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Genetic diversity and population structure of the vulnerable medicinal tree *Saraca asoca* in the Western Ghats India

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***Saraca asoca*, commonly known as 'Asoka' or 'Ashoka,' holds significant medicinal value in India. However, due to the escalating demand in the herbal market, the species has suffered a severe decline in naturally occurring populations, primarily caused by the unregulated extraction of its wood. Particularly within the Western Ghats, a global biodiversity hotspot, the species faces critically low population sizes. The study addresses a highly relevant conservation concern, focusing on an ecologically and culturally important species within a global biodiversity hotspot. Twelve populations in the Western Ghats were analyzed using five highly polymorphic and newly developed microsatellite markers. The results revealed a total of 78 alleles, with an average of 15.6 alleles per locus across the twelve populations. The AMOVA analysis indicated that the species exhibits higher diversity within populations (91.38% variation) compared to the variation observed among populations (8.62%). Further analysis employing Bayesian clustering identified six distinct genetic clusters within *Saraca asoca*. Based on these genetic findings, strategies for the development of an *in-situ* conservation plan for the species have been formulated. Overall, this study sheds light on the genetic characteristics of *Saraca asoca* populations in the Western Ghats and provides valuable insights for the implementation of effective conservation measures.**

Keywords Globally vulnerable tree, Microsatellite markers, Genotyping, Genetic diversity

Forests are integral components of ecosystems, covering approximately 31% of the Earth's land area and comprising an estimated 80,000–100,000 tree species globally, there are 73,000 tree species globally, among which ~9,000 tree species are yet to be discovered[1]. In tropical countries, forest tree species play a crucial role in supporting people's livelihoods, providing wood-based fuels, as well as non-timber products for nutrition, health, and income. Nonetheless, in most tropical countries, the forests are subject to high anthropogenic pressures and typically exhibit extensive fragmentation, leading to spatially scattered tree populations and isolated individual trees. Fragmentation leads to immediate repercussions for the reproductive processes of individual species[2]. The Western Ghats are recognized on a global scale as an ecosystem of immense importance for biological diversity protection. The 30 km Palghat Gap located around 11°N is the only break in the 1600 km long stretch of these mountains, that stretch an area approximately 140,000 km^2.

The Western Ghats' unique and perplexing effects on large-scale biophysical and ecological processes across the Indian peninsula, alongside its extraordinarily high level of biological diversity and endemism, indicate the region's Outstanding Universal Value. The Western Ghats are home to at least 325 species that are listed as globally endangered on the IUCN Red Data List. 129, 145, and 51 of the 325 globally threatened species occurring in the Western Ghats are listed as vulnerable, endangered, and critically endangered, respectively. The Ghats were declared a world heritage by UNESCO in 2012 for their exceptionally high level of biological diversity and endemism[3]. Moreover, there are genetic implications to consider, such as genetic drift and inbreeding[4,5], which have a recognized role in hastening the risk of extinction[6,7]. However, investigations into the impact of forest fragmentation and the quality of the surrounding areas on biodiversity in landscapes influenced by human activities seldom take into account genetic diversity, despite its crucial significance[8,9]. Understanding the extent and the role played by genetic diversity is needed to booster efforts in biodiversity conservation and the planning of forest restoration initiatives.

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Genetic diversity ensures the overall vitality of forests by enabling their resilience against diverse biotic and abiotic stresses in the face of changing and unpredictable environmental conditions. Genetic diversity empowers populations to mount effective evolutionary responses to unpredictable environmental challenges such as fluctuating weather patterns, disturbance events, and variations in resource availability and changes in the population sizes of competing species[10]. The level of genetic variation directly impacts a species' ability to cope with threats and adapt to environmental changes[11]. Populations with high levels of genetic variation are better equipped to respond to new environmental challenges. Conversely, low genetic variation restricts a species' short-term adaptability and long-term persistence[12]. Genetic diversity also serves as a valuable resource for tree breeding and improvement programs, enabling the development of well-adapted tree species and enhancing the genetic gain for various desirable traits[13]. It also supports the livelihoods of indigenous and local communities that rely on traditional knowledge and utilize forest resources. Therefore, the presence of high genetic diversity within and among plant species forms a vital foundation for maintaining food security and facilitating sustainable development[14].

Among all plant species, long-lived woody species exhibit the highest genetic diversity, characterized by a higher percentage of polymorphic loci and a greater number of alleles per locus. Genetic diversity within populations is generally highest (HE = 0.15) compared to other plant life forms (HE < 0.10). However, heterogeneity in genetic diversity exists among woody species taxa due to their different evolutionary histories. For instance, species originating from smaller founder populations or those that have experienced past population bottlenecks typically exhibit lower genetic diversity. Examples of species with high diversity include *Alseis blackiana, Picea glauca, Robinia pseudoacacia* and *Pinus sylvestris*, while *Acacia mangium, Pinus resinosa, P. torreyana,* and *Populus balsamea* show very low diversity[15,16].

Ashoka, known as *Saraca asoca*, is a rainforest tree primarily distributed in the evergreen forests of India, particularly in West Bengal, Assam, Odisha, Tamil Nadu, Karnataka, Kerala, Andhra Pradesh, Meghalaya, and Maharashtra[17–19]. It also has a wide distribution across the Western Ghats (both South and Central), the Sahyadri region, and throughout the Himalayas[20]. Despite its extensive range, the population of *Saraca asoca* is highly fragmented[21] The Northern Western Ghats, in particular, have experienced significant deforestation, resulting in the fragmentation of the natural populations of *Saraca asoca*[22]. *Saraca asoca* has also been indiscriminately exploited, and the rampant extraction of its wood has led to a sharp decline in naturally occurring populations in India[23]. Consequently, this species has recently been classified as "vulnerable" by the International Union for Conservation of Nature (IUCN) (IUCN 2011),[24–26]. Recently, the Planning Commission of the Government of India and the Medicinal Plant Board has included *Saraca asoca* in a list of 32 prioritized medicinal plants for further research and development[24,27]. The increase in deforestation, coupled with the high demand for *Saraca asoca,* has resulted in a limited supply and widespread adulteration of the species in the raw herbal trade market[28]. The primary reason for the global vulnerability and local endangerment of *Saraca asoca* is primarily the overexploitation of wild populations to meet the high commercial demand for its pharmaceutical value and traditional uses. The extract of *S. asoca* is used for leucorrhea and has an astringent but stimulative effect on endometrium and ovarian tissues[29,30]. The bark is used to cure dyspepsia, dysentery, piles, sores and irregular menstruation. The dried flowers are used for treatment of syphilis, hemorrhagic, diabetes and dysentery (Table 1). It also helps to get rid of the toxins from the body and is effective in purifying the blood naturally and in curing skin allergies. Seeds are used to treat bone fracture and vesicle calculi (Table 2). The plant is used in the treatment of dyspepsia, indigestion, blood disorders, tumours etc.[31]. There are various indigenous preparations available with Ashoka as a major constituent, of which the important ones include *Ashokarishta*, *Ashokaghrita*, *Ashoka kwath*[32,33].

Plant Parts	Biological activity	References
Leaves	Anti – oxidant	Sadhu et al.[34], Saha et al.[35], Jain et al.[36], Sarojini et al.[37], Pradhan et al.[33], Mishra et al.[38], Pandey et al.[39], Gahlaut et al.[40], Kalakotla et al.[41], Somani et al.[42]
	Antidiabetic	Pradhan et al.[33], Mishra et al.[38], Pandey et al.[39], Gahlaut et al.[40], Kalakotla et al.[41], Somani et al.[42]
	Cardio protective	Kalakotla et al.[41]
	Antimicrobial	Sadhu et al.[34], Shirolkar et al.[43], Kalakotla et al.[41], Ahmed et al.[44], Behari et al.[45]
	Anti-inflammatory	Shirolkar et al.[43], Kalakotla et al.[41], Ahmed et al.[44]
Bark	Anti-Cancer	Verghese et al.[46], Pradhan et al.[33], Mishra et al.[38], Saha et al.[35], Saha et al.[35], Samee et al.[47], Panchawat et al.[48], Pandey et al.[39], Yadav et al.[49], Somani et al.[42], Gupta et al.[50]
	Anti-bacterial	Annapurna et al.[51], Sadhu et al.[34], Shirolkar et al.[43], Kalakotla et al.[41], Ahmed et al.[44], Behari et al.[45]
	Anti-oxidant	Pradhan et al.[33], Ahmed et al.[44], Somani et al.[42], Panchawat et al.[48], Sadhu et al.[34], Shirolkar et al.[43]
	Anti-fertility activity	Suganthan and Santhakumari[52]
	Anti- Viral	Hattori et al.[53]
	Anti- HIV	Kusumato et al.[54]
	Oxytocic activity	Satyavati gowdagere[55]
Flowers	Anti-Cancer	Pradhan et al.[33], Mishra et al.[38], Saha et al.[35], Saha et al.[35], Samee et al.[47], Panchawat et al.[48], Pandey et al.[39], Yadav et al.[49], Somani et al.[42], Gupta et al.[50]
	Anti – Diabetic	Pradhan et al.[33], Ahmed et al.[44], Somani et al.[42]

Table 1. Biological activities of extracts obtained from leaf, bark and flower of *Saraca asoca.*

Site of Occurrence	Longitude	Latitude	Environmental variables	Altitude (in meter)
Jambani	74.5514	14.5983	ARF-1813.9 mm, AMT-26 °C	208
Patoli	74.5535	15.1878	AAP-459.8 mm	611
Urabail	74.7418	14.8038	ARF-2500-3500 mm	481
Palda	74.5604	14.5205	AMT25-32 °C	261
Devimane	74.3089	13.6179	ARF-2835 mm	0
Banavasi	74.9976	14.591	ARF -2835 mm	576
Kuppalli	74.8112	14.2231	ARF-1813.9 mm	472
Honnavara	74.8644	13.8781	ARF -2835 mm	637
Kakkalli	74.6583	14.7686	ARF-2835 mm	407
Subramanya	75.789	13.5492	ARF -3975 mm	953
Jog	74.5995	14.2701	ARF-1813.9 mm	42
Kodachadri	74.8216	13.8923	ARF-1813.9 mm	237

Table 2. Fitness of 12 occurrence sites under different environmental variables. ARF- Annual Rain fall, AAP-Annual Average Precipitation, AMT-Annual mean Temperature.

Name of primer	Acc. No. of primer	Repeat Unit	Primer sequence 5'-3'	Allele size range (bp)	Annealing Temp
SAR4	JQ406593	(TGG)6	F-AGGGGAAAGAATTTTACCTG R-ATGATTACGCCAAGCTCTAA	176–220	35
SAR5	JQ406594	(CT)8	F-AAAGTAATAGATCCCGCACA R-GATCCCAGAAACGATGAGTA	200–219	55
SAR6	JQ406595	(AG)15	F-TTGATCATCTCATTTCCCTTA R-GACTTGGTTCTTAGCAGTGG	251–298	55
SAR7	JQ406596	(AAAAG)4	F-GGAAAAAGTAGTGGCAGAGA R-ATGATTACGCCAAGCTCTAA	301–397	43
SAR10	JQ406599	(CT)10TT(CA)7	F-CAGACCTCTTGATTCTTGCT R-CGACTTCGAAGCTTGTTACT	305–340	55

Table 3. Fluorescent labelled primers sequences, annealing temperatures, allele size range summary for *S. asoca.*

The high medicinal value of *Saraca asoca* has led to its overexploitation across its natural range of distribution[23]. The adaptation of *Saraca asoca* to different *environmental* conditions depends on the genotype, ecotype, and phenology of the tree species, as well as various environmental factors such as temperature, precipitation, elevation, soil water availability, and day length[19]. Given the species' significance, the primary focus of this paper is to assess the population's genetic structure and gene flow across the fragmented populations in Western Ghats, India. This study aims to understand the genetic dynamics of *Saraca asoca*, providing valuable insights for conservation efforts and strategies to ensure its sustainable survival.

Results and discussion

Numerous research studies have been conducted on the valuable plant species *S. asoca,* encompassing various fields such as taxonomy, phytochemistry, and molecular genetics. These studies have aimed to explore its potential for enhanced utilization while emphasizing the need for conservation[23,28,33] due to high commercial demand in India[24]. Many bio-active, secondary metabolite compounds such as alkaloids, steroids, terpenoid, glycosides etc. were found predominantly in this species[56], also it is found to have antibacterial, antifungal and antioxidant, anti-depressant activities[57,58]. Recognizing the significance of *S. asoca,* the current research primarily focuses on understanding its population genetic structure in the Western Ghats across South India, with the aim of formulating effective conservation strategies.

Genetic diversity: In this study, we analysed five polymorphic SSR loci (Table 3), which generated a total of 78 alleles across 252 individuals representing 12 populations from the Central Western Ghats region. There was a significant level of heterozygosity among the populations sampled. Heterozygosity was quantified as observed (H_o) and expected (H_e) heterozygosity, across the 12 populations of *S. asoca,* H_o ranged from 0.63 to 0.88, while H_e ranged from 0.51 to 0.76, with mean values of 0.790 and 0.67 respectively (Table 4). Observed heterozygosity was found to be higher than expected heterozygosity among the 12 natural populations. Populations such as Patoli, Urabail, Palda, Banavasi, Kuppalli, Jog and Kodachadri exhibited higher H_o values, whereas Jambani, Urabail, Palda, Banavasi, Kuppalli, Jog and Kodachadri showed higher H_e values. Honnavara, Kakkalli, Subramanya, Devimane and Jambani populations displayed lower.

H_o and H_e, Urabail population measured the highest observed and expected heterozygosity, Kakkalli and Subramanya the less among the 12 populations respectively.

The total allele number by locus is a measure of genetic diversity, more sensitive to loss of genetic variation as consequence of small population size. The number of alleles in the 12 populations of *S. asoca* ranged from 2.6

Population Name	N	Na Mean ± SD	Ne Mean ± SD	H_o Mean ± SD	H_e Mean ± SD	A_R	A_P	% P	F_{ST}
Jambani	28	8.2 ± 1.35	3.77 ± 0.82	0.77 ± 0.27	0.67 ± 0.21	4.59	5	100	0.086
Patoli	25	6.0 ± 1.26	3.25 ± 0.67	0.83 ± 0.18	0.65 ± 0.15	3.98	0	100	0.086
Urabail	25	8.2 ± 1.32	4.21 ± 0.51	0.88 ± 0.06	0.76 ± 0.09	5.03	5	100	0.083
Palda	23	6.0 ± 0.95	3.55 ± 0.65	0.79 ± 0.16	0.69 ± 0.15	4.18	0	100	0.085
Devimane	25	7.0 ± 0.71	3.07 ± 0.56	0.76 ± 0.15	0.64 ± 0.13	4.11	3	100	0.087
Banavasi	24	7.0 ± 0.77	3.54 ± 0.74	0.81 ± 0.15	0.69 ± 0.11	4.40	0	100	0.086
Kuppalli	25	7.2 ± 1.07	3.86 ± 0.18	0.87 ± 0.13	0.75 ± 0.03	4.69	2	100	0.083
Honnavara	13	6.8 ± 1.11	4.06 ± 1.03	0.76 ± 0.25	0.64 ± 0.21	4.87	0	100	0.085
Kakkalli	29	7.6 ± 1.4	3.21 ± 1.02	0.63 ± 0.24	0.58 ± 0.22	4.03	4	100	0.089
Subramanya	06	2.6 ± 0.68	2.3 ± 0.53	0.77 ± 0.43	0.51 ± 0.31	2.6	0	80	0.092
Jog	15	6.4 ± 1.47	3.89 ± 0.76	0.83 ± 0.13	0.73 ± 0.12	4.72	2	100	0.084
Kodachadri	14	5.4 ± 0.93	3.13 ± 0.52	0.79 ± 0.20	0.68 ± 0.15	4.10	0	100	0.086
	252	**6.53 ± 0.35**	**3.49 ± 0.19**	**0.79 ± 0.10**	**0.67 ± 0.07**	**4.28 ± 0.6**		**98.33**	

Table 4. Genetic diversity parameters of 12 populations of *S. asoca* across South India. Bold values indicate moderate genetic differentiation. N- Number of samples, Na- Observed number of alleles, Ne- Effective number of alleles, H_o-Observed heterozygosity, H_e-Expected Heterozygosity, A_R-Allelic richness, A_p- Number of private alleles, %P- Percent polymorphic, F_{ST} – Weir and Cockerham estimates of Fixation index values.

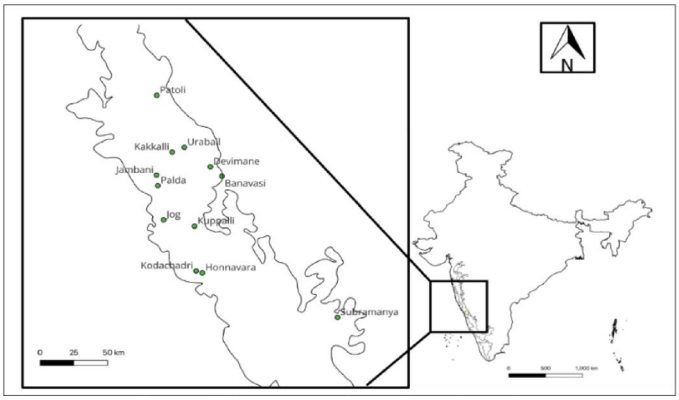

Fig. 1. Distribution map of 12 populations of *S. asoca* in Western Ghats regions of South India selected for assessment of genetic diversity and population structure.

to 8.2, while allelic richness ranged from 2.6 to 5.03. The mean number of alleles across the 12 populations was calculated as 6.53 (Table 4). Populations such as Jambani, Urabail, Devimane, Banavasi, Kuppalli, Honnavara, and Kakkalli exhibited a higher number of alleles. Urabail showed the highest allelic richness. The effective number of alleles (n_e), which inversely relates to expected homozygosity, was also measured. The mean number of effective alleles among the populations was found to be 3.48. In this study, Urabail and Honnavara displayed the highest effective number of alleles, with values of 4.20 and 4.05, respectively. Subramanya had a lower number of alleles, with 2.30.

Genetic polymorphism is a crucial aspect in assessing the existing genetic diversity among populations. Eleven populations showed 100% genetic polymorphism, except for Subramanya, which exhibited 80%. These results indicate that *S. asoca* wide genetic variability within populations, favouring higher heterozygosity. Private alleles (A_p), alleles exclusive to specific populations, were most abundant in Jambani and Urabail (5 each), while Kuppalli and Jog had the lowest number of private alleles (2 each) (Table 4). The F-statistics factors, including the fixation index (F_{is}) and F_{ST}, analyzed in this study for *S. asoca* populations, showed negative F values, indicating high heterozygosity. The F_{ST} value was highest in Subramanya (23%) and lowest in Urabail (6%), indicating significant genetic differentiation among the 12 populations (Fig. 1).

Population genetic structure: Dendrogram generated using microsatellite markers revealed distinct genetic distances among the 12 populations of *S. asoca* (Fig. 2). Jambani and Subramanya formed two extreme clades with the longest genetic distance, while the remaining nine populations (Kakkalli, Kuppalli, Kodachadri, Urabail, Honnavara, Patoli, Palda, Jog, and Banavasi) formed the largest cluster. Three distinct clusters were observed, one comprising Jambani and Devimane, another consisting of Subramanya alone, and the third cluster comprising the remaining nine populations. The molecular variance (AMOVA) analysis was conducted to assess the variation within and among populations of *S. asoca* in South India for 252 individuals (Table 5). The results showed a significant ($p > 0.01$) difference, indicating that the genetic diversity within populations (91.38%)

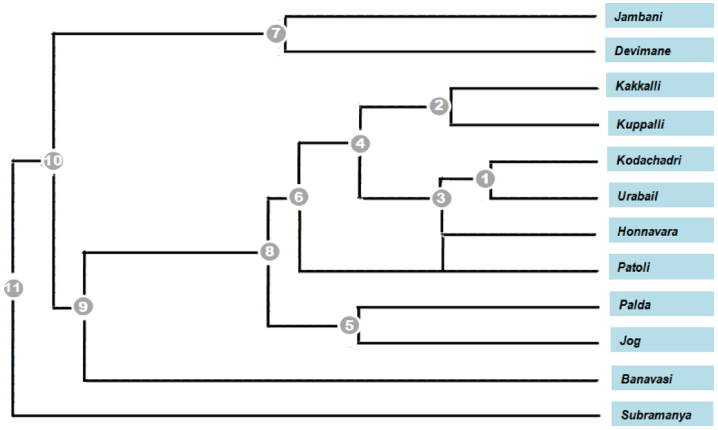

Fig. 2. Genetic distance of 12 different populations of *S. asoca* Dendrogram developed based on Nei's genetic Distance using PHYLIP Version 3.5.

Source of variation	Degrees of freedom	Sum of squares	Variance components	Total variance (%)	F-statistics
Among populations	11	90.12	0.158	8.62	$F_{ST} = 0.0862$
Within populations	492	822.15	1.671	91.38	P > = 0.01

Table 5. Molecular variance (AMOVA) analysis for 12 populations of *S. asoca* in Western Ghats South India.

was higher than the variation among populations (8.62%) with a permutation of 1023. The fixation index (Fst) value was low, measuring 0.0862. These findings suggest a strong gene flow between populations of *S. asoca* (Nm = 2.0353). According to Wright[59], a migration rate of Nm = 1.0 is theoretically required to counteract the effects of genetic drift. Gene flow is a micro evolutionary process that maintains the genetic exchange among local populations increasing population genetic diversity[60]. In our present study, the Nm value suggests that genetic drift may not have been the primary factor influencing the genetic structure of *S. asoca* populations. Additionally, the low level of genetic differentiation with a GST value of 0.109 (considered high if > 0.15, according to Nei, 1978) indicates that these populations are generally less prone to 'environmental stress. In the present study, high level of gene flow value (2.035) and the inbreeding coefficient (0.227) was found among the 12 population of *S. asoca*, indicating the greater possibility of genetic differentiation. In the present study, the geographical distance among the population of *S. asoca* influenced the flow of alleles and genetic diversity significantly among all the 12 populations of *S. asoca*. Increase in geographical distance between the populations restricted the gene flow significantly.

The genetic diversity analysis, based on maximum likelihoods using a Bayesian model with alternative values of K, and population structure analysis for the 12 populations of *S. asoca*, revealed the presence of four genetic clusters (Fig. 3a). The length of the vertical lines represents the proportion of admixture attributed to the inferred clusters. The principal component analysis performed revealed the scatter plot for individuals in populations showing 13%%(coordinate axis 1), 11% (coordinate axis 2) and 10% (coordinate axis 3) variance and for 12 populations are placed according to values of first two principal coordinates determined during the PCoA. This plot showed 5 distinct clusters among individuals of populations and along with populations distributed in negative coordinates whereas Subramanya population is present in positive coordinate suggesting distinct subgroup having 41%(coordinate axis 1), 18%(coordinate axis 2) and 12% (coordinate axis 3) variance among first 3 axes based on genetic and genotypic distances with negative Eigen values indicating less variability in each dimension (Fig. 3b&c). These analysis provides insights into the population structure and genetic relationships among the different populations of *S. asoca.* High level of gene flow (2.035) and inbreeding coefficient (0.227) observed among the 12 populations of *S. asoca*, indicates a greater possibility of genetic differentiation. The geographical distance between these populations significantly influenced the flow of alleles and genetic diversity. As the geographical distance increased, the gene flow was restricted. Among the 12 populations studied, five populations (Patoli, Jambani, Subramanya, Jog, and Urabail) were identified as genetically diverse and suitable candidates for *"in-situ"* -

-conservation. "*In-situ*" conservation of forest species is a promising and feasible method for conserving the genetic diversity. This approach involves identifying source populations[61,62] that contribute to genetic diversity and sink populations that benefit from this diversity. Based on the genetic parameters calculated, populations such as Patoli, Jambani, and Urabail were considered as sources, while Subramanya and Kakkalli are identified as sinks. The remaining populations were found to have medium genetic diversity and were highly vulnerable, the populations with low level of genetic variation are generally less genetically stable and more vulnerable to pathogenic infections and harmful changes of environmental conditions. Artificial cultivation may be an effective approach for securing medicinal and bark supply which generates beneficial ecological effect and considerable

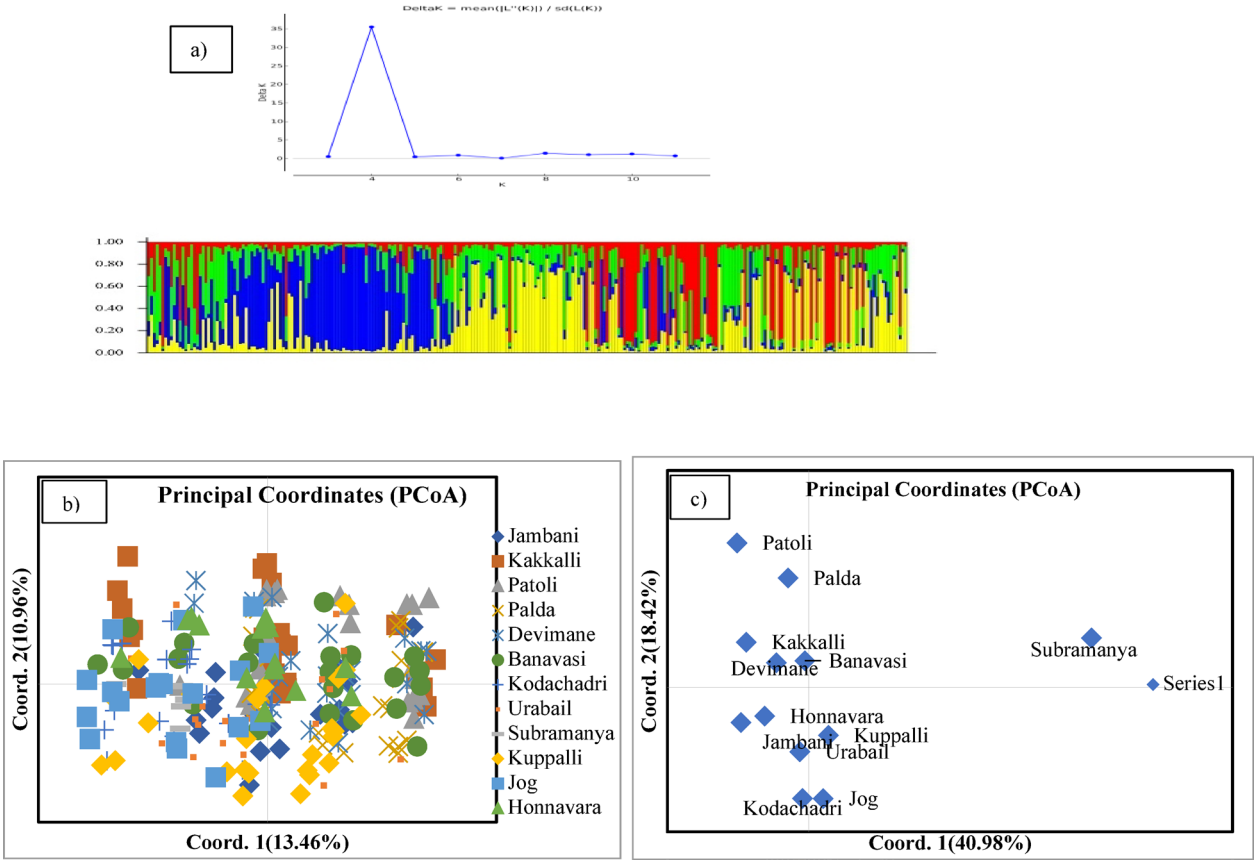

Fig. 3. (**a**) Bayesian clustering analysis of 12 populations of *S. asoca* based on five microsatellite primers. The peak of the graph indicates the delta K value to represent the possible clustering, the Posterior probability value of 12 different populations, and estimated population structure and (**b**) Principal Component analysis for individuals and (**c**) populations on percentage of coordinates.

economic benefit. However, to maintain local variation, plant materials used in reforestation efforts should be obtained within the same conservation unit to maintain the genetic uniformity and also to avoid the dilution of original genetic characteristics by introducing inappropriate gene pools. Some populations with higher genetic variation, eg Urabail, Jambani, could be given high priority for in-situ conservation as a source of genes to bring genetic introgression in order to develop new genetic dimension in *S. asoca* for its overall genetic improvement.

Conclusion

Many approaches to the conservation of genetic diversity, knowledge on the amount and distribution of genetic diversity within *S. asoca* populations through genetic information contained in DNA, particularly in microsatellite sequences, offers valuable input to the in situ and ex situ conservation strategies and forest-genetic resources. Populations in sink areas are of utmost importance and require "*in-situ*" conservation efforts. However, the genetic pool of these populations can be augmented by incorporating genetic diversity from the source populations. The understanding of population structure and molecular diversity of *S. asoca* obtained in this study not only contributes to "*in-situ*" conservation strategies but also holds potential for overall genetic improvement. Furthermore, the identification of donor and source populations through various genetic parameters can be instrumental in developing viable strategies for establishing gene banks for *S. asoca*. The results regarding population diversity and genetic structure of *S. asoca* provide a foundation for further research on this valuable plant species.

Experimental design

***Study species*:** *S. asoca,* a small evergreen tree reaching heights of up to 10 m, demonstrates a distribution ranging from longitude 72.930°E to 91.5°E and latitude 8.33°N to 30.44°N. This species is found across various geographic locations with minimal phenotypic variations. Despite its wide distribution, it exhibits a high degree of restriction to specific fragmented habitat types, such as moist evergreen patches and streamlines.

Study site: The study was carried out in the Central Western Ghats, a mountain range located along the west coast of India. Twelve localities with varying levels of protection, ranging from wildlife sanctuaries to reserve forests, were selected and mapped spatially to develop a distribution map. Leaf samples of *S. asoca* were collected from approximately 252 individual trees across 12 natural populations (Fig. 1 and Table 2) belonging to

three niches become the primary source of materials as these three niches were more prominent in nature with highest population size. The samples were stored at -20 °C in a deep freezer and served as the source material for genomic DNA isolation throughout the study. Genomic DNA was extracted from fresh leaf samples of *S. asoca* using the CTAB method with modifications based on the procedure suggested by Glenn and Schable[63]. The quantification of DNA was performed using a bio-photometer (Eppendorf) and by comparing it with a known quantity of DNA standard (Lambda DNA) through electrophoresis on 1% agarose gels with ethidium bromide.

Genotyping/Molecular data: The genotyping process involved preparing a polymerase chain reaction (PCR) mixture of 20 µl for each aliquot. The composition of the mixture included 10X PCR reaction buffer (2.5 µl), forward primer (5 pmol/µl, 1.0 µl) labeled with a fluorescent dye, reverse primer (5 pmol/µl, 1.0 µl), 1 mM dNTPs (2.5 µl), distilled water (12.0 µl), Taq DNA polymerase enzyme (0.20 µl), and DNA template (25 ng/µl, 1.50 µl) for amplification. The PCR profile consisted of an initial denaturation step at 94 °C for 3 min, followed by denaturation at 94 °C for 1 min, annealing at the primer's specific temperature for 1 min 30 s, extension at 72 °C for 2 min, final extension at 72 °C for 10 min, and a hold at 4 °C. The PCR products containing fluorescent dye-labeled primers were electrophoresed on 2.5% agarose gels in 1 × TAE buffer. Five microliters of the PCR product from each individual representing different populations of *S. asoca* were loaded onto the gels along with a pre-stained ethidium bromide 100 bp ladder. The gels were then documented using a Gel Documentation System. The remaining 15 µl of amplified products were sent for genotyping analysis using the ABI 310 Genetic Analyzer (Chromous Biotech, Bangalore), and the chromatograms of the genotyped samples were analyzed.

Genetic diversity measures are calculated for each population using data from five loci developed newly for this study species. These five primers were proven high resolution polymorphic bands, degree of polymorphism and number of alleles than other primers in preliminary screening for analyzing population genetic structure of *S. asoca*. These measures included the mean number of alleles per locus (N_a), the effective number of alleles per locus (N_e), observed heterozygosity (H_o), expected heterozygosity (H_e), and fixation index (F_{IS}). The genetic variation among the twelve populations, such as allele distribution and private alleles (alleles present in only one population and not shared by any other population), quantified using the Peakall and Smouse GENAlex 6.5 software[64], 2012). The mean effective number of alleles (N_e) per population was adjusted to the smallest number of individuals genotyped to account for uneven sampling. The standardized estimate of allelic richness (A_r) per locus, averaged across each locus and per population, adjusted to sample size, was calculated using the FSTAT 2.9.3 program. F_{ST} was calculated by Arlequin version 3.11 with 1000 dememorization steps and 10,000 MCMC chains[65]. Dendrogram was constructed based on Nei's genetic distance data using PHYLIP version 3.5 software. The molecular variations within and among the twelve populations of *S. asoca* were examined using the analysis of molecular variance (AMOVA) method implemented in GENAlex 6.5. To detect population structure and assign individuals to groups, a model-based Bayesian clustering method implemented in STRUCTURE version 2.3.3 was employed[66]. The parameters were set to an admixture model with correlated allele frequencies between populations, without prior population information. The cluster number (K) was varied from 1 to 12 (K = 1 to K = 12), and the model was run as 20 independent simulations for each K. A burn-in length of 1000 and a run length of 10,000 MCMC iterations. Evanno et al.[67] method was used for determining most likely value of K by plotting log probability (L (K)) and ΔK of data over multiple runs as implemented in STRUCTURE HARVESTER[68].

Data availability

The data that support the findings of this study are not openly available due to reasons of sensitivity and are available from the corresponding author upon reasonable request.

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Declarations

Competing interests
The authors declare no competing interests.

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