, Karnataka and kerala) in southern part of India Figure 1.

b) Sampling: Total 177 samples representing three distinct states of Southern India were selected (Table 1). To precede the genetic diversity study matured leaves of sandalwood randomly collected from healthy trees based on heartwood content Figure 2 and put it into desiccated silica gel containing zip lock covers to absorb the moisture content. The samples were kept for drying at least 3 to 4 days and then stored the dried samples to −20˚C cryogenic freezer to maintain the quality of the leaves for further analysis.

c) Total genomic DNA isolation: Total genomic DNA was extracted from stored silica dried mature leaves of *S. album* L. accessions 177 trees grown in 14

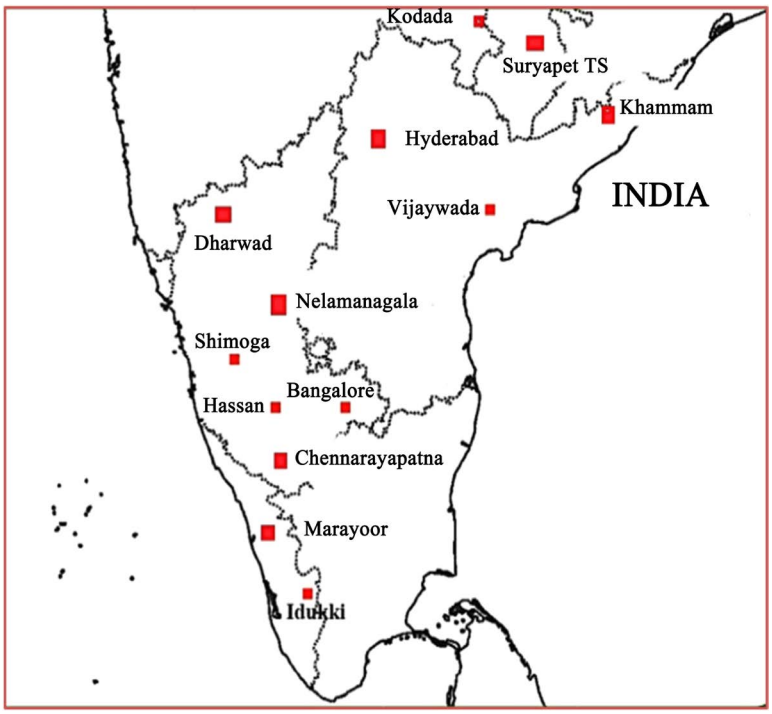


Figure 1. Map of Southern India showing the geographic location of *S. album* sample collection regions in Karnataka, Kerala and Telangana state.

Table 1. Details of *S. album* samples collected locations in Southern Part of India (Karnataka, Kerala and Telangana state).

Sl No.	Provenances	Latitude (N)	Longitude (E)	Origin	Sample size (N)
1.	Kodada TS	10˚13'22.1"	77˚09'18.5"	Plantation	22
2.	Suryapet TS	17˚07'36.2"	079˚50'45.2"	Plantation	22
3.	Khammam TS	17˚13'4.08"	79˚84'9.01"	Plantation	10
4.	Hyderabad TS	17˚55'05.05"	78˚44'48.2"	Plantation	10
5.	Marayur 1 KL	10˚14'47.9"	77˚9'23.4"	Natural	12
6.	Marayur 2 KL	10˚16'39.6"	77˚09'30.9"	Natural	10
7.	Marayur L3 KL	10˚16'41.3"	77˚09'27.8"	Natural	10
8.	IWSKA	13˚00'41.3"	77˚34'17.6"	Natural	10
9.	Nelamangala KA	13˚00'39.9"	77˚34'14.9"	Plantation	20
10.	Dharwad KA	15˚27'36.90"	75˚0'37.02"	Natural	10
11.	Hassan KA	13˚00'29.9"	76˚06'10.8"	Plantation	11
12.	Chennarayapatna KA	13˚04'35.1"	77˚20'57.6"	Natural	10
13.	Gottipura KA	13˚23'0.96"	76˚21'38.4"	Plantation	10
14.	Shimogga KA	13˚55'47.70"	75˚34'5.16.0"	Plantation	10

Figure 2. 15 years old plantation of Sandalwood in Suryapet Telangana state showing girth of stem and tree bearing leaves.

populations (natural and plantation) in Southern India including Karnataka, Telangana state and Kerala by using modified CTAB method [6] [7]. The yield of extracted DNA was quantified by Nano drop at 260/280 nm wavelength in basic Eppendorf spectrophotometer. The purity of DNA was checked by running the samples on 0.8% agarose gel.

d) Screening and selection of microsatellite markers: Total 44 developed primers for other sandalwood species (*O. lanceolata*, [8], *S. austrocaledonicum* [9] *S. insulare* [10] and *S. spicatum* [11] were screened for cross amplification of extracted DNA of *S. album.* 25 primers (**Table 2**) were amplified and generated polymorphism with the selected genotypes for the genetic diversity study.

e) PCR Standardization of PCR (Polymerase Chain Reaction): Polymerase chain reaction (PCR) was performed according to the protocol developed by [12]. The PCR amplifications were carried out in 0.2 mL tube in Mastercycler gradient (Thermo scientific, Germany) in 13 µL reaction volume. The reaction mixture containing 2 µL (30 ng/µL) of genomic DNA as template DNA, 1.5 µL of 10× PCR buffer, 1.5 µL 15 mM $MgCl_2$, 2 µL 10 mM SSR primers (Eurofins Pvt. Ltd.), 10 mM dNTPs and 0.2 µL (3 U/µL) Taq polymerase (Genie, Bangalore Pvt. Ltd.) and 4.4 µL double distilled water to maintain the volume. Amplification reactions were carried out with cycle profiles viz; initial denaturation at 94˚C for 3 minutes, followed by 30 cycles of denaturation at 94˚C for 30 sec, primer annealing at 50˚C - 65˚C (depends on the primer melting temperature) for 1 min, extension at 72˚C for 2 min and a final extension of 10 min at 72˚C temperature.

f) Separation and detection of amplified PCR products: The amplification products were size separated by gel electrophoresis in 4% agarose [13] gel in 1× TAE buffer at a constant 80 V current for 4 h and stained in ethidium bromide solution and gel mixture was observed in UV light provided by a trans-illuminator in (Syngene G: Box) gel documentation system to visualize the bands. The size of the amplification products generated was estimated by using a standard molecular weight ladder depends upon the product size (50 bp and 100 bp Fermentas Thermofisher pvt. Ltd.).

g) Microsatellite marker Statistical data analysis: Due to bi-allelic nature of *S. album* the allele information for 25 loci from selected individuals was assembled as a co-dominant data. The samples were categorized according to sample wise of all populations, and region wise. GenAlex v 6.5 [14] [15] was used to estimates the following genetic diversity parameters at individual level and population level; Number of alleles (Na), Effective number of alleles (Ne), Observed heterozygosity (Ho), Expected heterozygosity (He), Shannon information index (I), F statistics (F_{IS} F_{IT} & F_{ST}), Polymorphic information content (PIC) (Cervus 3.0.3) and Nie's genetic distance (1978) were estimated at the population level. Gene flow was calculated by using the formula $Nm = 0.25\ (1 - F_{ST})/F_{ST}$. Analysis of molecular variance (AMOVA) used to determine the genetic variations in between the populations and among the populations. A phylogenetic tree distance-based cluster analysis was performed and dendrogram based on the unweighted pair group method of arithmetic mean (UPGMA) was constructed using Jaccard's similarity coefficient was based on neighbour joining method constructed in popgen version 3.2 [16] at 0.05 significance level.

h) STRUCTURE and PCA (Principle Component Analysis): An unbiased Bayesian approach using Markov chain Monte carlo (MCMC) clustering of

Table 2. List of selected SSR primers for *Santalum album*. F: Forward; R: Reverse primers.

SI. No.	Locus	Accession No.	Primer sequences (5'-3')	No. of bp.	Repeat motif	Tm (˚C)	Allele range size in species (bp)	Allele size range in *S. album* (bp)
			Osyris lanceolata					
1.	KFOL2	LC126834	**F:** AGAATGTCATTTGAAGGCTCGA **R:** CCTTTCCTCCGTTCTCCTCG	22/20	CGTC	57	178 - 194	170 - 190
2.	KFOL8	LC154966	**F:** GCTGCTTCTACGGTCACTGT **R:** GTGGTGGATATGGAGGTGGC	20/20	CCG	58	120 - 130	150 - 170
3.	KFOL28	LC126839	**F:** ATAAAGGCCCACGAGCTCAG **R:** AACATCGCCATGCAGAACAG	20/20	CT	60	320 - 350	330 - 350
4.	KFOL42	LC126843	**F:** AGGTCCTCCTGCCTGAGAAT **R:** CATAGGGCTGTGATGCCTCA	20/20	TG	61	270 - 300	300 - 315
5.	KFOL16	LC154968	**F:** TGGAGCCCATTCTCTTTCCTT **R:** TGCACGTATTCCACATTTCCA	21/21	GT	59	130 - 160	140 - 165
6.	KFOL19	LC154969	**F:** GGTAGCGAGCGGTGATATGT **R:** ACCTAACAACTTGAAGCTCTCCC	20/23	TC	57	200 - 230	275 - 292
7.	KFOL29	LC154961	**F:** GCTGAATCAGGGACAGGCAT **R:** GGCCTCGAACAAAGTGCATG	20/20	GA	56	230 - 250	159 - 170
8.	KFOL30	LC126840	**F:** CTAAACTGTCAGGGCTTGCT **R:** ATACCTTAGCTCCCGTTGCG	20/20	TC	55	270 - 250	195 - 208
9.	KFOL37	LC126841	**F:** TTTCTAGAGCTAACATACCTCTGAA **R:** ATGACCTGGGTGCTTTGCTG	25/20	TG	59	270 - 306	265 - 310
10.	KFOL17	LC12836	**F:** CATTGACGAATTGCATCCCGT **R:** CGTGAAGTTCAGTGCAAACC	21/20	AG	60	160 - 175	165 - 178
11.	KFOL24	LC126838	**F:** CAACTCGATCGTGCATTGGC **R:** TCCGCATATCCATTTGGCCG	20/20	CT	61	200 - 220	205 - 218
12.	KFOL13	LC126835	**F:** TCCGAGGAACAGGGACTCTT **R:** AGCGAAGAACTCATGAGCGAA	20/21	AC	60	140 - 155	100 - 115
13.	KFOL27	LC154970	**F:** CTAAACTGTCAGGGCTTGCT **R:**ATACCTTAGCTCCCGTTGCG	20/20	ATG	61	135 - 150	200 - 211
14.	KFOL15	LC154967	**F:** CATTGACGAATTGCATCCCGT **R:** CGTGAAGTTCAGTGCAAACC	21/20	CGC	60	170 - 185	180 - 200
15.	KFOL7	LC154965	**F:** CTGTGCAATGGAGAAGGCCA **R:** CGCGGGATTGGGATGTCATA	20/20	ATT	61	250 - 350	260 - 320
			Santalum austrocaledonicum					
16.	mSaCIRE09	AJ831397	**F:** GGAAAGGGTTGACAGGAAAGAAAA **R:** TGCCAGTGAGTGGGAAAGTAGA	24/22	$(CT)_{16}$	59	179 - 190	174 - 187
17.	mSiCIR153	AM113984	**F:** ATGCTTTTGTGGTGATTC **R:**GCTTGGAGGTATCTTGTTGG	18/20	$(CA)_2GA$	58	210 - 220	180 - 215
18.	mSaCIRH10	AJ831403	**F:** AAGCCCGATAACGAGAAAAGAAA **R:** ATGAATAGGGATGGCGAGAGGAT	23/23	$(GA)_{27}$	59	117 - 130	118 - 132
19.	mSaCIRH09	AJ831402	**F:** GCCTCTGCTTCCTCCCATTGTAG **R:** AACTCCATTTGTGATTCCTCCCA	23/23	$(GA)_{20}$	59	150 - 170	200 - 212
20.	mSaCIRG10	AJ831401	**F:** GTGCTACCTGCTACCCTTTTT **R:** CCAATAACGGCTTCAACTTCA	21/21	$(CA)_7$	50	160 - 170	159 - 168
			Santalum insulare					
21.	mSiCIR33	AM113978	**F:** GAAGTTGAAGTTGTTGATGC **R:** AAATGAGAGACCTGAGTGAAG	20/21	$(TC)_{19}$	58	176 - 199	169 - 198
22.	mSiCIR42	AM113980	**F:** CGCACAAACTAAAAACCCT **R:** TCGTAATGGATGGCTTCTTCTA	19/22	$(GA)_{11}$	58	122 - 135	230 - 250
23.	mSiCIRF10	AJ831398	**F:** TTAGGAAACATAGCACACT **R:** GAGCACTTCACCACCATTAC	19/20	$(GA)_{17}$	59	200 - 220	149 - 162

Continued

			Santalum. Spicatum					
24.	SsB112	EU287768	**F:** GGAGCAAGCTAAGCACAC **R:** GCAGCCAAGAAAAATTACTAC	18/21	$(TC)_{16}AC)_{11}$	60	213 - 238	220 - 233
25.	SsB122	EU287769	**F:** AGGTGCGTCTCTTTCATACTA **R:** CAGTCGTTTCGGTCACA	21/17	$(CT)_{19}$	60	234 - 273	240 - 252

samples was conducted via the STRUCTURE v2.2.3 software (http://www.stats.ox.ac.uk/) [17]. Parameters were set as diploid data for each individual and assessed for values of K ranging from 1 to 11 Burn in MCMC iteration setting were 50000 and 100000 respectively. Allele frequencies were correlated for each value of K, 10 replicate simulations using a model allowing for admixture used correlated allele frequencies. Data from each sample analysis were combined andΔK statistics calculated using Structure harvester [18] as for the microsatellite marker analysis. The appropriate value forΔK was estimated from LnP (K) value described byΔK = m (|L"K|)/s[L(K)] [19] and an adhoc method based on the second rate order rate of change of the likelihood (ΔK) described by [20]. Principal component analysis was performed to show the differentiation of the *S. album* accessions in two-dimensional array of eigen vector using the variance and eigen module [21]. PCA is a prominent development in genotypes collections from different geographical locations that allows a better understanding of the entirely different populations. PCA was executed for all the accessions by using Minitab v.18. by using the selected microsatellite markers. PCA used to represent genetic relations among the accessions from different geographical locations.

3. Results

i) DNA quantification: The quality of extracted DNA was quantified by running the 3 µL DNA on 0.8% agarose gel in 1X TAE buffer stain containing 4 µL of 0.3% ethidium bromide solution checked the purity of DNA **Figure 3**.

ii) SSR marker amplification and polymorphism: All markers produced polymorphic amplification bands in selected genotypes **Figure 4**, which ranged from 120 - 350 bp. 15 to 6 number of alleles was generated at each locus with total 172 alleles. High polymorphism was found in LC154966, LC126838, LC126839, LC154969 and LC154965 which were specifically belongs to *O. lanceolata* and AJ831401, AJ831403, EU287768 was showing low polymorphism. AJ831397 AJ831398 primers were gradient polymorphic and AM113980, LC154967, LC154970, LC126840 and LC126843 was non-repeatable stable monomorphic in each population.

iii) Genetic diversity: In this study highest number of alleles (Na) (10.05) with the average of 8.98 was found in Karnataka state followed by Kerala and Telangana 8.83 8.03 respectively. In Kerala state the observed and expected heterozygosity was 0.168 and 0.847, which revealed the very low genetic diversity in Kerala populations (**Table 3**). Total numbers of alleles were ranged from (16.5 - 6.7) LC154969 in LC154961 followed by Kodada Telangana state and Marayur

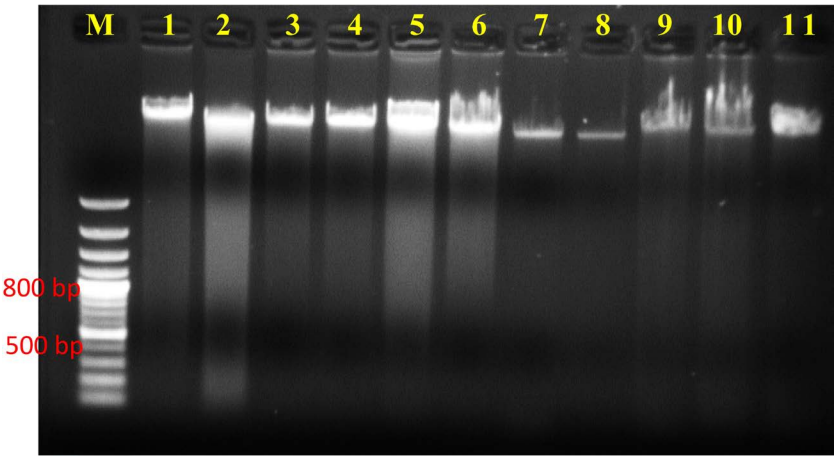
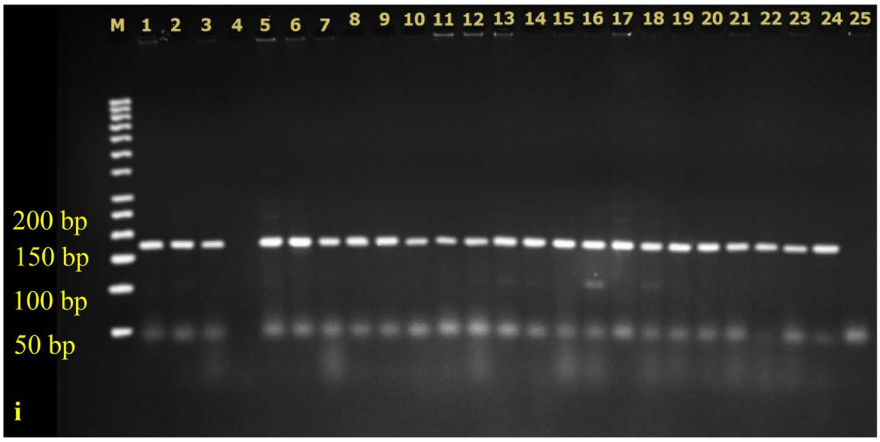
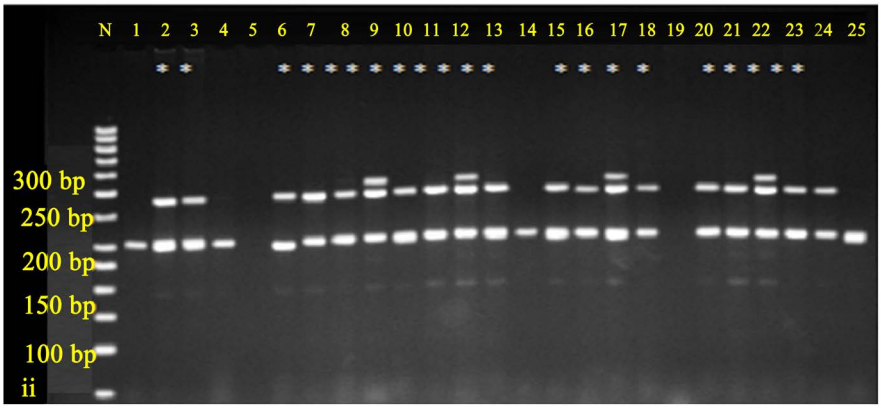
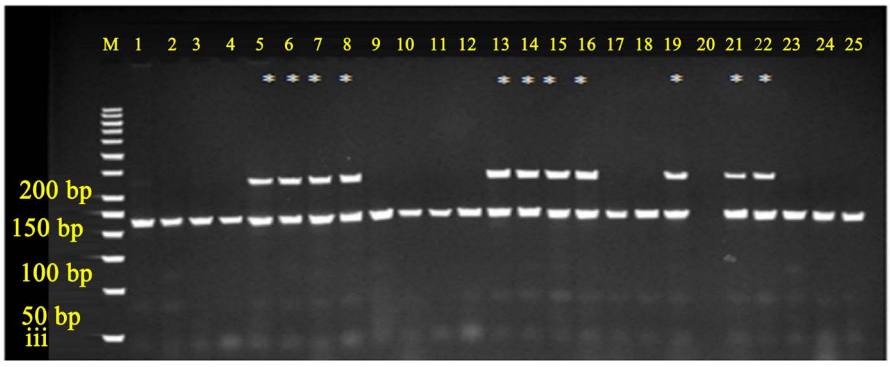


Figure 3. Sandalwood genomic DNA extracted by modified CTAB protocol.







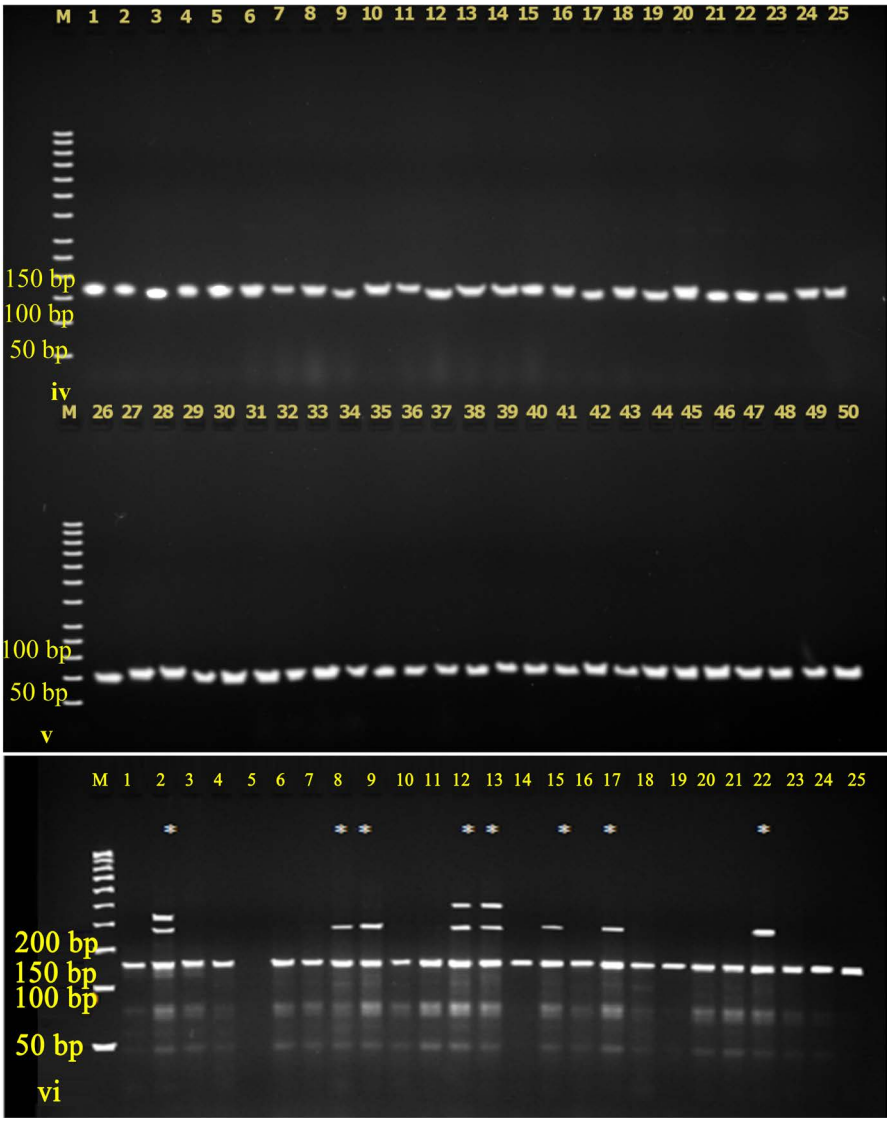


Figure 4. Amplification and polymorphism profile of *S. album* L. accessions using SSR markers. M: Ladder (50 bp. Fermentas Thermofisher pvt. ltd.) i. LC154967 (*O. lanceolata*) ii. AM113984 (*S. austrocaledonicum*) iii. AJ831401 (*S. austrocaledonicum*) iv. AJ831397 (*S. austrocaledonicum*) v. AJ831398 (*S. insulare*) vi. AJ831403 (*S. austrocaledonicum*).

Table 3. Mean and standard error of Na, Ne, Ho, He, F and I for over all three states using microsatellite markers.

State	Na	Ne	Ho	He	I	F
Karnataka	10.05	8.09	0.187	0.861	2.03	0.89
Kerala	8.83	7.17	0.168	0.847	2.005	0.81
Telangana	8.03	7.43	0.186	0.85	2.12	0.78
Average	8.98	7.56	0.180	0.85	2.052	0.81

location 2. Effective number of alleles was ranged from (0.50 - 0.294) LC154969 and AJ831401 followed by Kodada Telangana followed by Hassan Karnataka. In *O. lanceolata*, 17 microsatellite markers were developed in which 12 were poly-

morphic and 5 (LC154965, LC154966, LC154967, LC154970 and LC154961) were monomorphic. In this study out of 15 primers, five were polymorphic (LC154966, LC126834, LC126835, LC126838, LC126839, LC126841, LC154961, LC154968, LC154969 and LC154965) and monomorphic (LC126843, LC12836, LC126840, LC154967 and LC154970). *O. lanceolata* the no. of alleles per locus was ranged from (2 - 17) with the average 8.05 where as in this study total no. of alleles per locus in *S. album* was observed by these markers were ranged from (6 - 16.55) with the average of 9.33. The no. of alleles across the total 14 populations ranged from Marayur location 2 Kerala 7.90 to 11.65 Kodada Telangana. In *O. lenaceolata* the expected heterozygosity was ranged from 0.00 - 0.77 while in *S. album* it was found 0.54 (Marayur Kerala, Chennarayapatna and Shimogga Karnataka) to 0.927 Kodada, Suryapet Telangana and IWST Bangalore Karnataka [8]. Highest observed heterozygosity (Ho) was found in AM113984 and lowest was found in LC154967 surveyed by Chennarayapatna Karnataka and Marayur location 2 Kerala. Highest expected heterozygosity was found in LC154969 and lowest was obtained in LC126834 and in population it was Suryapet Telangana and lower was in Marayur location 1 Kerala. Shannon information index was ranged from (2.435 - 1.69) followed by Marayur location 3 and in lowest in IWST Karnataka (1.93 - 2.2). Fixation index was highest in 968 and lowest in - 0.082 followed by Dharwad and Hassan Karnataka (0.77 - 0.83) **Table 4** and **Table 5**.

Table 4. Mean and Standard error (SE) for each Locus, Sample Size (N), no. of alleles (Na), effective no. of alleles (Ne), Shannon Information Index (I), Observed heterozygosity (Ho), Expected heterozygosity (He) and Fixation Index (F) over all populations using microsatellite markers.

Population	N	Na		Ne		Ho		He		F		I	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Kodada TS	22	11.650	1.139	8.778	0.754	0.191	0.088	0.869	0.012	0.795	0.095	2.224	0.091
Suryapet TS	22	11.200	0.99	8.59	0.70	0.173	0.083	0.87	0.008	0.81	0.094	2.21	0.07
Khammam TS	10	8.500	0.74	7.37	0.65	0.180	0.084	0.84	0.011	0.80	0.89	2.00	0.65
Hyderabad TS	10	8.750	0.63	7.61	0.56	0.200	0.092	0.85	0.009	0.78	0.092	2.056	0.56
Marayur 1 KL	12	9.50	0.74	7.86	0.64	0.183	0.084	0.85	0.010	0.79	0.100	2.104	0.64
Marayur 2 KL	10	7.90	0.59	6.60	0.49	0.150	0.082	0.83	0.009	0.83	0.092	1.93	0.49
Marayur L3 KL	10	8.15	0.60	7.07	0.54	0.170	0.082	0.84	0.010	0.81	0.091	1.97	0.54
IWST Bangalore KA	20	11.500	0.94	9.05	0.80	0.200	0.092	0.87	0.008	0.78	0.090	2.2	0.80
Nelamangala KA	10	8.75	0.699	7.52	0.58	0.150	0.092	0.85	0.009	0.78	0.098	2.04	0.58
Hassan KA	11	8.05	0.651	6.94	0.60	0.160	0.082	0.84	0.010	0.83	0.100	1.95	0.60
Chennarayapatna KA	10	8.10	0.59	6.913	0.521	0.200	0.082	0.85	0.012	0.82	0.090	1.96	0.52
Dharwad KA	10	8.30	0.73	7.264	0.644	0.200	0.092	0.84	0.011	0.78	0.089	1.98	0.64
Gottipura KA	10	8.45	0.65	7.16	0.534	0.200	0.092	0.84	0.08	0.77	0.101	2.00	0.53
Shimogga KA	10	8.65	0.72	7.16	0.621	0.184	0.083	0.84	0.011	0.78	0.101	2.00	0.62
Overall pop	177	9.10	0.743	7.56	0.616	0.181	0.0864	0.84	0.015	0.796	0.151	2.044	0.523

Table 5. Mean and SE over populations for each locus, no. of alleles (Na), no. effective alleles, Shannon Information Index (I), observed heterozygosity (Ho), expected heterozygosity (He), Fixation Index (F) and Polymorphic information content (PIC).

	Locus	834	978	968	401	403	980	397	984	961	969	402	398	835	840	967	970	836	838	843	839	768	769	966	841	965
Na	Mean	6.0	13.42	8.571	7.429	7.214	7.500	8.500	15.92	6.786	16.500	7.286	7.929	8.143	8.214	8.214	7.857	7.786	7.786	7.571	13.42	10.65	10.76	8.34	11.24	13.56
	SE	0.32	1.329	0.510	0.291	0.366	0.572	0.374	0.642	0.318	0.747	0.450	0.385	0.376	0.447	0.334	0.361	0.408	0.447	0.359	0.796	0.980	0.56	0.45	0.76	0.65
Ne	Mean	5.18	10.25	7.116	6.104	6.304	6.127	7.554	13.3	5.619	13.944	6.123	6.555	6.903	6.931	7.288	6.457	6.616	6.471	6.042	10.32	8.764	8.56	6.57	9.49	11.27
	SE	0.33	0.918	0.324	0.294	0.367	0.336	0.371	0.427	0.264	0.507	0.391	0.350	0.357	0.286	0.320	0.296	0.326	0.330	0.276	0.666	0.556	0.17	0.29	0.26	0.34
I	Mean	1.69	2.397	2.040	1.888	1.883	1.887	2.067	2.673	1.802	2.709	1.872	1.960	1.999	2.004	2.034	1.947	1.953	1.937	1.898	2.435	1.38	2.45	1.78	1.98	2.09
	SE	0.06	0.101	0.050	0.045	0.059	0.060	0.046	0.035	0.046	0.039	0.059	0.049	0.048	0.048	0.042	0.043	0.049	0.056	0.041	0.058	0.0484	0.035	0.045	0.026	0.098
Ho	Mean	0.00	0.647	0.00	0.00	0.00	0.00	0.00	1.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.089	0.065	0.032	0.015	0.087
	SE	0.00	0.103	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.00	0.00	0.00	0.00
He	Mean	0.79	0.890	0.855	0.830	0.833	0.831	0.863	0.924	0.817	0.927	0.829	0.841	0.849	0.852	0.859	0.841	0.844	0.840	0.830	0.898	0.7985	0.657	0.621	0.546	0.743
	SE	0.01	0.011	0.007	0.010	0.012	0.008	0.007	0.002	0.008	0.003	0.009	0.009	0.009	0.007	0.006	0.007	0.007	0.009	0.007	0.006	0.003	0.005	0.006	0.003	0.001
F	Mean	0.99	0.290	1.000	1.000	1.000	1.000	1.000	0.082	1.000	-0.079	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.114	-0.031	0.87	0.065	0.045	0.002
	SE	0.00	0.110	0.000	0.000	0.000	0.000	0.000	0.003	0.000	0.003	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.008	0.009	0.007	0.005	0.00	0.000
PIC		0.82	0.95	0.893	0.857	0.868	0.856	0.899	0.94	0.85	0.94	0.86	0.87	0.89	0.90	0.86	0.88	0.88	0.86	0.92	0.932	0.876	0.76	0.654	0.581	0.710

From Figure 5, it was clearly depicted that IWST Bangalore Suryapet, Kodada populations were showing high expected heterozygosity whereas Kerala populations were presenting very low expected heterozygosity which indicated the low genetic diversity and high homozygosity in Kerala populations than Telangana and Karnataka Populations.

AMOVA revealed that 3% of the total variation was due to differences among populations and 97% due to differences within the populations in 25 microsatellite markers in selected populations. The genetic differentiation among populations (F_{ST}) 0.012 at $p < 0.001$ was significant Table 6.

Analysis of the dividing of the genetic variability in *S. album* indicated that it mainly occurred due to within populations and among the individuals variation. In population variability was found only 3% of the total variation in southern India (Fst = 0.027). Gene flow is the movement of genes into or out of the populations. This flow may be due to migration of pollens or genetic drift in different populations. The estimates of gene flow among the populations of Kodada Telangana (Nm = 15.109); IWST Karnataka (Nm = 13.62) than across other geographical populations (Nm = 9.40). Additionally, the estimates of gene flow between Telangana and Kerala (Nm = 10.178) was greater than Telangana and

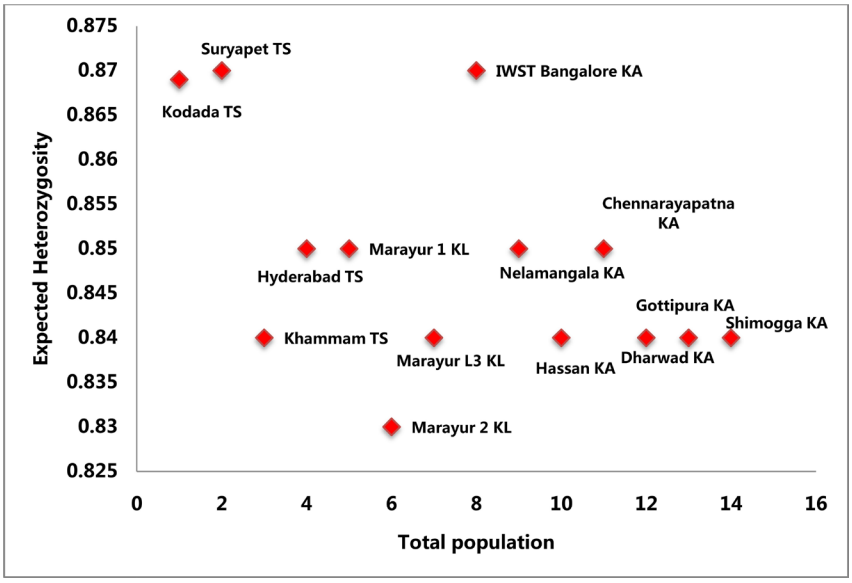


Figure 5. Relationship between Expected heterozygosity and 14 *S. album* populations of southern India.

Table 6. Analysis of Molecular Variance (AMOVA) of *S. album* accessions based on genotypes with SSR markers.

Source of Variation	df	SS	Variance Component	Estimated variation	% of variation
Among populations	13	152.588	11.738	0.11	3%
Within populations	340	3047.59	8.964	8.964	97%
Total	353	3200.18	20.702	9.074	100%

Karnataka (Nm = 9.78) followed by Kerala and Karnataka (Nm = 8.49). These results suggested that there was a high exchange of genepool between Telangana populations were observed genetically more related to Kerala than Karnataka populations Table 7.

iv) Distribution of Genetic variation: F_{IS} and F_{IT} reflects the degree of deviation from Hardy Weinberg equilibrium within the populations and among the populations. F_{IS} of 14 populations with the selected genotypes of *S. album* ranged from -0.114 of locus LC126839 to 1.00 of several loci (LC126843, LC54968) belongs to *O. lanceolata*, AJ831397, AJ831398 *S. austrocaledonicum* and *S. insulare* with the average 0.80 (Table 8) indicated that significant heterozygosity deficiency at LC126839 and AM113984 (*O. lanceolata* and *S. austrocaledonicum*). F_{ST} as the population differentiation coefficient that measures the degree to genetic differentiation among populations. The lowest F_{ST} was obtained in AM113984 and the highest F_{ST} was found in 966. F_{ST} values of the genetic distance among all the populations were found significant. The evaluations of gene flow into the populations by microsatellite markers was 5.32 *S. album* populations. In *S. austrocaledonicum* markers gene flow was observed (Nm = 6.79); in *O. lanceolata* (Nm = 5.164); *S.* insulare (Nm = 4.492) and *S. spicatum* (Nm = 4.85). This result indicated that the interchange of genetic pools of *S. album* was more related to *S. austrocaledonicum* and *O. lanceolata*.

Nei's genetic distance revealed that the nearest genetic distance was found in between IFB Hyderabad Telangana state and Marayur location 3 populations Table 9. The longest genetic distance was found in between Kodada Telangana

Table 7. F statistics and Nm (Gene flow) of *S. album* populations; Nm = [(1/Fst) − 1]/4.

SI No.	Populations	Fst	Nm
1.	Kodada TS	0.016	15.109
2.	Suryapet TS	0.019	12.729
3.	Khammam TS	0.023	10.686
4.	Hyderabad TS	0.027	9.081
5.	Marayur 1 KL	0.032	7.537
6.	Marayur 2 KL	0.037	6.494
7.	Marayur L3 KL	0.025	9.610
8.	IWSKA	0.032	13.624
9.	Nelamangala KA	0.018	7.565
10.	Dharwad KA	0.028	8.710
11.	Hassan KA	0.032	7.469
12.	Chennarayapatna KA	0.030	8.224
13.	Gottipura KA	0.033	7.396
14.	Shimogga KA	0.026	7.428
	Average	0.027	9.405

Table 8. F statistics and Nm (Gene flow) of *S. album* microsatellite markers; Nm = [(1/Fst) − 1]/4.

Loci	F_{IS}	F_{IT}	F_{ST}	Nm
KFOL2	0.99	0.99	0.06	3.38
KFOL8	0.87	0.76	0.076	5.61
KFOL28	−0.11	−0.08	0.029	8.31
KFOL42	1.00	1.00	0.096	4.48
KFOL16	1.00	1.00	0.050	4.79
KFOL19	−0.07	−0.05	0.021	**11.85**
KFOL29	1.00	1.00	0.057	4.16
KFOL30	1.00	1.00	0.055	4.25
KFOL37	0.85	0.43	0.065	3.46
KFOL17	1.00	1.00	0.058	4.03
KFOL24	1.00	1.00	0.06	3.70
KFOL13	1.00	1.00	0.062	3.77
KFOL27	1.00	1.00	0.040	6.05
KFOL15	1.00	1.00	0.059	3.97
KFOL7	0.35	0.26	0.064	5.65
mSiCIR33	0.27	0.32	0.064	3.62
mSiCIR42	1.00	1.00	0.041	5.90
mSiCIR153	−0.08	−0.04	0.022	**10.86**
mSaCIRE09	1.00	1.00	0.048	4.97
mSiCIRF10	1.00	1.00	0.046	5.28
mSaCIRH10	1.00	1.00	0.054	4.40
mSaCIRH09	0.87	0.65	0.053	4.16
mSaCIRG10	1.00	1.00	0.051	3.65
SsB112	1.00	1.00	0.045	4.98
SsB122	1.00	1.00	0.053	4.72
Average (=SE)	0.80 (0.093)	0.80 (0.09)	0.050 (0.003)	5.32 (0.52)

state and Dharwad Karnataka populations that indicated that the populations of Telangana state mostly belong to Kerala populations rather than Karnataka populations. These genetic distance results were also justified by the gene flow (Nm) Table 7.

The dendrogram grouped the selected populations into three major clusters. Cluster I involved all Telangana state populations. Cluster II divided into 2 sub-clusters. Sub-cluster I Marayur location 1, IWST KA and Nelamangala KA populations were included and in sub-cluster II included Marayur location 3, Chennarayapatna KA and Gottipura KA. Cluster III divided into 3 sub clusters: sub-cluster I—Shivamogga, sub-cluster II—Marayur location 3 and in sub-cluster III Dharwad, Hassan were included (Figure 6).

Table 9. Pairwise population matrix of [22] unbiased Nei's genetic distance.

KOTS	SY TS	Kham TS	Hyd TS	MKE1	MKE2	MKE3	IWKA	NelKA	HasKA	CheKA	DharKA	ShiKA	GoKA	
***														KOTS
0.081	***													SY TS
0.095	0.012	***												Kham TS
0.081	0.067	0.049	***											Hyd TS
0.045	0.000	0.000	0.060	***										MKE1
0.142	0.128	0.088	0.000	0.148	***									MKE2
0.198	0.099	0.111	0.057	0.095	0.193	***								MKE3
0.093	0.051	0.013	0.000	0.004	0.098	0.110	***							IWKA
0.182	0.106	0.053	0.005	0.118	0.078	0.092	0.007	***						NelKA
0.125	0.016	0.007	0.038	0.074	0.123	0.164	0.000	0.109	***					HasKA
0.075	0.098	0.037	0.000	0.056	0.064	0.169	0.045	0.103	0.035	***				CheKA
0.222	0.184	0.074	0.120	0.178	0.286	0.179	0.101	0.117	0.045	0.114	***			DharKA
0.149	0.056	0.096	0.030	0.033	0.153	0.137	0.025	0.041	0.090	0.135	0.222	***		ShiKA
0.146	0.108	0.123	0.104	0.130	0.171	0.084	0.042	0.106	0.080	0.082	0.133	0.136	***	GoKA

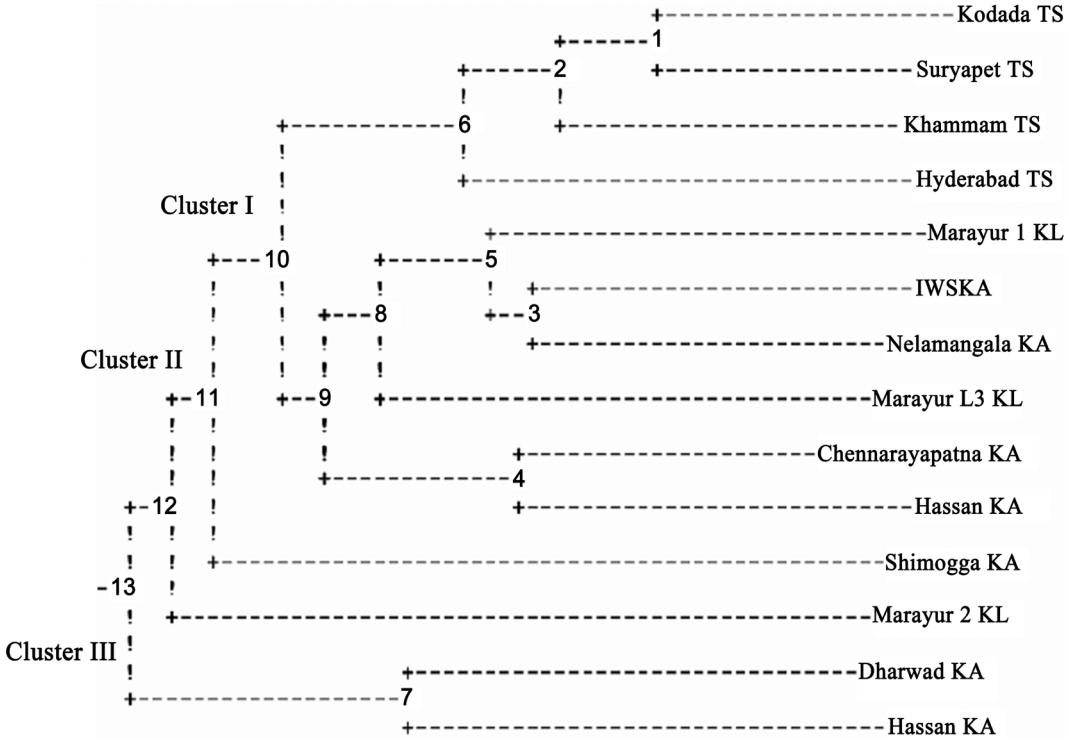


Figure 6. UPGMA Dendrogram for selected populations of *S. album* based on [23] genetic distance method using SSR markers.

Based on UPGMA cluster analysis the 177 accessions were divided into 5 major groups. Group 1 included majority of sandalwood accessions from Karnataka and Telangana state. Within this group 4 sub-groups were observed. Sub group 1

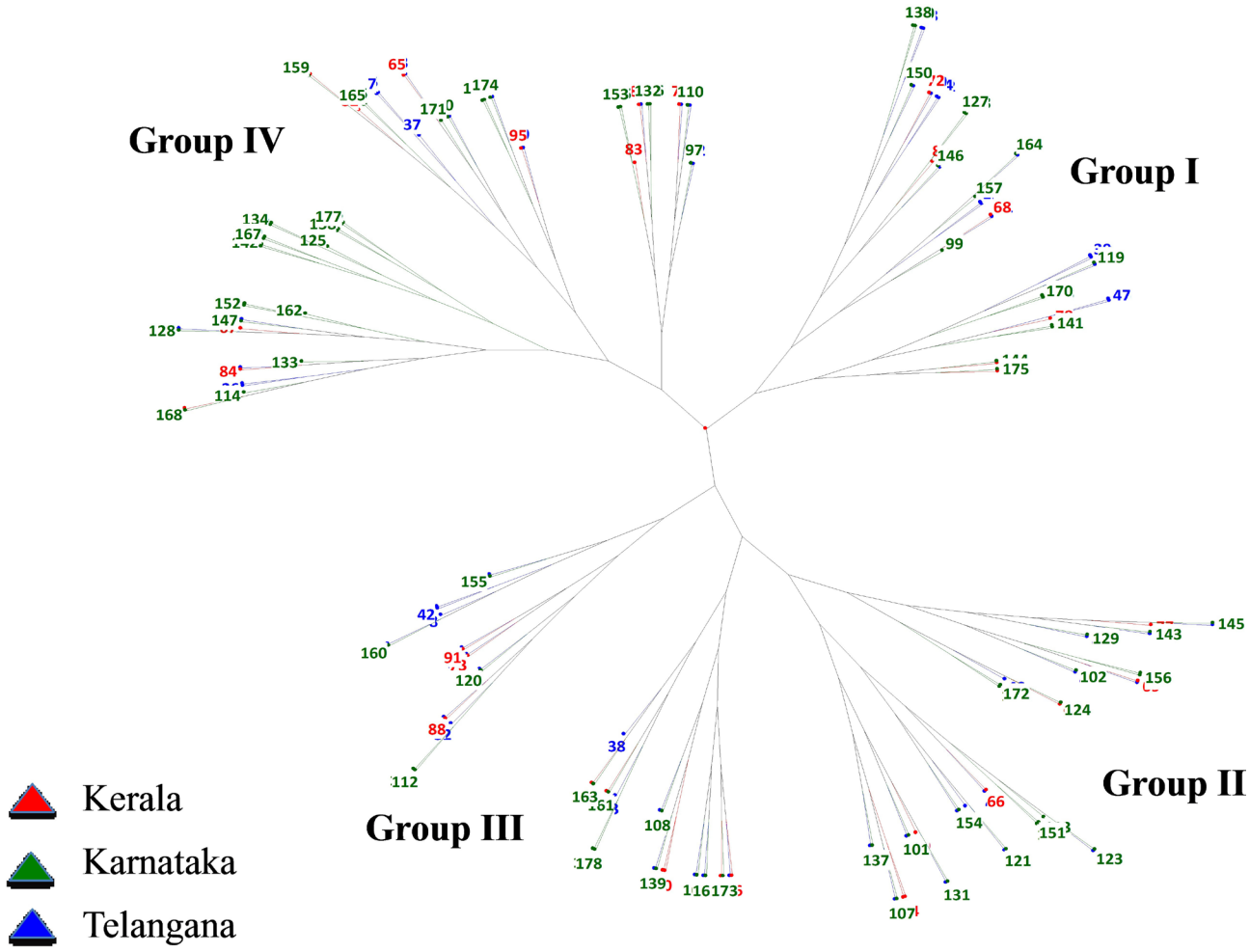


Figure 7. Unweighted pair group methods using arithmetic average algorithm dendrogram (Darwin v. 6.0.17) based on 25 microsatellite (SSR) markers for 177 *S. album* genotypes.

included Chennarayapatna, Dharwad, Shimogga Karnataka and Kodada Telangana state. In subgroup 2 Chennarayapatna, Nelamangala, IWST Bangalore and Kerala location 1 accessions were grouped. Subgroup 3 included accessions of Nelamangala, Dharwad, Chennarayapatna and few accessions from Kerala and Telangana state. In subgroup 4 included accessions belongs to Nelamangala and Gottipura Hoskote Karnataka. Group 2 included Hassan, Nelamangala, Chennarayapatna, Gottipura, Dharwad and few accessions from Kerala. Group 3 contained within all the accessions from Karnataka state except one genotype from Marayur location 3. Group 4 divided into 3 sub-group in which group 1 included IWST Bangalore, Nelamangala and Gottipura were grouped together and in subgroup 2 included Telangana state *S. album* accession. Mixed accessions of Kerala and Telangana were observed in subgroup 3. Group 5 included Shimogga and Dharwad Karnataka accessions mixed with Telangana and Kerala (Figure 7). Several subgroups were observed in *S. album* accessions indicated the genetic variability within and among the accessions in each population.

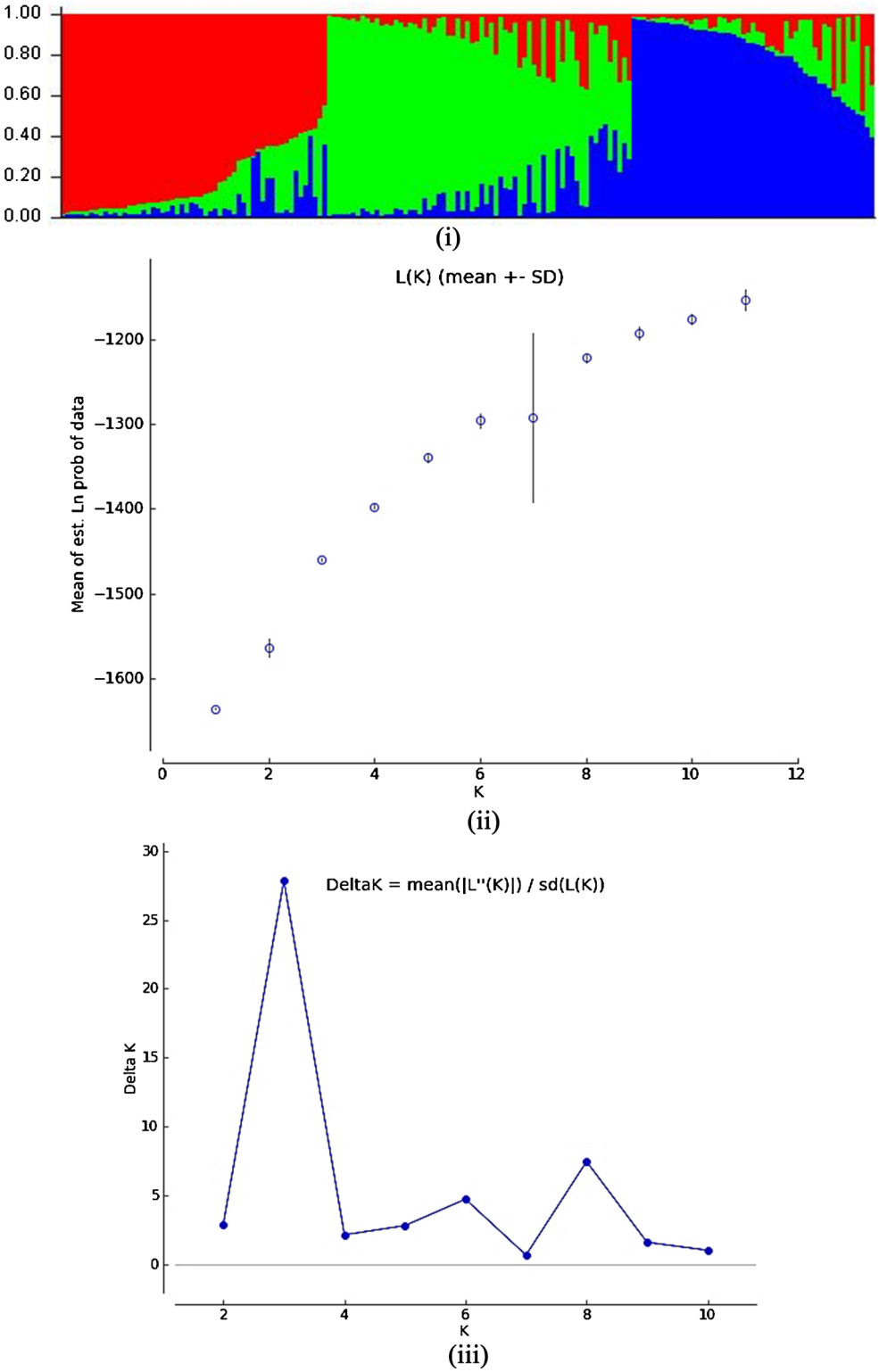


Figure 8. Genetic structure plot of 14 *S. album* populations based on the Structure analysis Red, Green and Blue demonstrating different clusters. **i.** Model based origin of accessions with cluster number 3 K = 3. Membership coefficients (Q_s) are denoted vertically for each sample **ii.** Likelihood plots **iii.** ΔK from the structure analysis of the full set of 14 populations. Only the maximum log-likelihood from the 11 iterations runs performed at each K included in the log-likelihood plots.

Structure clustering at Ks = 3 highlighted three distinct groups with the inferred clusters i. 0.369, ii. 0.304, iii. 0.327. The estimated mean value of Ln probability likelihood was found -160.16 with the variance of ln likelihood was 851.5. In group I the total percentage of genetic structure variation was 28.4% (*red lines) in which 46% belongs to Karnataka followed by few Kerala and Telangana admixtures. In group II, 26.55% (*green lines) and group III, 20.33% (*blue lines) with 21.46% of admixtures. Structure analysis revealed that genetic structure of Kerala and Karnataka and Telangana populations were admixtures and classified in three groups Figure 8 and it is varying among the individuals with 25 primers and the structure lines were divided into three groups I, II, III and admixtures.

Table 10. Principal component analysis among different microsatellite markers of selected *S. album* Accessions.

PCs	Eigenvalue	Variation%	Cumulative percentage (%)
PC1	3.16	7.9	7.9
PC2	3.01	7.5	15.4
PC3	2.8	7.1	22.6
PC4	2.5	6.3	28.9
PC5	2.4	6.2	35.1
PC6	2.2	5.7	40.7
PC7	2.2	5.5	46.2
PC8	1.9	4.9	51.1
PC9	1.9	4.9	56.0
PC10	1.8	4.5	60.6
PC11	1.6	4.2	64.8
PC12	1.6	4.1	68.9
PC13	1.4	3.7	72.5
PC14	1.4	3.6	76.1
PC15	1.3	3.3	79.4
PC16	1.1	2.8	82.3
PC17	0.98	2.5	84.7
PC18	0.91	2.3	87.0
PC19	0.90	2.3	89.3
PC20	0.87	2.2	91.5
PC21	0.79	2.0	93.5
PC22	0.74	1.9	95.3
PC23	0.66	1.7	97.0
PC24	0.65	1.6	98.6
PC25	0.544	1.4	100

Level of significance *$p < 0.05$; **$p < 0.01$.

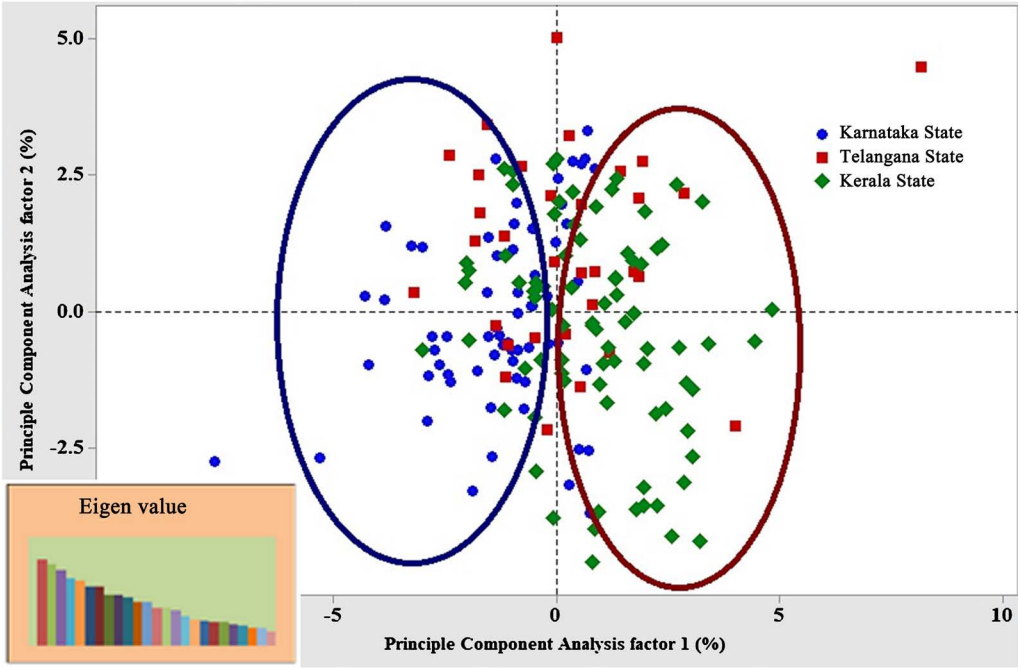


Figure 9. Primary Principle Component analysis (PCA) of genetic distances among the *S. album* accessions from 14 populations (Colored vertical bars in the Eigenvalues histogram.

PCA analysis grouped Sandalwood populations into two mixed groups of Karnataka, Kerala and Telangana populations. PCA showed similar results as for UPGMA dendrogram clustering. A three-dimensional scatter plot based on the first and second principal components of the 177 accessions indicated different levels and directions. So that the degree of relationship between the SSR markers could be discriminated. Eigenvalue of each principal component analysis was greater than 1 (except 9 PCs) showed high variability and performed the importance for the selection of the genotypes. The above results showed the combined variation 82.2% of these markers. Eigenvalues and variance associated with each principal components gradually decreased and cumulative percentage increased **Table 10**. The PCA analysis further illustrates the geographical differences between the populations. Analysis revealed that Kerala populations and Telangana populations were more similar than Karnataka and Telangana populations with few admixtures among individuals. Total populations were clustered into 2 discrete groups (I) Karnataka (II) Kerala state and Telangana with the overlapping of few samples of Karnataka, Kerala and Telangana. The PC1 (Component 1) 7.9% and PC2 (Component 2) 7.5% of the total variance 81.1 respectively **Figure 9**.

4. Discussion

The main aim of this study was to explore the genetic diversity and determining the genetic structure of *S. album* based on their geographic location and allows the selection of superior genotypes for further genetic improvement in *S. album*. [24] used allozymes to identify the genetic variability in *S. album* that could be

used for *in situ* conservation and revealed the high level of heterozygosity with average no of alleles and no of effective alleles (Na and Ne) was (2.174 and 1.602) in sandalwood populations in peninsular India. In this research we found high level of polymorphism by using microsatellite markers with average No. of alleles and no of effective alleles per locus (Na and Ne) was (9.10 and 7.56). In *S. austrocaledonicum* a native species of New Caledonia and Vanuatu genetic diversity study by 8 developed SSR markers, estimated the total number of alleles per locus (Na) was ranged from 3 - 33 [9] while in *S. album* (Na) was ranged from 7.25 - 8.50 by these selective markers. These results suggested that *S. austrocaledonicum* was highly polymorphic than *S. album* accessions. High polymorphism was found in *S. spicatum* with the number of alleles per locus ranged from (2 - 10) and in *S. album* it was ranged from (9 - 10) with the range of 9.5. [25] In *S. lanceolatum* (Northern sandalwood) and *S. leptocladum* (Southern sandalwood) a native species of Australia characterized eight nuclear microsatellite markers and tested against [9] [10] for amplification. Three microsatellite markers Lanc03, MSaCIR09 and MSaCIRH10 (AM113978 AJ831397 AJ831403) were produced total number of alleles (Na) and expected heterozygosity in *S. lanceolatum* was Na (9, 16 and 10); He (0.37, 0.76 and 0.56) respectively. In *S. leptocladum* Na (2, 8 and 6); He (0.11, 0.83 and 0.61). In our study for above mentioned primers in *S. album* were showing more polymorphic no of alleles, Na (13.42, 8.500 and 7.21); Ho (0.890, 0.833 and 0.863). These results indicated that more polymorphism in *S. album* populations than other sandalwood species. In *S. spicatum* the expected heterozygosity (Ho) was ranged from 0.00-0.884 with the mean value 0.756 [12]. Whereas in this study the expected heterozygosity was observed from (0.657 - 0.798) with the mean value 0.727 in *S. album* in naturally and plantation population growing in Southern India. The above results indicated the similarity of *S. album* and *S. spicatum* due to presence of similar number of alleles and expected heterozygosity. Higher genetic diversity was found in nine *Lolium* species by using of Thirty-two nuclear SSR markers and the average PIC value 0.83 which revealed the outcrossing species than inbreeding species [26]. In this study the average PIC value 0.87 was obtained for selected markers respectively which also revealed the outcrossing performance of *S. album.* Sandalwood performs largely outcrossing mating and to avoid inbreeding depression they required abundant population size for gene flow. Cluster analysis of the selected sandalwood populations suggested that gene flow and outcrossing opportunities might have been restricted [27] and the above results showed that lowest gene flow was obtained in LC126834 primer and highest were found in LC126839 with an average 5.29. The genetic differentiation among population is low when the coefficient of degree of differentiation (F_{ST}) is less than 0.25 [28]. The F statistics results of the present study showed that the genetic differentiation among 14 sandalwood (natural and plantation) populations was relatively low (F_{ST} max—0.104 and F'_{ST}—0.118, $P > 0.001$) and the genetic variation was mostly found in within populations and within indi-

viduals. The low degree of genetic variability within sandalwood populations might be due to the fragmentation of a previously large origin population, discrimination of attributions due to random genetic drift and minimum amount of gene flow between the populations [29]. The average of F_{ST} was 0.003 that denoted the 3% of the genetic variation existed among the populations. The average of Nm was 5.32 > 1 implied that the high rate of gene flow occurred within populations than among the populations. Comparison of the UPGMA dendrogram with the two-dimensional principal component analysis plot provided a greater understanding of the complexity of relationship of the selected microsatellite markers, *S. album* accessions and the germplasm origin. This study revealed that gene flow was high in natural populations as compared to plantation populations. The nearest genetic distance was found in between Telangana and Kerala. The longest genetic distance was found in between Telangana state and Karnataka populations that indicated that the populations of Telangana state mostly belongs to Kerala populations rather than Karnataka populations. In the present study, the gene flow of *S. album* populations was relatively high in Telangana state as compared to Kerala and Karnataka. In *S. album* isozyme study indicated a high level of gene flow among the populations than across the geographical regions [3]. The gene flow within *S. album* populations in selected states of Southern India was relatively high in Telangana state as compared to Kerala and Karnataka state (mean 5.295). Among the 25 SSR markers LC126834 (*O. lanceolata*) showed highest polymorphism. A Marker with high He and PIC value is useful for genetic diversity and distinguish the genotypes belongs to different populations. [30]. The genetic transferability of the SSR markers in genus *S. album* is highly conserved [31]. Though in this study we found that 84% transferability of *O. lanceolata*, 81% of *S. spicatum*, 72% *S. insulare* and 78% *S. austrocaledonicum* in *S. album* that is quite higher than the other genus transferability. Cross species transferability of SSR indicated the conservation of primer and compared to less reproducible marker [32]. In this research we found that the high polymorphism in *S. album* by these selected SSR markers. This result indicated that genetic structure of *S. album* mostly similar to *S. spicatum* and *O. lanceolata.*

5. Conclusion

The present study identified the high profile of genetic diversity population of Sandalwood such as Kodada Telangana, Suryapet Telangana, IWST Bangalore, Dharwad and Hassan Karnataka in southern India. Identification of these hot spot populations would be helpful in collecting and maintaining germplasm for sandalwood. These findings of the present study can have important implications for the *ex-situ*, *in-situ* conservation, selection for genetic improvement program (Micropropagation, cloning, tissue culture). It was concluded that Sandalwood populations with high genetic diversity should be given preferential for developing *in situ* conservation strategies. Additionally, these markers would

be a useful tool for investigating genetic diversity, genetic structure of other regions natural and plantation populations of *S. album* or other sandalwood species.

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Author Contributions

All author equally contributed.

Author Statement

All authors read, reviewed, agree and approved the final manuscript.

Conflicts of Interest

None declared.

References

[1] IUCN (2007) IUCN Red List of Threatened Species. https://www.iucnredlist.org/

[2] Kumarvelu, G., Bharathi, R.K., Jainaldeen, A. and Balraj, V.C. (2007) Resurrection of the Rare Aromatic *Santalum album* Population in Tamil Nadu, India. *Proceedings of National Seminar*, 23-31.

[3] Rao, N.M., Ravikanth, G., Ganeshaiah, K.N., Rathore, T.S. and Shaanker, R.U. (2007) Assessing Threats and Identifying the Ecological Niche of Sandal Resoures to Identify "Hot-Spots" for *in Situ* Conservation in Southern India. *Sandal Proceedings of National Seminar*, 23-31.

[4] Jones, C.G., Plummer, J.A. and Barbour, L. (2007) Non-Destructive Sampling of Indian Sandalwood (*Santalum album* L.) for Oil Content and Composition. *Journal of Essential Oil Research*, **19**, 157-164. https://doi.org/10.1080/10412905.2007.9699250

[5] Azeez, S.A., Nelson, R., Prasadbabu, A. and Rao, M.S. (2009) Genetic Diversity of *Santalum album* Using Random Amplified Polymorphic DNAs. *African Journal of Biotechnology*, **8**, 2943-2947.

[6] Murray, M.G. and Thompson, W.F. (1980) Rapid Isolation of High Molecular Weight Plant DNA. *Nucleic Acid Research*, **8**, 4321-4325. https://doi.org/10.1093/nar/8.19.4321

[7] Fatima, T., Srivastava, A., Hanur, V.S. and Rao, S.M. (2018) An Effective Wood DNA Extraction Protocol for Three Economic Important Timber Species of India. *American Journal of Plant Sciences*, **9**, 139-149. https://doi.org/10.4236/ajps.2018.92012

[8] Otieno, O.J., Omondi, S.F., Perry, A., Odee, W.D., Makatiani, E.T., Kiplagat, O. and Cavers, S. (2016) Development and Characterization of Microsatellite Markers for

Osyris lanceolata Hochst. & Steud., an Endangered African Sandalwood Tree Species. *Tropical Plant Research* (*An International Journal*), 701-703. http://www.tropicalplantresearch.com/archives/2016/vol3issue3/092.pdf

[9] Bottin, L., Vaillant, A., Sire, P., Cardi, C. and Bouvet, M. (2005) Isolation and Characterization of Microsatellite Loci in *Santalum austrocaledonicum*, Santalaceae. *Molecular Ecology Notes*, **5**, 800-802. https://doi.org/10.1111/j.1471-8286.2005.01067.x

[10] Emeline, L., Alexandre, V., Francoist, B.J. and Marc, B.J. (2006) Isolation and Characterization of Microsatellite Loci in *Santalum insulare*, Santalaceae. *Molecular Ecology Notes*, 653-655. https://doi.org/10.1111/j.1471-8286.2006.01279.x

[11] Millar, M.A., Byrne, M. and Barbour, E. (2012) Characterization of Eleven Polymorphic Microsatellite DNA Markers for Australian Sandalwood (*Santalum spicatum*) (R. Br.) A. D.C. (Santalaceae). *Conservation Genetics*, **4**, 51-53. https://doi.org/10.1007/s12686-011-9473-9

[12] Williams, J.G.K., Kubelik, A.R., Livak, K.J., Rafalski, J.A. and Tingey, S.V. (1990) DNA Polymorphisms Amplifies by Arbitrary Primers Are Useful as Genetic Markers. *Nucleic Acid Research*, **313**, 101-105. https://www.ncbi.nlm.nih.gov/pubmed/1979162.

[13] Ipek, A., Barut, E., Gulen, H. and Ipek, M. (2012) Assessment of Inter and Intra Cultivar Variations in Olive Using SSR Markers. *Scientia Agricola*, **69**, 327-335. https://doi.org/10.1590/S0103-90162012000500007

[14] Peakall, R. and Smouse, P.E. (2012) GENALEX 6.5: Genetic Analysis in Excel. Genetic Analysis in Excel. Population Genetic Software for Teaching and Research—An Update. *Bioinformatics* (*Oxford*, *England*), **28**, 2537-2539. https://doi.org/10.1093/bioinformatics/bts460

[15] Peakall, R. and Smouse, P.E. (2006) GENALEX 6: Genetic Analysis in Excel. Population Genetic Software for Teaching and Research. *Molecular Ecology Notes*, **6**, 288-295. https://doi.org/10.1111/j.1471-8286.2005.01155.x

[16] Yeh, F.C. and Yang, R. (1999) Microsoft Window-Based Freeware for Population Genetic Analysis (POPGENE Ver. 1.31). University of Alberta, Alberta.

[17] Pritchard, J.K., Stephens, M. and Donelly, P. (2000) Inference of Population Structure Using Multilocus Genotype Data. *Genetics*, **155**, 945-959.

[18] Earl, D.A. and Vonholt, B.M. (2012) STRUCTURE HARVESTER: A Website and Program for Visualizing STRUCTURE Output and Implementing the Evanno Method. *Consevation Genetic Resources*, **4**, 359-361. https://doi.org/10.1007/s12686-011-9548-7

[19] Rosenberg, N.A., Burke, T., Elo, K., Feldman, M.W. and Freidlin, P.J. (2001) Empirical Evaluation of Genetic Clustering Methods Using Multilocus Genotypes from 20 Chicken Breeds. *Genetics*, **159**, 699-713.

[20] Evanno, G., Regnaut, S. and Goudet, J. (2005) Detection the Number of Clusters of Individuals Using the Software STRUCTURE: A Simulation Study. *Molecular Ecology*, **14**, 2611-2620. https://doi.org/10.1111/j.1365-294X.2005.02553.x

[21] Ma, M., Lie, E., Meng, H., Wang, T., Xie, L., Shen, D., Zhou, X. and Lu, B. (2017) Cluster and Principal Component Analysis Based on SSR Markers of Amomum Tsao-Ko in Jinping Country of Yunnan Province. *AIP Conference Proceedings*, **1864**, Article ID: 020070.

[22] Nie, M. (1973) Molecular Evolution in Search of Itself. *Molecular Evolutionary Genetics*, *Cell*, **51**, 343-344.

[23] Nei, M. (1978) Estimation of Average Heterozygosity and Genetic Distance from a

Small Number of Individuals. *Genetics*, **89**, 583-590.

[24] Rao, N.M., Ganeshaiah, K.N. and Shaankar, R.U. (2007) Assessing Threats and Mapping Sandal Resources to Identify Genetic "Hot-Spot" for *in Situ* Conservation in Peninsular India. *Conservation Genetics*, **8**, 925-935. https://doi.org/10.1007/s10592-006-9247-1

[25] Jones, B.L., Waycott, M., Heather, L.A., Calladine, R.A. and Page, T. (2010). Isolation and Characterization of Microsatellite Loci in *Santalum lanceolatum* and *Santalum leptocladum* (Santalaceae). *American Journal of Botany*, **97**, e97-e98. https://doi.org/10.3732/ajb.1000213

[26] Guan, X., Yuyama, N., Stewart, A., Ding, C., Xu, N., Kiyoshi, T. and Cai, H. (2017) Genetic Diversity and Structure of Lolium Species Surveyed on Nuclaer Simple Sequence Repeat and Cytoplasmic Markers. *Frontiers in Plant Sciences*, **8**, 584. https://doi.org/10.3389/fpls.2017.00584

[27] Ratnaningrum, Y.W.N., Indrioko, S., Faridah, E. and Syahbudin, A. (2017) Gene Flow and Selection Evidence of Sandalwood (*Santalum album*) under Various Population Structures in Gunung Sewu (Java, Indonesia), and Its Effects on Genetic Differentiation. *Biodiversitas*, **18**, 1493-1505.

[28] Wright, S. (1965). The Interpretation of Population Structure by F-Statistics with Special Regard to Systems of Mating. *Evolution*, **19**, 395-420. https://doi.org/10.1111/j.1558-5646.1965.tb01731.x

[29] Suma, T.B. and Balasundaran, B. (2003) Isozyme Variation in Five Provenances of *Santalum album* in India. *Australian Journal of Botany*, **51**, 243-249. https://doi.org/10.1071/BT02094

[30] Mohammad, N., Mahesh, S., Kumar, P. and Ansari, S.A. (2012) Genotyping of *Santalum album* L. Accessions through Cross-Species Transferability of SSR Markers of *Santalum austrocaledonicum* and *Santalum insulare*. Sandalwood Research Newsletter, 1-4.

[31] Byrne, M., Marquez-Garcia, M.I., Uren, T., Smith, D.S. and Moran, G.G. (1996) Conservation and Genetic Diversity of Microsatellite Loci in the Genus Eucalyptus. *Australian Journal of Botany*, **44**, 331-341. https://doi.org/10.1071/BT9960331

[32] Patel, D.M., Fougat, R.S., Sakure, A.A., Kumar, S., Kumar, M. and Mistry, J.G. (2016) Detection of Genetic Variation in Sandalwood Using Various DNA Markers. 3 *Biotech*, **6**, 55. https://doi.org/10.1007/s13205-016-0391-0

Abbreviations

Ho: Observed heterozygosity

He: expected heterozygosity

Na: Number of alleles

Ne: Effective number of alleles

Nm: Number of migrants (Gene flow)

PIC: Polymorphic information content

I: Shannon information index

F: Inbreeding coefficient