

Genetic diversity and population structure analysis of the endangered endemic and economically important plant, Red Sanders, distributed in the Eastern Ghats. India

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Abstract

Pterocarpus santalinus, or Red Sanders, is an Indian native tree species that is under threat of decline in natural populations due to illicit felling in Eastern Ghats. In the present study, we assessed the genetic variation and population structure across 22 natural populations 16 highly polymorphic SSR markers in 361 individuals. The average number of alleles (N_a) was 7.79, with an expected heterozygosity (H_e) of 0.65, which is lower than that of other woody plants. Interestingly, the Tirupati base-Sadashiva Kona population presented the greatest genetic diversity ($H_e = 0.87$), whereas the Chitaleti Pati base Camp population presented the least genetic diversity ($H_e = 0.44$). The analysis revealed that extensive genetic variation among populations (72%) contrasted with that within populations (28%). The Tirupati circle ($H_e = 0.93$) and Chittor divisions ($H_e = 0.91$) presented high genetic diversity. The F_{ST} values revealed considerable genetic differentiation among the populations, with a value of 0.31 and poor gene flow ($N_m = 0.82$). Cluster analysis of 361 samples from 22 populations revealed three main genetic groups. Populations located at lower latitudes presented greater genetic diversity than those located at higher latitudes did, and geographical and genetic distances were positively correlated. The population as a whole presented moderate level of genetic diversity, with clear variation between the populations at lower and higher latitudes and positive geographical and genetic correlations. These results indicate the importance of conserving *P. santalinus*.

1. Introduction

Pterocarpus santalinus, commonly known as Red Sanders, is a forest tree species that is found exclusively in the Eastern Ghats of Andhra Pradesh, India. It is an endangered species with a naturally limited distribution range. *P. santalinus*, a member of the Fabaceae family, is a slow-growing tree species that relies on cross-pollination and insects for reproduction [54]. In natural forests, it typically takes between 50 and 60 years for a pole-sized tree (measuring 30 cm girth at breast height or 173 cm from the ground) to achieve a harvestable girth of 70 cm. This species has been prized for centuries for its dyeing properties, traditional medicinal uses, and use as a source of timber. The heartwood of the tree boasts a reddish-brown hue, a distinctive wavy grain, and exceptional durability. Presently, *P. santalinus* heart wood is highly valued on the global market [2, 53, 67]. The legal trade of seized wood is currently limited to occasional sales through the Forest Departments of Andhra Pradesh (APFD), Tamil Nadu, and Karnataka (NDF, 2019). This includes a range of products, such as wood chips, extracts, timber, and wood carvings. According to a report by the APFD, the auction of 8179.85 MT of seized wood from 2005–2018-19 generated revenue of Rs.1688.24 crores. However, there is still a balance of 6285.909 MT of wood waiting for auctions in the APFD alone [26]. This species is highly vulnerable because of the overharvesting of mature trees from the wild due to the continued high demand for timber [2]. In 1988, the species was listed as endangered and categorized under the A2cd criterion [28, 3], which explains habitat loss and population size reduction in the natural distributions of Eastern Ghats, India. The species is listed in Appendix II of the Convention for International Trade in Endangered Species of Wild Fauna and Flora (CITES) in 1995 because of its declining population and restricted distribution. Recently, a drastic decline (7.8–3.9%) in the number of harvestable trees (GBH > 70 cm) has been observed within the last six years [3, 25]. One reason for the decline of this species is the hot and dry conditions of its natural habitat, which put its pollination ecology at risk. The tree blooms during the dry season. Its yellow flowers attract honey bees, especially *Apis dorsata*, which are the primary pollinators. The other two species, *A. indica* and *A. florea*, visit only in the early morning. This tree mainly prevents the growth of fruits from self-pollinated flowers, and only 6% of flowers grow into fruits due to various factors [55].

Genetic diversity plays a crucial role in biodiversity, influencing how well a species can adapt to environmental challenges and changes [22, 40]. By measuring the genetic diversity within a species, we gain valuable insights into its evolution and the variations in its genetic makeup [4, 44]. Genetic variation among and within populations of various plant species has been studied extensively molecular markers over the last three decades. In recent years, advanced molecular tools and next-generation sequencing (NGS) technologies have helped us discover robust molecular markers and use these markers to elucidate genetic variation in tree species [15]. Among the molecular markers available, microsatellites or simple sequence repeats (SSRs) are often preferred because they are highly mutated, generate multiple allelic forms and mutations per locus per generation, and are codominant [18, 69]. The combination of both characteristics allows them to be used as a sensitive tool for assessing genetic diversity among species, determining population structure, reconstructing phylogenetics, genetic mapping, evolutionary analyses, and molecular breeding [59]. In practice, SSRs are popular, simple to implement, and reproducible in most research environments because they require minimal resources (i.e., technical skills, laboratory equipment, and consumables) [46]. Despite being a highly economically valuable tree species, very few studies have been reported on *P. santalinus* via the use of DNA molecular markers. However, recent advancements in next-generation sequencing (NGS) have made it possible to sequence transcriptomes and whole genomes *de novo* [1, 63]. Previous genetic diversity studies have used molecular markers such as RAPD and ISSRs, which are dominant, nonlocus-specific, and have poor reproducibility. Moreover, past studies have used small sample sizes that do not represent the entire range of species distributions. However, this study emphasized covering the entire species distribution range of red sanders in close association with field officers of the Andhra Pradesh Forest Department. This study analyzed the genetic variation and population structure of *P. santalinus* across 22 populations using 16 SSR markers and identified genetic diversity hotspots of high conservation importance.

2. Materials and Methodology

2.1 Study area: We conducted a literature survey and consulted with the forest department officials of Andhra Pradesh to sample pristine forest tracts in each forest range within the natural habitat of *P. santalinus*. The state of Andhra Pradesh has been divided into 5 territorial circles, one wildlife circle, and one project tiger circle for administration and forest management. At the divisional level, there are 32 territorial divisions, two wildlife divisions, and 13 social forestry divisions. There are also 10 social forestry divisions. The Andhra Pradesh Forest Department (APFD) has a

total of 2,312 beats, 996 sections, and 295 ranges according to the available data [26]. The study was conducted in the Middle to Southern Eastern Ghats and covered five districts of Andhra Pradesh, located between latitude 13°32'14.1"N and longitude 79°36'04.1"E to 15°29'77.4"N and 79°12'81.7"E. We selected 22 forest ranges/populations under the Kurnool, Tirupati, and Guntur Circles in Andhra Pradesh, India, for sample collection (Table 1, Fig. 1). These circles include the endemic region of *P. santalinus*, which is categorized under dry deciduous forests [13]. The forests in these regions have both pure stands of *P. santalinus* and mixed associations, and we considered the distinctness of the forest patch in terms of altitude and the edaphic nature of the locality when sampling the closely located forest tracts. To facilitate efficient study management and organizational ease, we established each range (Table 1 and Fig. 1) as a distinct population unit. Leaf samples of *Pterocarpus santalinus* were collected from the Eastern Ghats of Andhra Pradesh, India. The plant material was identified by Divakara, BN, a scientist at IWSST in Bengaluru, and a voucher specimen has been deposited at TDU/IWSST, Bengaluru (IWSST-NBA-01 to 400). Only leaf samples were collected without harming the tree, and their reproducibility. Collected samples were stored in deep freezers at IWSST, Bengaluru.

2.2. Study material: From each forest range, an undisturbed natural forest area was selected for sampling. Fresh leaf samples were collected from four different girth classes. Seedlings and saplings with girth at breast height (GBH 1.37 m from the ground) up to 10 cm; young trees with a GBH of 10 to 20 cm; trees with a GBH of 20 to 30 cm; and mature trees with a GBH greater than 30 cm. A total of 4 to 5 plants were randomly selected for leaf sampling from each girth class. Eight to ten young leaves were selected per sample from each selected tree, and stored in a zip-lock cover containing silica gel. We collected ~16 to 20 individuals per population. A total of 361 individual leaf samples were collected from 22 populations. After the surface moisture in the silica gel was removed, the samples were transferred to -800°C for further storage and use. Geo-coordinates and altitudes were recorded using hand-held GPS instruments. The presence of the species at different altitudes and soil types was taken into account whenever the sampled forest patches of two different ranges were situated in proximity. Each sampled population was designated with acronyms formed with the first letter from the respective names of Forest Division, Forest Range, and Forest Beat (Table 1).

2.3. DNA isolation, PCR, and genotyping of SSR markers: We used the CTAB protocol described by Doyle and Doyle [16] to isolate DNA. For DNA amplification, we used 50–100 ng of genomic DNA and 5 picomoles of M13-tailed markers in a 20 µl reaction mixture. The amplified samples were subsequently subjected to fragment analysis. Our previous publication [63] described in detail the protocols for DNA isolation, PCR conditions, and fragment analysis. In the present study, 16 highly polymorphic SSR loci (Table S1) were used to analyze the 361 samples from 22 different populations of *P. santalinus*. These loci had a polymorphism information content (PIC) greater than 0.9 [63].

2.4. Genetic diversity and population structure analysis: The raw genotyping data were analyzed via Peak Scanner software (Applied Biosystems, California, USA) [61]. The samples were categorized into different groups on the basis of their forest circle, division, population, girth class, and geographic distance (determined by their latitude and longitude). The allelic data for each group were then analyzed statistically. GenAlEx v.6.5. [49] was used to calculate various parameters, such as the number of alleles (Na), the number of effective alleles (Ne), the polymorphism information content (PIC), the observed heterozygosity (Ho), the expected heterozygosity (He), Shannon's information index (I), the coefficient of genetic differentiation (F_{ST}), the gene flow (Nm) and analysis of molecular variance (AMOVA), to analyze genetic variation among the populations. Within populations, principal coordinate analysis (PCoA) based on the genetic distance matrix and the Mantel test were used to determine the correlation between geographic and genetic distances [49].

We applied Bayesian model-based cluster analysis via STRUCTURE 2.3.4 software [51] to determine population structure. The number of subgroups (K) was estimated to be 2 to 10 runs. Ten runs were performed on each K value, with a 10,000 iteration burn-in period followed by 10,000 Monte Carlo Markov chain (MCMC) replicates. The best K value was selected by Structure Harvester [17] based on the maximum ΔK [20] value. Cluster analysis was carried out by DARwin 6 software to construct a dendrogram via the unweighted neighbor-joining (UNJ) method with 10,000 bootstraps [50]. AMOVA was used to analyze the genetic variation among and within populations for all grouped data. Principal coordinate analysis (PCoA) was performed on the basis of the genetic distance matrix across 22 populations. A Mantel test was conducted using GenAlEx v.6.5 to determine the correlation between geographic and genetic distances [49].

2.5. Genetic diversity combined with ecological factors: To comprehensively understand the ecological factors influencing genetic diversity and population clusters, we carefully examined several key factors. This involved considering factors such as elevation, soil type, annual precipitation, and temperature during the sampling process. We recorded GPS location and elevation data for each sample and determined the soil type through ground truth observations and a literature review. Additionally, we gathered information from NASA [29] regarding the mean annual precipitation, mean annual temperature, mean annual relative humidity, and mean annual wind direction for each population from 1981–2023. To identify the soil type in 22 sampling populations, we utilized a soil map available at <https://www.fao.org> [27]. Furthermore, we conducted a comparative analysis of genetic diversity parameters across environmental factors.

3. Results

3.1. Genetic variation: Twenty-two populations of *P. santalinus* from Eastern Ghats were analyzed for genetic diversity and population structure via 16 highly polymorphic SSR markers [63] (Table S1). Among these loci, PIN-82 presented the highest value for all diversity indices: number of alleles (Na) = 12.82, effective number of alleles (Ne) = 8.85, expected heterozygosity (He) = 0.87, and observed heterozygosity (Ho) = 0.70. On the other hand, the PSSSR-2 locus had the lowest values for the same indices: Na=3.77, Ne=2.34, He=0.36, and Ho=0.12. Across all the loci, the average expected value for He was 0.65, whereas the average Ho was 0.34. Additionally, the PSSSR-32 locus had the highest PIC value (0.96), whereas the

PSSSR-2 locus had the lowest PIC value among the 16 loci (0.91) (Table 2). The CPV population presented the highest Na value at 13.44, whereas the TCT population presented the lowest Na value at 4.63. On the other hand, TTP had the highest Ne value at 9.21, whereas NRA had the lowest at 3.27. The average He was 0.65, with the CPV again having the highest value of 0.87 and the PVT having the lowest value of 0.44. The Ho values ranged from 0.673 (CPV) to 0.10 (TCT), with an average of 0.34 (Table 3).

3.2. Rare and private alleles across different populations: This study revealed rare alleles, which are common in less than 25% of populations, as well as population-specific alleles or private alleles (PAs), which are unique to a single population (Fig. 2). Loci PSSSR-24 and PSSSR-26 presented the greatest number of rare alleles (Fig S1). Among the populations, CVP had the highest number of rare alleles at 6.62, followed by TTP and CSM at 6.50 and 6.625, respectively. Across 16 highly polymorphic loci, 236 Pa were detected. The maximum number of Pa was found in the PSSSR-31, and the minimum was found in the PSSSR-18. On average, 0.67 private alleles (PAs) were found from 22 populations, ranging from 0.188 to 2.125. The NAG, TTP, CSM, and CPV populations contained the maximum number of Pa, with values of 2.12, 1.50, 1.44, and 1.31, respectively (Table 3). This study demonstrated clear genetic variation among populations from varying latitudes. Three primary groups, L1 (14.5–15.3°N), L2 (14.0–14.5°N), and L3 (13.0–13.9°N), were identified in this study, with each group having a cluster of alleles unique to the given latitude. The L1 group was composed of 105 alleles specific to this latitudinal group. These alleles are genetic variations that do not exist in other populations. The L2 group, in a similar vein, presented characteristics of 124 such alleles at this latitude, whereas the L3 group presented 142 alleles specific to its latitude. Compared with the other two groups, the L3 group presented a greater number of private alleles (Table S2; Fig S2).

3.3. Genetic differentiation: The inbreeding coefficient (Fis) ranged between 0.80 (PSSSR-72) and 0.19 (PIN-82), with a mean of 0.50. F statistics for each locus in the species revealed significant differences in the genetic differentiation coefficient (FST) and gene flow (Nm) at the locus level (Table 2). Furthermore, when the genetic differentiation coefficient (FST) and gene flow (Nm) among populations were compared, the average FST was 0.31, indicating moderate genetic differentiation. The Nm value ranged from 0.32 to 2.92, with a mean of 0.82, indicating a low rate of gene flow (Table 2). Analysis of molecular variance (AMOVA) with 999 permutations revealed 73% genetic variation within populations and 27% genetic variation between populations (Table 4). Additionally, FST = 0.27 (P=0.001) indicated a significant genetic difference among the 22 populations of *P. santalinus*. The pairwise FST ranged between 0.049 and 0.427. The highest level appeared between PVT and TBB, whereas the lowest was between populations of TTP and CVP (Table S3).

3.4. Genetic Structure and Cluster Analysis: The structural analysis revealed a maximum $\Delta K=3$. All 22 populations were grouped into three distinct genetic clusters (Fig. 3). Cluster I (red) contained 136 individuals from 8 populations (NRA, KOD, TBB, TCT, TTP, CSM, CPV, and NAK). Cluster II (green) contained 115 individuals from 7 populations (KVP, KSM, KRG, KKM, RRS, RCV, and NVV). Cluster III (blue) contained 110 individuals from 7 populations (PVT, PPT, PBB, RSJ, RKK, GGS, and NVD). UNJ tree analysis also grouped the 361 individuals from 22 populations into three main clusters. Compared with the structure analysis, there was little difference in population grouping. Cluster I included four populations, Cluster II had seven, and Cluster III contained eleven populations (Fig. 4). An analysis of the codominant alleles via PCoA was conducted to compare the structural and UNJ analyses. The first two principal coordinates captured 35.56% of the information, with individual contributions of 16.04% and 9.95% (Fig. 5). Mantel tests were conducted to analyze the relationships between geographic distance and pairwise Fst (Fig. 7), uNeiP (Fig. S3a), and genetic distance (Supplementary Fig. 3 b, c and d) in 22 *P. santalinus* populations. The results indicated that genetic distance (90%) contributed more substantially to the genetic differentiation among the populations than did geographic distance (70%). Therefore, it is evident that there was no clear geographic origin-based structuring or predominant isolation by distance among the studied populations (Fig 6).

3.5. Genetic variation among the different girth classes: Genetic diversity analysis was conducted across four girth classes: seedlings and saplings, young trees with a GBH of 10–20 cm, young trees with a GBH of 20–30 cm, and mature trees (>30 cm). The purpose was to observe differences in genetic fitness among the four girth classes potentially impacted by the illegal harvesting of adult trees. The data revealed that the number of alleles (Na) ranged from 30.69 to 32.81, whereas the number of effective alleles (Ne) varied between 16.30 and 16.73 (Table 5). The Ho values ranged from 0.34 to 0.36, and He remained constant at 0.94. Additionally, the data revealed a mean fixation index (F) of 0.63. These findings suggest that there is no noteworthy difference in genetic diversity among the four girth classes.

3.6. Correlations between genetic diversity and environmental factors:

A statistical analysis (Table 6) was used to explore the relationships between genetic diversity and environmental factors. Data collected on four environmental variables during the period 1980–2022 were plotted against populations (<https://power.larc.nasa.gov/data-acce>). The annual mean temperature varied from 26.23°C (RRS and RSJ) to 27.68°C (NAK) (Fig. S4a). There was a wide range of relative humidities between 62.86% (KKM/KOD/KRG/KSM/KVP) and 69.19% (TTP and CPV) (Fig. S4b). The wind direction varied from 222.4 (GGS) to 264.6 degrees (TTP and CPV) (Fig. S4c). Between 0.32 mm (KOD) and 1.67 mm (TTP and CPV) of precipitation were recorded (Fig. S4d). Populations with high genetic diversity experienced mean temperatures ranging from 26.5°C (CPV and TTP) to 26.90°C (CSM). The altitude and latitude were not significantly correlated with most of the genetic parameters studied (Fig. S5a-f: Fig. S6 a-f). Similarly, the annual mean temperature did not significantly correlate with the genetic parameters (Fig. S7 a-f). The population of Na increased with increasing relative humidity ($R^2=0.30$, $p<0.05$) and annual precipitation ($R^2=0.43$, $p<0.05$) (Fig. S9a, 10a). Additionally, Shannon's information index (I) was correlated with relative humidity ($R^2=0.22$, $p<0.05$) and annual precipitation ($R^2=0.34$, $p<0.05$) (Fig. S9b, 10b). Between 0.32 mm (KOD) and 1.67 mm (TTP and CPV) of precipitation were recorded (Fig. S4d). Populations with high genetic diversity experienced mean temperatures ranging from 26.5°C (CPV and TTP) to 26.90°C (CSM). The altitude and latitude were not significantly correlated with most of the genetic parameters studied (Fig. S5a-f: Fig. S6 a-f). Similarly, the annual mean temperature

did not significantly correlate with the genetic parameters (Fig. S7 a-f). The population of Na increased with increasing relative humidity ($R^2=0.30$, $p<0.05$) and annual precipitation ($R^2=0.43$, $p<0.05$) (Fig. S9a, S10a). Additionally, Shannon's information index (I) was correlated with relative humidity ($R^2=0.22$, $p<0.05$) and annual precipitation ($R^2=0.34$, $p<0.05$) (Fig. S8b, 9b). The Na population increased in correlation with both increasing relative humidity ($R^2=0.30$, $p<0.05$) and annual precipitation ($R^2=0.43$, $p<0.05$; Figures 8a and 9a). Similarly, the Shannon's information index (I) was correlated with relative humidity ($R^2=0.22$, $p<0.05$) and annual precipitation ($R^2=0.34$, $p<0.05$; Fig. S8b and S9b). However, there was no significant relationship between the Ho or fixation index and either the mean relative humidity or the annual precipitation (Fig. S8c, S9c; Fig. S9e). On the other hand, He was significantly related to annual precipitation ($R^2=0.25$, $p<0.05$; Fig. S8d and S9d). Additionally, Pa was significantly correlated with both relative humidity ($R^2=0.21$, $p<0.05$) and annual precipitation ($R^2=0.46$, $p<0.05$; Figures S8f and 9f; Table 6). An analysis of the locations of different populations with a soil map revealed that 20 out of 22 populations were situated on Lithosols (Fig. 7a), with one population each on Chromic Luvisols (PPT) and VerticCambisols (TTP). A noticeable pattern emerged, revealing an increase in genetic diversity from Lithosols to VerticCambisols (Fig. 7 b-g). Interestingly, the three populations with the highest genetic diversity consisted of two populations (CPV and CSM) situated on Lithosols and one population (TTP) on VerticCambisols. This trend in genetic diversity parameters from Lithosols to VerticCambisols provides valuable preliminary insights (Fig. 7b-g).

3.7. Genetic diversity analysis was performed on the basis of the different forest classifications. The Forest Department of Andhra Pradesh classified the distribution of *P. santalinus* into different circles and divisions in Eastern Ghats (Fig. S11 and S12). Analysis of genetic diversity by circle revealed that the Tirupati circle had the highest Na (35.38), whereas the Guntur circle had the lowest (22.94). However, Guntur had a greater Ne of 11.60 than the Kurnool did, despite the latter having a slightly greater Ne (Table 7, Fig. S11). This trend was also observed in Ho. Despite the small difference in sample size between the Kurnool and Tirupati populations, the private alleles found differed significantly (5.69–10.88). Additionally, although the population size of the Guntur circle was half that of the Kurnool, all the genetic diversity parameters were nearly equal to or greater than those of the Kurnool circle. These results clearly indicate that Tirupati has the highest diversity, followed by the Guntur and Kurnool circles. There was a significant difference in the number of individuals (N) among the different divisions (Table 8, Fig. S12). Kadapa had the greatest population size, with 78 individuals, whereas Giddalur and Nandyal had only 14 individuals. Divisions such as Chittoor, Nellore, and Rajampet had high N values, leading to higher allele frequencies than those of other divisions. As expected, Giddalur and Nandyal presented relatively low Ne values. Among all the divisions, Chittoor had the highest He value (0.91), whereas Nandyal (0.53) had the lowest genetic diversity. The Chittoor division had a low (fixation index) F value of 0.36, whereas the Giddalur division had a high F value of 0.75. The Nellore and Rajampet division populations had a high number of private alleles, which indicates a unique set of alleles in their genetic makeup.

The Forest Department of Andhra Pradesh classified the distribution of *P. santalinus* into different circles and divisions in the Eastern Ghats (Figs. S11 and S12). Through genetic diversity analysis, it was found that the Tirupati circle had the highest Na (35.38), whereas the Guntur circle had the lowest (35.38). However, Guntur had a greater Ne (11.60) than did Kurnool, despite Kurnool having a slightly greater Ne (Table 7; Fig. S11). Despite the small difference in sample size between the Kurnool and Tirupati populations, the private alleles found differed significantly (5.69–10.88). This trend was also observed in Ho. Even though the population size of the Guntur circle was half that of the Kurnool, all the genetic diversity parameters were nearly equal to or greater than those of the Kurnool circle.

Therefore, Tirupati clearly has the highest diversity, followed by the Guntur and Kurnool circles. There was a significant difference in the number of individuals among the different divisions (Table 8, Fig. S12). Kadapa had the greatest population size, whereas Giddalur and Nandyal had the lowest. The Chittoor division had the highest He value (0.91), whereas the Nandyal division (0.53) had the lowest genetic diversity. The Chittoor division had a low F value of 0.36, whereas the Giddalur division had a high F value of 0.75. The Nellore and Rajampet division populations had a high number of private alleles, indicating a unique set of alleles in their genetic makeup.

4. Discussion

4.1. Genetic diversity of *P. santalinus*: In India, *P. santalinus* is a valuable tree species that is in decline due to its valuable heartwood, compromising its genetic diversity. Genetic diversity is crucial for long-term survival and genetic improvement of a species through breeding [19, 34, 57]. To protect and preserve this species, effective conservation and management strategies have been developed using molecular markers [7, 5, 67]. Earlier studies focused on understanding genetic variations via dominant markers such as RAPD. Micropropagated *P. santalinus* individuals showed no genetic variation with RAPD markers [10], and significant DNA polymorphisms were detected in natural accessions [47] and nursery-grown plants [68]. These studies used small sample sizes, and dominant markers may have limitations in accurately capturing the total genetic diversity of the species. In this study, 16 highly polymorphic simple sequence repeats (SSRs) were used to assess the genetic diversity of *P. santalinus* across different populations. The present study revealed a total of 749 alleles among 361 individuals, which is considerably greater than the number of alleles obtained from 13 EST-SSRs. The genetic diversity values in this study were greater than those reported via EST-SSRs in *P. santalinus* and SSRs in *P. erinaceus* [1]. Two hundred thirty-seven alleles obtained from 17 SSRs were amplified across 365 individuals of *P. erinaceus* [31], as were the 54 alleles detected from 19 SSRs in 42 individuals of *Dalbergia odorifera* [38].

Our study revealed moderate genetic diversity values, with observed and expected heterozygosity values of 0.34 and 0.65, respectively. These values are relatively low compared with those of widely spread hardwood species such as *Swietenia macrophylla* ($H_e=0.78$) [36], *Eucalyptus globulus* Labill ($H_e=0.82$) [66], *E. grandis* ($H_e=0.77$) [64], *Tectona grandis* ($H_e=0.77$) [21], and *T. grandis* L. ($H_e=0.94$) [39]. Low values coincide with the general

belief that narrow-ranged species generally have lower genetic diversity than widespread congeners do [14]. These values were higher than those reported in studies within the genus *Pterocarpus*, specifically EST-SSRs in *P. santalinus* ($H_e=0.322$) [1] and SSRs in *P. erinaceus* ($H_e=0.57$) [31]. Among the 22 populations studied, the CPV population presented the highest genetic diversity, whereas the TCT population presented the lowest genetic diversity. The low genetic diversity values in the TCT can be attributed to forest fragmentation, the presence of agricultural land near this population, and anthropological activities, which led to genetic bottlenecks and reduced genetic diversity due to the loss of natural habitat and gene flow barriers. Importantly, the value of H_o was much lower than that of H_e , indicating heterozygote deficiency in the populations of *P. santalinus*. The potential reasons for these findings could be attributed to the entomophilous cross-pollinating nature and wind dispersal of seeds [54, 55, 67], which restrict long-distance dispersal in *P. santalinus*. Additionally, this species is recognized for its abundant regeneration through coppice formation [30]. Similar characteristics have also been observed in the related genus *Dalbergia* [38, 43]. Alternatively, in smaller populations, there is a greater occurrence of mating between relatives than in larger populations [43].

4.2. Rare and private alleles across the different populations: The frequency range of private alleles (PAs) varied significantly among different populations within the Eastern Ghats, with values ranging from 0.188 to 2.125. This wide range of variation suggests that some populations have a greater degree of genetic uniqueness than others do [24]. The unique alleles discovered in these populations could represent distinct evolutionary paths, as observed in rare plant species [32]. The presence of private alleles in a population is of evolutionary importance, and the evolutionary importance of a population is determined by both the number of alleles and the presence of private alleles [33, 48, 51]. Notably, the population with the highest P_a frequency (NAK) indicates significant genetic differentiation. These populations stand out as genetic reservoirs of unique alleles, indicating a greater degree of isolation or historical divergence that has led to the accumulation of distinctive genetics [32, 65], which might explain the long survival of *P. Santalinus* despite the enormous anthropological pressure. Hence, these trees are important genetic resources that can be used in tree breeding programs.

4.3. Genetic Differentiation and Population Structure

According to the AMOVA, the majority (73%) of the genetic variation detected was within populations, whereas only 27% was between populations. This suggests that the primary contributor to total genetic variation can be attributed to variation within populations. Similar findings were reported in a recent study on *P. marsupium* [41], which used intersimple sequence repeat (ISSR) markers and reported that 91% of the genetic variation was within populations and that only 9% was found among populations. Amri and Mamboya [6] reported that genetic variation in *P. angolensis* 77.13% within the population and 28.86% among distinct populations. This aligns with previous research on long-lived, outcrossing, late-succession plant taxa, which have been shown to exhibit high levels of genetic diversity within populations [35]. The Shannon's information index (I) of 1.52 and Nei's gene diversity (2.31) obtained in the present study revealed high levels of genetic variation within the populations (Table 3). These findings are greater than those reported for *P. angolensis* ($I=0.2769$) [6], *P. marsupium* ($I=0.46$) [41] and *D. odorifera* ($I=0.65$ and Nei's genetic diversity 0.38) by Liu et al. [38]. Similarly, a significant pairwise F_{ST} of 0.05 to 0.43 was observed among the *P. santalinus* populations (Table S2), which was greater than the F_{ST} (0.042 to 0.115) of *D. odorifera* [38], indicating moderate genetic differentiation among the 22 *P. santalinus* populations. The highest level of genetic differentiation was found between the PVT and TBB populations (0.427), which were isolated at a distance of 150 km between them, and the lowest was found between the TTP and CPV populations (0.049), which were located at 34 km (Table S2). This finding revealed a decrease in gene flow with increasing geographic distance. Gene flow is vital for determining genetic differentiation and genetic drift among populations [58]. Gene flow in plant species occurs through the exchange of pollen, seeds, and spores, influencing the genetic variation in the population [72, 73]. A gene flow value greater than 1 can overcome genetic drift, whereas a value less than one indicates that genetic drift is the predominant factor affecting a population's genetic structure [62]. A mean N_m of 0.82 was observed in the present study, indicating low gene flow among the populations and indicating the occurrence of genetic drift in the populations. If not addressed, this will lead to the isolation of populations, leading to inbreeding depression, genetic bottlenecks and the extinction of populations/species from natural habitats. *P. santalinus* is pollinated mostly by *Apis dorsata* only during the night [54, 55]. The short distance of these bees from hives might be the primary reason for the low gene flow between populations and the fragmentation of the populations.

An admixture model-based analysis was used to evaluate population structure. The genetic structure revealed the distribution pattern of genetic diversity within and between populations. Structural analysis revealed a maximum ΔK of 3, with 361 genotypes assigned to three clusters (Fig. 3). Cluster I included the populations in the Tirupati and Chittoor Circles, Cluster II included the populations in the Kadapa and Rajampet regions, and Cluster III included the populations from the Guntur and Nellore Circles. Some of the geographically close populations were placed in different clusters and vice versa, but the percentage of individuals with admixture was very low. The UNJ and PCoA results also supported the STRUCTURE analysis, which was attributed to differences in population clustering. Although all three analyses were successful in grouping 22 populations of *P. santalinus* into three subgroups, there were differences in the clustered populations and the number of populations. Overall, no apparent population clustering was observed, and a significant correlation was not found between genetic and geographic distances. The Mantel test results revealed that 68% of the genetic differences among the investigated populations were attributed to geographical distance (Fig. 6). The principle of isolation by distance, as described by Wright in 1943 [71], predicts that a positive correlation can be expected between genetic and geographic distances when populations reach equilibrium between gene flow and genetic drift. An analysis of the data revealed that there was a positive correlation between geographic distance and population differentiation, but it was not significant (Fig. 6). This differentiation is mostly attributed to genetic distance (Fig. 7b) rather than geographic distance. These findings indicate that human interference and the limited foraging distance of bees are the main factors influencing pollination differentiation. In the present study, low gene flow was detected even though the natural populations are located

within a maximum distance of 250 km. This phenomenon eventually results in inbreeding and affects the fitness of the population. A possible reason could be habitat loss and fragmentation of populations by human interference, as reported by the NDF survey.

4.4. Genetic variation among the Girth classes: The analysis revealed that there were no significant differences in genetic diversity parameters such as Na, Ne, He, and Ho among the four girth classes (Table 7). This finding suggests that the genetic makeup of the population remained consistent regardless of the girth class. However, a noticeable upward trend in all genetic diversity parameters was observed from higher to lower girth classes. These results align with findings from other studies on tree species such as *Pinus ponderosa* [37], *Koompassia malaccensis* [45], and *Dysoxylum malabaricum* [12], which also revealed a similar pattern of genetic diversity between seedlings and adult plants. One possible explanation for this trend could be the occurrence of genetic drift or gene flow within the population [11]. In contrast, a study conducted on *Manilkara multifida* [70] in a highly fragmented habitat revealed a significant difference in Ho between adult individuals (EVC = 0.720, UBR = 0.736) and juveniles (EVC = 0.463, UBR = 0.560). Similarly, *D. binectariferum* reported a high level of allelic richness in adults compared with juveniles [11, 42]. These findings indicate that population fragmentation may have an impact on genetic diversity. However, our study did not find any such differences in genetic diversity parameters despite the selective pressure of logging on the population. The forest department's tremendous efforts to protect the species and the good amount of natural regeneration might be the factors influencing the maintenance of genetic diversity among girth classes.

4.5. Effects of ecological factors and edaphic factors:

The genetic diversity of plant species depends on different factors, such as ecological, geographical, breeding system, and anthropogenic factors [56]. Higher genetic diversity is expected to reflect better adaptation to the changing environment of a species [23]. The *P. santalinus* distribution in Andhra Pradesh is restricted mainly to the Cuddapah landscape (13° 30' and 15° 00' North latitude and between 78° 45' and 79° 39' East longitude) [52]. Geographically, the region occupies approximately 5160 km², and its geographical spread includes the Chittoor, Cuddapah, and Nellore districts of the Seshachalam Hill Range, which has altitudes ranging from 150–900 m [8]. Physiographically, the most favorable altitude is reported to be from 300–800 m [52]. However, our study included altitudes ranging from 124 m to 838 m (Table 1). The CSM (124 m), CPV (318 m), and TTP (602 m) populations presented high genetic diversity and were located in low- and medium-altitude regions. There was no clear pattern observed in genetic variation with increasing elevation. Overall, in this study, we observed a significant correlation between genetic diversity according to annual precipitation and relative humidity. The genetic diversity of *P. santalinus* is influenced by the type of soil and topography. This plant species grows on dry, hilly, and often rocky terrain, and it prefers lateritic and gravelly soil [60]. Approximately 80% of the red-sander growing area is occupied by outcrops of quartzites, whereas the remainder is occupied by shale geological formations [52]. The high potassium content of the quartzite soil could be a major factor in the localization of *P. santalinus* [52]. According to the soil distribution map of Eastern Ghats, which was created from data from bhukosh (<https://bhukosh.gsi.gov.in/Bhukosh/Public>), 20 out of 22 populations were found to be located on lithosols. One population was found on chromic luvisols (PPT), and one was found on vertic cambisols (TTP) (Fig. 8a). Additionally, we observed an increasing trend in genetic diversity parameters from Lithosols to vertic cambisols (Fig. 8b-g). Furthermore, to investigate the relationships between environmental factors and genetic diversity, detailed soil nutrient data and other environmental information are necessary. Further investigations of soil pH, nutrient levels, and microbial associations may help in understanding the influence of soil and other environmental factors on genetic diversity.

4.6. Genetic diversity analysis based on different forest classifications: The genetic diversity of *P. santalinus* in the Eastern Ghats of Andhra Pradesh shows significant variation across different circles and divisions. The analysis revealed notable variations in allele frequencies and diversity parameters. Compared with the other populations, the Tirupati circle presented the greatest genetic diversity, with the greatest number of alleles (Na) and private alleles having high precipitation, humidity, and low temperatures. Interestingly, despite differences in population size, the Guntur circle presents comparable or greater adequate population size (Ne) and diversity metrics than do larger circles such as Kurnool. Similarly, within divisions, genetic diversity correlates with population size, with greater Ne observed in larger populations. Divisions such as CTR are notable for their elevated genetic diversity, whereas others such as NLR and RJT have unique sets of private alleles. These findings highlight the need to consider population size and local environmental factors in conservation strategies for *P. santalinus* in the Eastern Ghats. Despite the stringent efforts of the APFD to combat unlawful deforestation practices, a noteworthy decline in the population of mature *P. santalinus* bearing trees available for harvesting has been detected in various forests [3]. This decline is most prominent in three forest department circles and eight divisions, where the Tirupati circle and Chittoor division exhibit significant variations. This could be due to *P. santalinus* natural populations prevailing in dry areas [9]. These populations hold immense potential as germplasms for future conservation initiatives aimed at safeguarding the species.

5. Conclusion

This study represents the first comprehensive genetic diversity analysis covering the entire natural range of *P. santalinus* in Eastern Ghats, India. We used 16 highly polymorphic SSR markers to assess 22 natural populations in the region. Our findings revealed moderate genetic diversity, which is relatively low for a woody tree species, with a few populations displaying high levels of diversity, whereas others presented alarmingly low genetic variation. Most of the genetic variation was observed within populations, with moderate genetic differentiation and low gene flow among them. Among the 22 populations, Tirupati base-Sadashiva Kona (CPV), followed by Tirupati–Papanashini (TTP) and Chittoor East-Srikalahasti-Melachur (CSM), presented the highest genetic diversity (He=0.869), and the lowest was Chitaleti Pati Base Camp (PVT) (He=0.436). Additionally, many private alleles have been identified in populations Nellore Atmakuru-Kadarinaidupalli (NAK), Tirupati–Papanashini; (TTP), and Chittoor East-Srikalahasti-Melachur; (CSM), presenting opportunities for selective breeding to increase adaptation and resistance to changing environments. At the forest circle and division levels, the Tirupati circle and Chittoor divisions were identified as highly diverse, which is consistent with the findings at

the population level. Despite habitat fragmentation and anthropogenic pressure on forest areas, no notable differences in genetic diversity parameters were observed between seedlings, saplings, and mature trees. The study also revealed significant correlations between genetic diversity parameters, annual precipitation, and relative humidity. Moreover, soil type and topography likely play major roles in species distributions. Notably, populations located at lower latitudes presented significant genetic diversity across their distribution range. These results underscore the need for further investigations into the effects of terrain, soil, and environmental factors to better understand the interaction between the environment and genetics on heartwood formation. In conclusion, the SSR loci identified in this study could serve as valuable tools for future tree improvement programs. Populations with high genetic diversity and unique alleles should be leveraged as germplasm resources and for seed material in both *in situ* and *ex-situ* conservation efforts, particularly to address the low gene flow rate among populations and manage those with low genetic diversity.

Abbreviations

- APFD – Andhra Pradesh Forest Department
- CITES - Convention on International Trade in Endangered Species of Wild Fauna and Flora
- RAPD-Random Amplified Polymorphic DNA
- ISSR-Inter-Simple Sequence Repeat
- SSR- simple sequence repeats
- EST-SSR - expressed sequence tag-derived simple sequence repeat marker
- NGS- Next-Generation Sequencing
- DNA- Deoxyribonucleic Acid
- CTAB- Cetyltrimethylammonium bromide
- PCR- Polymerase chain reaction
- Na-Number of different alleles
- Ne-number of effective alleles
- Ho- observed heterozygosity
- He-Expected Heterozygosity
- PIC- Polymorphic Information Content
- I- Shannon's information index
- F- Fixation Index
- Fis- Inbreeding coefficient within an individual relative to the total
- Fit- Inbreeding coefficient within an individual relative to the total
- Fst- Inbreeding coefficient within a subpopulation relative to the total
- Nm-Gene Flow
- AMOVA - Analysis of Molecular Variance
- UNJ- Unweighted neighbor-joining
- PCoA-Principal Coordinate Analysis
- MCMC-Monte Carlo Markov Chain
- NDF- Non-Detrary Findings
- GBH- Girth at Breast Height

GC – Girth class

SiO₂-Silicon dioxide

K -Potassium

Fe- Iron

Ca- Calcium

Na-Sodium

A2cd- Declining population (past, present and/or projected)

A2-Population reduction observed, estimated, inferred, or suspected in the past where the causes of reduction may not have ceased OR may not be understood OR may not be reversible.

(c) Declines in area of occupancy (AOO), extent of occurrence (EOO) and/or habitat quality

(d) Actual or potential levels of exploitation

Declarations

Source of Funding: National Biodiversity Authority (NBA) (No. Tech./Genl./22/149/17/18-19/382).

Conflict of interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as potential conflicts of interest. All the authors declare that there are no conflicts of interest.

Clinical trial number: Not applicable.

Consent to Participate: Not applicable. This study does not involve any human or animal trials, nor does it include any genetic modification of the plant.

Ethics statement: The plant material was identified by Divakara, BN, a scientist at IWS in Bengaluru, and a voucher specimen has been deposited at TDU/IWS, (IWS-NBA-01 to 400), FRLHT, Bengaluru. We have collected samples with the gracious permission of the Forest Department of Andhra Pradesh, India. Reference No. EF02-20051/33/2020 - Research SEC PCCF dated 10/20/2020 from the Forest Department, Government of Andhra Pradesh, India. We adhered to both local and national guidelines during the sample collection.

Data Availability Statement: All data generated or analyzed during this study are included in this published article [and its supplementary information files].

Consent to publish: Consent to publish were obtained from all authors, and all the authors declare that there are no conflicts of interest.

Plant reproducibility: Leaf samples of *Pterocarpus santalinus* were collected from the Eastern Ghats of Andhra Pradesh, India. Only leaf samples were collected without harming the tree, and ensuring their reproducibility.

Author contribution statement: The project was conceptualized by MKP, PHR, and DBN. Sample collection was carried out by MKP, PHR, and DBN. Sample preparation, experiments, and data analysis were carried out by SMV, MAH, PHR, and MKP. Discussion of the data and MS preparation **are performed by** SMV, MAH, PHR, CJ, and MKP. The finalization of the manuscript was performed by PHR, CJ, SK, DBN, and MKP.

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Tables

Table 1: Sample collection data for 22 populations of *Pterocarpus santalinus* from various locations in Eastern Ghats, Andhra Pradesh, India.

Forest Circle	Division	Range	Beat	Location	Population ID	Latitude (N)	Longitude (E)	Altitude (m)	Sample size
Guntur	Giddalur	Giddalur	Singasanipalli	PallugaoduThoka and Eluguddu shola	GGS	15.2977	79.12817	512	16
	Nellore	Udayagiri	Devammacheruvu	Anasagarabodu and Badithala Bodu	NUD	14.90691	79.16839	300	19
	Nellore	Atmakur	Kadarinaidupalli	Urlukonda Camp 410	NAK	14.67652	79.19965	157	20
	Nellore	Venkatagiri	Vembaluru	Mekalaguntha Camp 248 and Chinnakona Base Camp	NVV	14.09043	79.47644	162	16
Kurnool	Nandyal	Rudravaram	Ahobilam North	Chinnakuchala	NRA	15.15379	78.71711	305	16
	Proddatur	Porumamilla	Tekurpeta	Yerratichala	PPT	14.99517	79.07484	398	16
	Proddatur	Vanipenta	Tippireddypalli	ChitaletiPati Base Camp	PVT	14.96653	78.75828	315	16
	Proddatur	Badvel	Balayapalli	Bodabodu Camp 283	PBB	14.62871	78.97646	177	14
	Kadapa	Siddavatam	Maddur	Chinnadoddikona	KSM	14.51345	79.00589	190	16
	Kadapa	Vempalli	Polathala	Bandennapadalu	KVP	14.36888	78.67371	404	16
				Camp 582					
	Kadapa	Kadapa	Maddimadugu East	Inaparala gutta Camp 519	KKM	14.3371	78.78028	238	16
	Kadapa	Rayachoti	Guvalacheruvu west	Mangalam Bayalu road	KRG	14.31187	78.76187	462	16
	Kadapa	Ontimitta	Dasarala Doddi	Utumora	KOD	14.30738	78.99724	211	16
Tirupati	Rajampet	Rajampet	Sri RangarajaPalem	RollamadujuRastha Camp 889	RRS	14.12091	79.03636	367	16
	Rajampet	Sanipaya	Jandrapenta	Kuntathuvalu	RSJ	14.12925	79.00255	351	16
	Rajampet	Chitvel	Venkatarajpalli	EtikuntoduBadava	RCV	14.11746	79.39793	187	16
	Rajampet	Kodur	K. V. bhavi (North)	Bangla board	RKK	13.91702	79.26148	151	16
	Tirupati	Balapalli	Balapalli West	Pangalachala	TBB	13.78834	79.38422	355	16
	Tirupati	Chamala	Talacona centra	Rondavanka	TCT	13.7736	79.17559	602	16
	Tirupati	Tirupati	Papanashini and Timmala	Papanashini Dam Camp 128 and Yerriganthalu Camp 127	TTP	13.71216	79.35739	790	16
	Chittoor East WL	Srikalahasti	Melachur	Savadugunta Camp 464	CSM	13.83461	79.49982	124	16
	Chittoor East	Puttur	Vadamala Peta	Sadashiva Kona	CPV	13.53716	79.60114	318	20
GGS-GiddalurGiddalurSingasanipalli;NUD-Nellore Udayagiri Devammacheruvu; NAK- Nellore AtmakurKadarinaidupalli ; NVV- Nellore Venkatagiri Vembaluru; NRA- NandyalRudravaramAhobilam North; PPT- ProddaturPorumamillaTekurpeta; PVT- ProddaturVanipentaTippireddypalli; PBB- ProddaturBadvelBalayapalli; KSM- Kadapa SiddavatamMaddur; KVP- Kadapa VempalliPolathala; KKM- Kadapa KadapaMaddimadngu; KRG- Kadapa RayachotiGuvalacheruvu; KOD- Kadapa OntimittaDasarala Doddi; RRS- RajampetRajampet Sri RangarajaPalem; RSJ- RajampetSanipayaJandrapenta; RCV- RajampetChitvelVenkatarajpalli; RKK- Rajampet Kodur K. V. bhavi; TBB- Tirupati BalapalliBalapalli West; TCT- Tirupati ChamalaTalacona centra; TTP- Tirupati TirupatiPapanashini; CSM- Chittoor East SrikalahastiMelachur; CPV- Chittoor East Puttur Vadamala Peta.									

Table 2: Genetic diversity indices across 16 microsatellite loci for 22 populations of *Pterocarpus santalinus*

Locus	N	Na	Ne	I	Ho	He	PIC	F	F _{IS}	F _{ST}	N _m
PIN-82	16	12.82	8.85	2.29	0.7	0.87	0.94	0.18	0.19	0.08	2.92
SSR-3	19	4.91	2.71	0.94	0.17	0.44	0.92	0.68	0.61	0.52	0.23
SSR-6	20	11.55	7.83	2.13	0.61	0.84	0.95	0.31	0.28	0.12	1.85
SSR-8	16	9	5.89	1.84	0.45	0.78	0.94	0.42	0.42	0.17	1.2
PSSSR-2	16	3.77	2.34	0.72	0.12	0.36	0.91	0.73	0.65	0.61	0.16
PSSSR-5	16	5.36	3.45	1.13	0.28	0.54	0.94	0.57	0.47	0.43	0.33
PSSSR-6	16	6.36	4.23	1.24	0.24	0.54	0.95	0.62	0.56	0.44	0.32
PSSSR-10	14	6.18	3.77	1.35	0.31	0.64	0.94	0.58	0.52	0.32	0.53
PSSSR-18	16	8.95	5.71	1.8	0.59	0.76	0.94	0.27	0.22	0.19	1.06
PSSSR-20	16	5.5	3.16	1.17	0.17	0.55	0.92	0.71	0.69	0.4	0.37
PSSSR-24	16	10.41	6.81	1.9	0.38	0.75	0.95	0.55	0.5	0.21	0.96
PSSSR-26	16	7.68	5.23	1.54	0.19	0.66	0.95	0.73	0.71	0.31	0.55
PSSSR-29	16	8.86	5.78	1.69	0.5	0.71	0.93	0.35	0.31	0.23	0.82
PSSSR-30	16	8.82	5.2	1.61	0.39	0.67	0.95	0.49	0.41	0.29	0.61
PSSSR-31	16	7.14	4.09	1.48	0.25	0.66	0.94	0.66	0.62	0.3	0.58
PSSSR-32	16	7.36	4.71	1.54	0.14	0.68	0.96	0.82	0.8	0.3	0.59
Mean	16	7.79	4.99	1.52	0.34	0.65	0.94	0.54	0.5	0.31	0.82
N: mean number of samples, Na: number of alleles, Ne: number of effective alleles, I: Shannon's Information Index, Ho: observed heterozygosity, He: expected heterozygosity, PIC: polymorphic information content, F: fixation index, F _{IS} : inbreeding coefficient within individual relative to total, F _{ST} : inbreeding coefficient within subpopulation relative to total, N _m : gene flow.											

Table 3: Analysis of genetic diversity in 22 populations of *Pterocarpus santalinus* using 16 microsatellite markers.

Population	N	Na	Ne	I	Ho	He	F	Pa	PPL
GGS	16	5.88	3.92	1.23	0.20	0.57	0.75	0.19	100%
NUD	19	8.63	5.05	1.59	0.51	0.69	0.30	0.38	100%
NAK	20	10.00	6.03	1.81	0.20	0.75	0.72	2.13	100%
NVV	16	8.06	4.32	1.51	0.42	0.64	0.38	0.56	100%
NRA	16	5.19	3.27	1.11	0.16	0.53	0.70	0.19	88%
PPT	16	8.13	4.77	1.65	0.34	0.71	0.54	0.56	100%
PVT	16	5.56	3.59	1.00	0.20	0.44	0.67	0.25	75%
PBB	14	6.56	3.95	1.44	0.43	0.66	0.34	0.75	100%
KSM	16	7.50	4.45	1.54	0.37	0.67	0.50	0.25	94%
KVP	16	6.69	4.42	1.36	0.29	0.59	0.62	0.50	94%
KKM	16	7.63	4.50	1.60	0.45	0.71	0.40	0.44	100%
KRG	16	7.63	4.06	1.53	0.33	0.68	0.55	0.75	100%
KOD	16	5.81	3.80	1.21	0.27	0.57	0.63	0.56	94%
RRS	16	7.31	4.40	1.53	0.41	0.67	0.41	0.25	94%
RSJ	16	6.81	3.99	1.32	0.29	0.57	0.63	0.19	94%
RCV	16	9.94	6.82	1.96	0.52	0.81	0.34	0.75	100%
RKK	16	7.00	4.64	1.28	0.27	0.55	0.67	1.13	94%
TBB	16	4.94	3.55	1.03	0.13	0.49	0.81	0.25	81%
TCT	16	4.63	3.46	1.01	0.10	0.49	0.88	0.44	88%
TTP	16	12.31	9.21	2.27	0.52	0.86	0.40	1.50	100%
CSM	16	11.81	9.12	2.20	0.46	0.84	0.47	1.44	100%
CVP	20	13.44	8.39	2.29	0.67	0.87	0.22	1.31	100%
Mean	16	7.79	4.99	1.52	0.34	0.65	0.54	0.67	0.95

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, Pa: number of private alleles, PPL: percent polymorphic loci.

GGS-GiddaluruGiddaluruSingasanipalli;NUD-Nellore Udayagiri Devammacheruvu; NAK- Nellore AtmakurKadarinaidupalli ; NVV- Nellore Venkatagiri Vembaluru; NRA- NandyalRudravaramAhobilam North; PPT- ProddaturPorumamillaTekurpeta; PVT- ProddaturVanipentaTippireddypalli; PBB- ProddaturBadvelBalayapalli; KSM- Kadapa SiddavatamMaddur; KVP- Kadapa VempalliPolathala; KKM- Kadapa KadapaMaddimadngu; KRG- Kadapa RayachotiGuvalacheruvu; KOD- Kadapa OntimittaDasarala Doddi; RRS- RajampetRajampet Sri RangarajaPalem; RSJ- RajampetSanipayaJandrapenta; RCV- RajampetChitvelVenkatarajpalli; RKK- Rajampet Kodur K. V. bhavi; TBB- Tirupati BalapalliBalapalli West; TCT- Tirupati ChamalaTalacona centra; TTP- Tirupati TirupatiPapanashini; CSM- Chittoor East SrikalahastiMelachur; CPV- Chittoor East Puttur Vadamala Peta.

Table 4: Analysis of molecular variance (AMOVA) for 361 individuals of *Pterocarpus santalinus* populations.

Source	Degree of freedom	Sum of squares	Mean of squares	Variance components	% of variation
Among Populations	21	1591.32	75.77	2.14	28%
Within Populations	700	3862.87	5.51	5.51	72%
Total	721	5454.19		7.66	100%

Table 5: Genetic variation in different girth classes of *Pterocarpus santalinus*.

Girth Class (cm)	N	Na	Ne	I	Ho	He	F
GC-1 (0-10)	88	32.81	16.62	3.09	0.35	0.94	0.63
GC-2 (10-20)	88.44	32.44	16.73	3.08	0.35	0.94	0.63
GC-3 (20-30)	86.19	32.38	16.68	3.08	0.36	0.94	0.62
GC-4 (30->)	83.38	30.69	16.30	3.05	0.34	0.94	0.64
Mean	86.53	32.08	16.58	3.08	0.35	0.94	0.63

N: mean 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 6: Pearson correlation analysis between genetic diversity and environmental factors.

Variable	Pearson's Coefficient	Latitude	Altitude(m)	Annual Temperature	Annual Precipitation	Relative Humidity
Na	r	0.405	0.036	0.318	0.655	0.544
	p	0.061	0.874	0.149	0.001	0.009
Ho	r	0.328	0.136	0.284	0.308	0.283
	p	0.136	0.546	0.200	0.163	0.202
He	r	0.320	0.083	0.206	0.502	0.391
	p	0.146	0.713	0.357	0.017	0.072
I	r	0.381	0.036	0.286	0.580	0.473
	p	0.080	0.873	0.198	0.005	0.026
F	r	0.193	0.217	0.187	0.146	0.130
	p	0.391	0.332	0.403	0.517	0.564
Pa	r	0.360	0.100	0.085	0.675	0.454
	p	0.100	0.657	0.706	0.001	0.034

Na: number of alleles, Ho: observed heterozygosity, He: expected heterozygosity, I: Shannon's Information Index, F: fixation index, Pa: number of private alleles.

Table 7: Genetic diversity analysis of *Pterocarpus santalinus* based on the different forest circlewise distributions in Eastern Ghats, Andhra Pradesh, India.

Circle	N	Na	Ne	I	Ho	He	F	Pa
Guntur	68	22.94	11.6	2.63	0.34	0.9	0.63	3.63
Kurnool	137	26.63	10.95	2.67	0.31	0.9	0.65	5.69
Tirupati	140	35.38	14.86	3.01	0.38	0.93	0.59	10.88
Mean	115	28.31	12.47	2.77	0.35	0.91	0.62	6.73

N: mean 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, Pa: number of private alleles.

Table 8: Genetic diversity analysis of *Pterocarpus santalinus* based on the different forest divisionwise distributions in Eastern Ghats, Andhra Pradesh, India

Division	N	Na	Ne	I	Ho	He	F	Pa
CTR	33.44	19.69	11.68	2.67	0.58	0.91	0.36	3.19
NLR	53.50	20.62	10.44	2.52	0.37	0.88	0.58	3.37
GDR	14.50	5.87	3.92	1.23	0.20	0.57	0.75	0.19
KDP	78.06	18.56	8.00	2.32	0.34	0.86	0.60	3.19
NDL	15.56	5.19	3.27	1.11	0.16	0.53	0.70	0.19
PDT	44.12	15.69	7.50	2.18	0.32	0.82	0.63	1.69
RJT	61.50	19.56	9.30	2.46	0.37	0.88	0.59	2.44
TPT	45.49	17.62	6.92	2.12	0.25	0.77	0.69	2.50
Mean	43.27	15.35	7.63	2.08	0.32	0.78	0.61	2.09

CTR-Chittoor; NLR-Nellore; GDR-Giddalur; KDP-Kadapa; NDL- Nandyal; PDT-Poddratur; RJT-Rajampet; TPT-Tirupati

N: mean 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, Pa: number of private alleles.

Figures

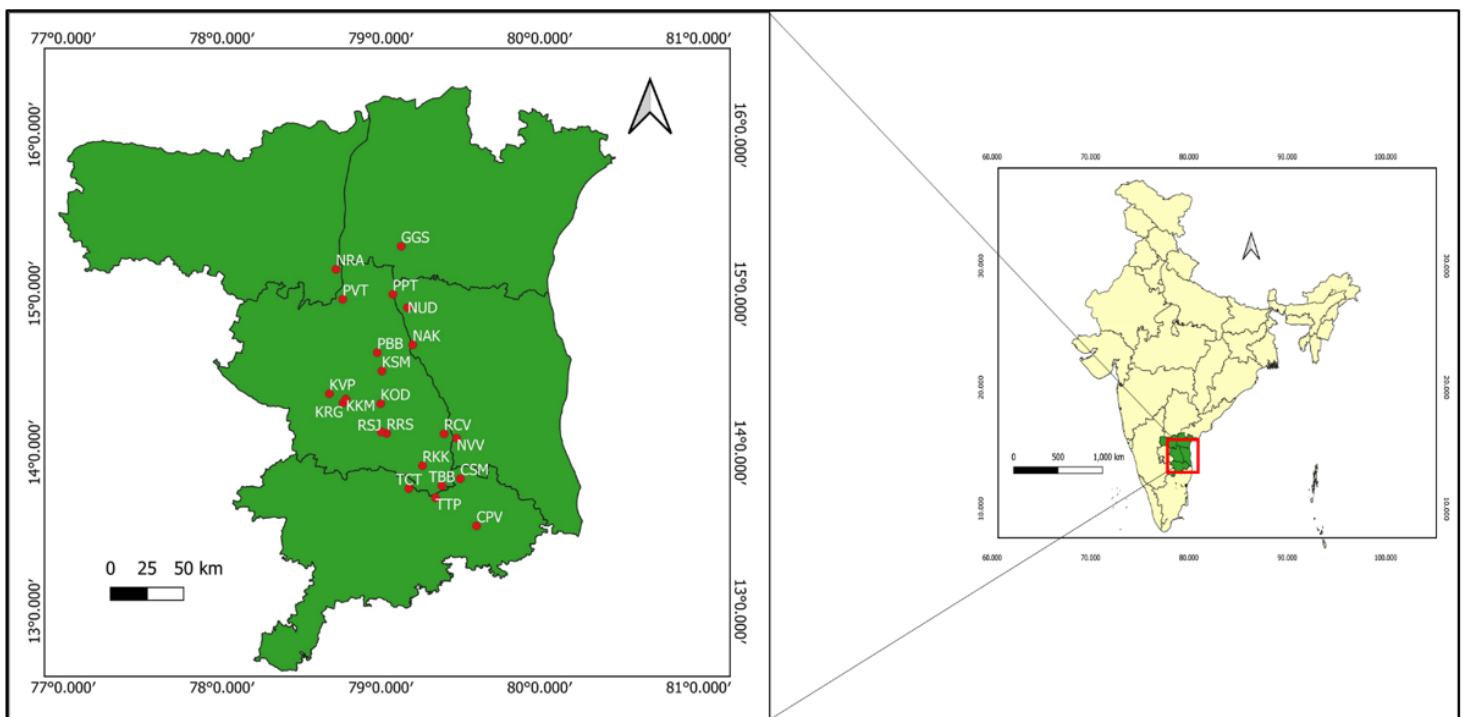


Figure 1

Geographical distribution of 22 populations of *Pterocarpus santalinus* in the Eastern Ghats, Andhra Pradesh, India

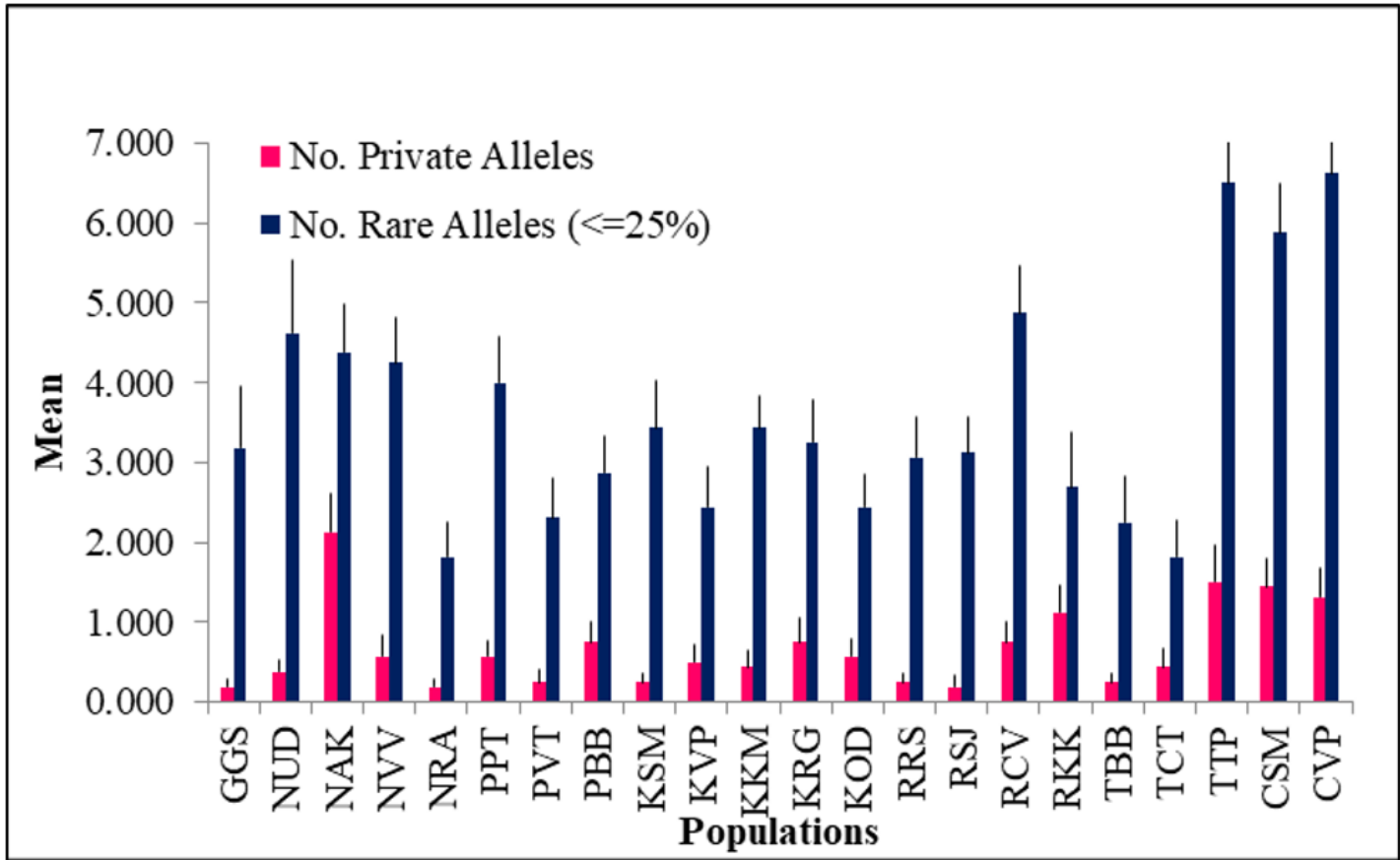


Figure 2

Number of rare and unique alleles among the 22 populations of *Pterocarpus santalinus*

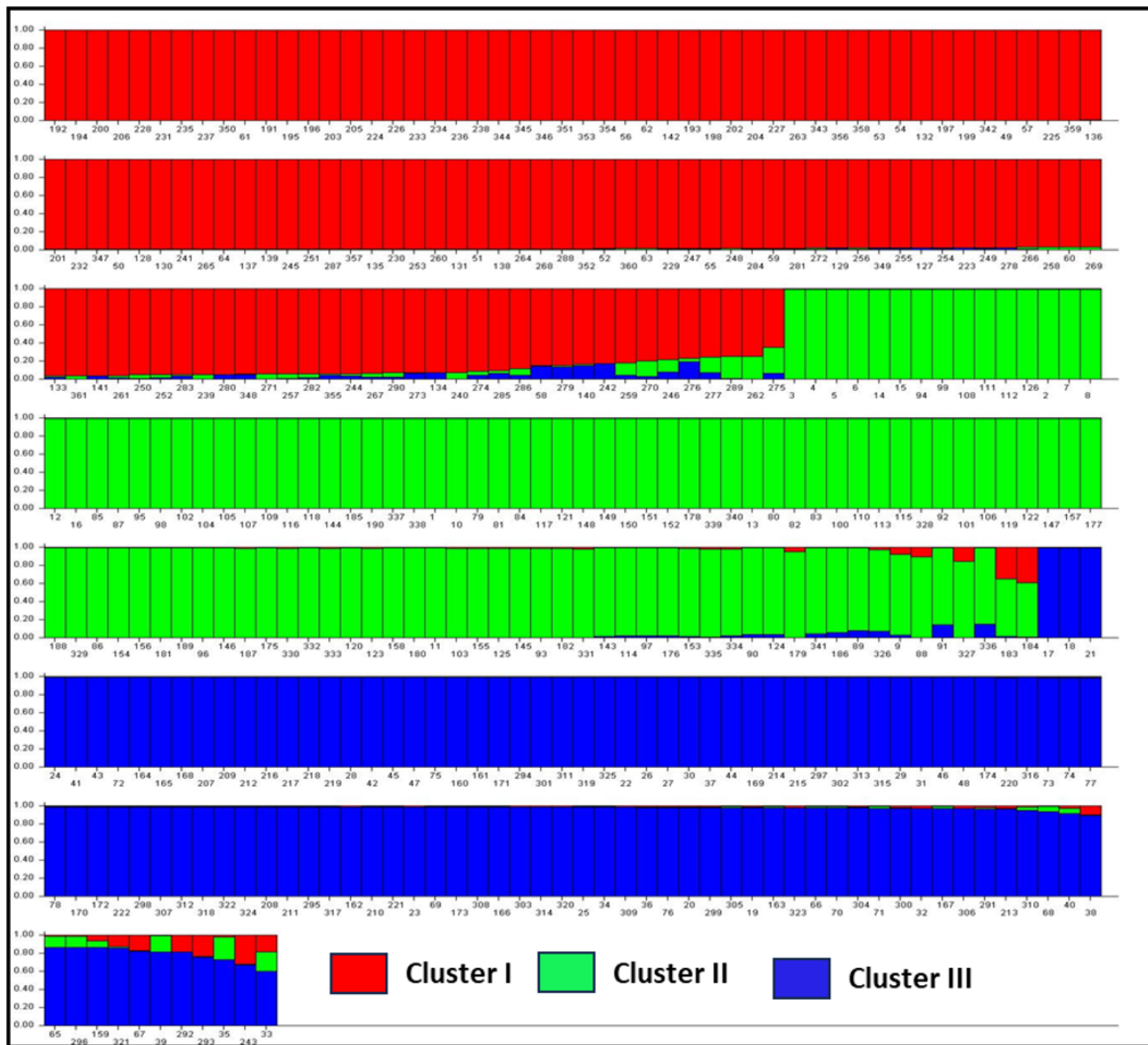


Figure 3

STRUCTURE bar graph representing three genetic clusters ($\Delta K = 3$) among 22 populations of *Pterocarpus santalinus*. Each vertical bar represents an individual sample, and the color of the bar indicates the assignment probability of that individual belonging to one of the three identified clusters (designated by different colors).

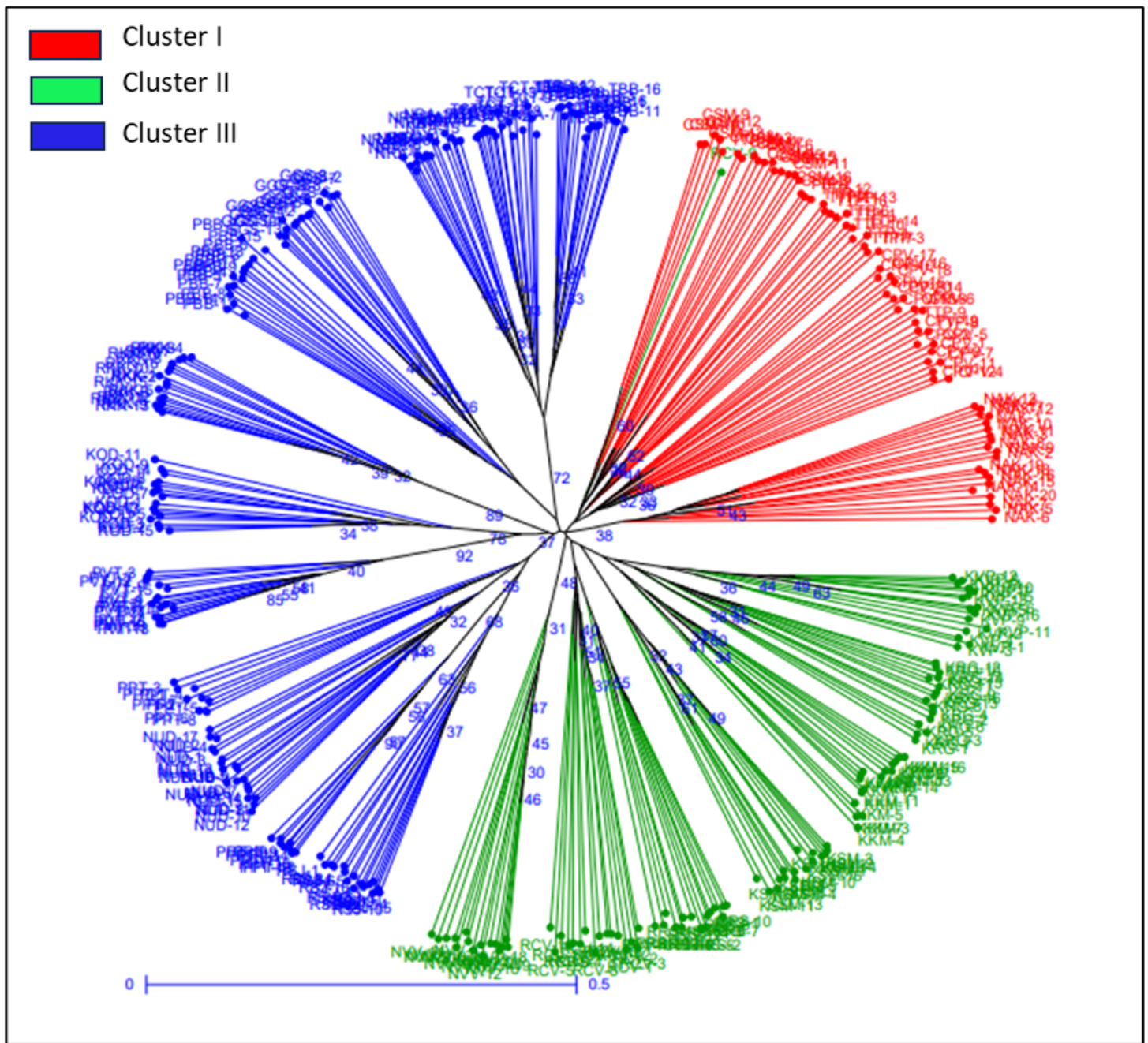


Figure 4

Neighbor-joining tree for 361 individuals of *Pterocarpus santalinus*. Each color depicts a cluster. The numbers indicate the percentage of bootstrap support using 10000 replications.

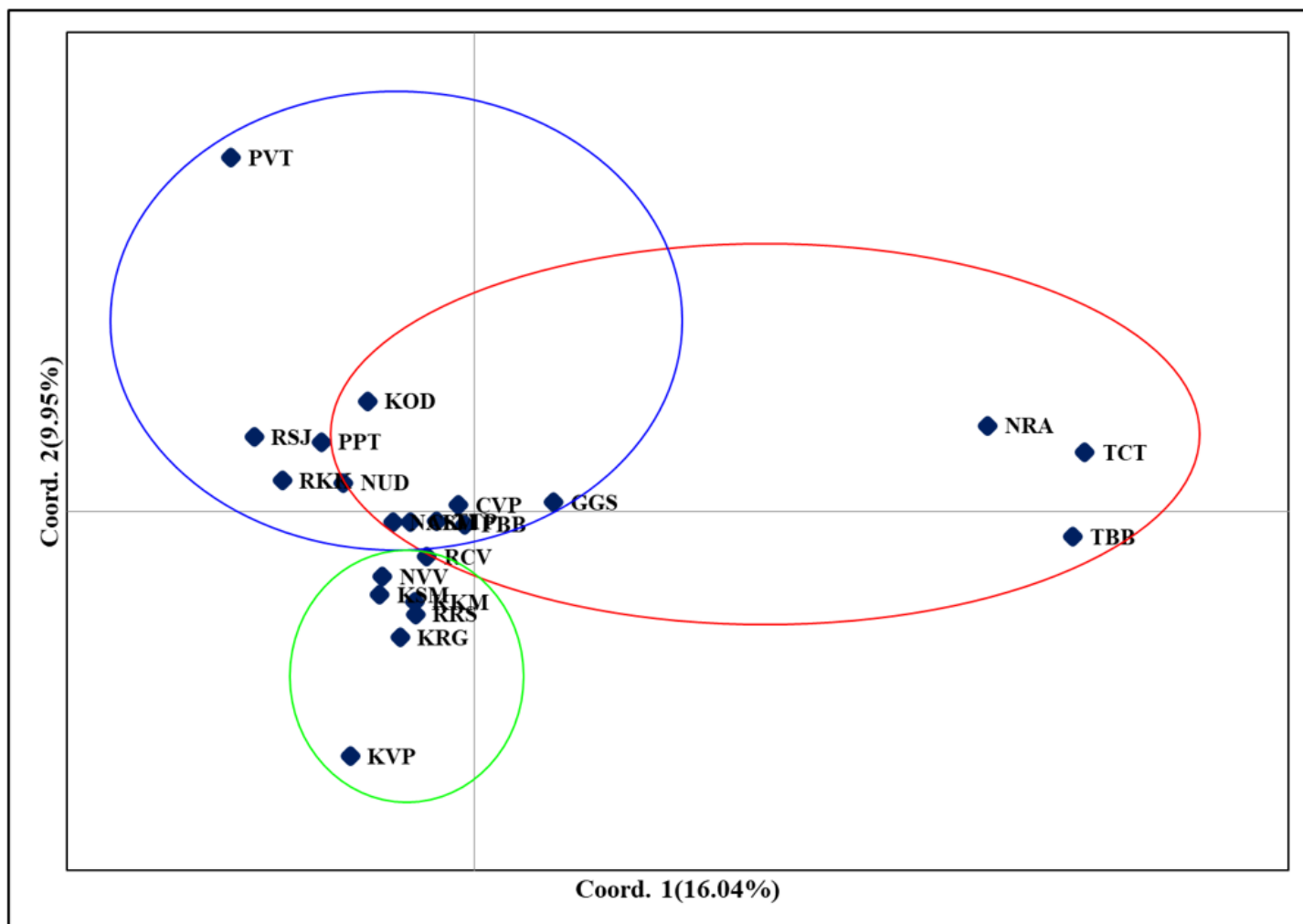


Figure 5

Principal coordinate analysis (PCoA) based on pairwise F_{ST} . Coord.1 (16.04%): The first principal coordinate explained 16.04% of the variation; Coord.2 (9.95%): The second principal coordinate explained 9.95% of the variation.

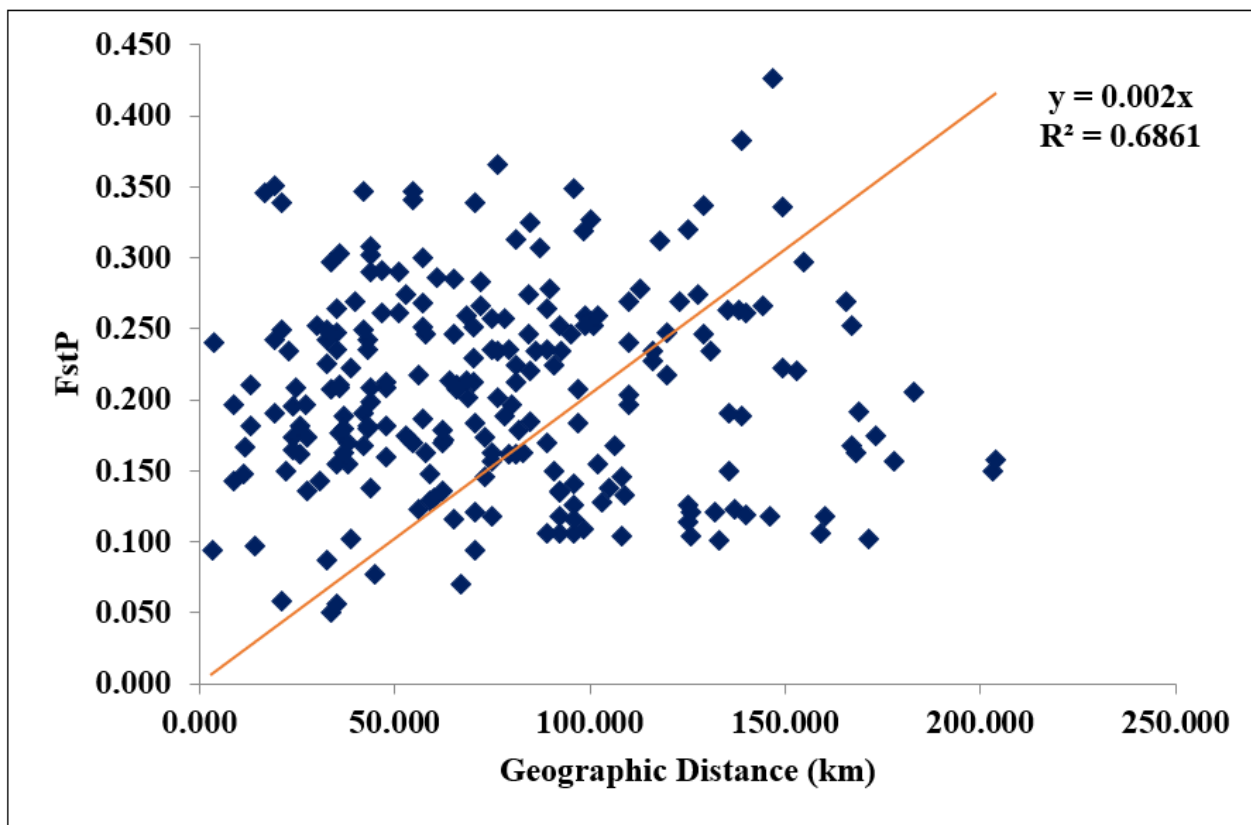


Figure 6

Mantel tests for relationships between pairwise F_{ST} matrix and geographic distance for *Pterocarpus santalinus* natural populations ($R^2 = 0.6861$, $p > 0.05$).

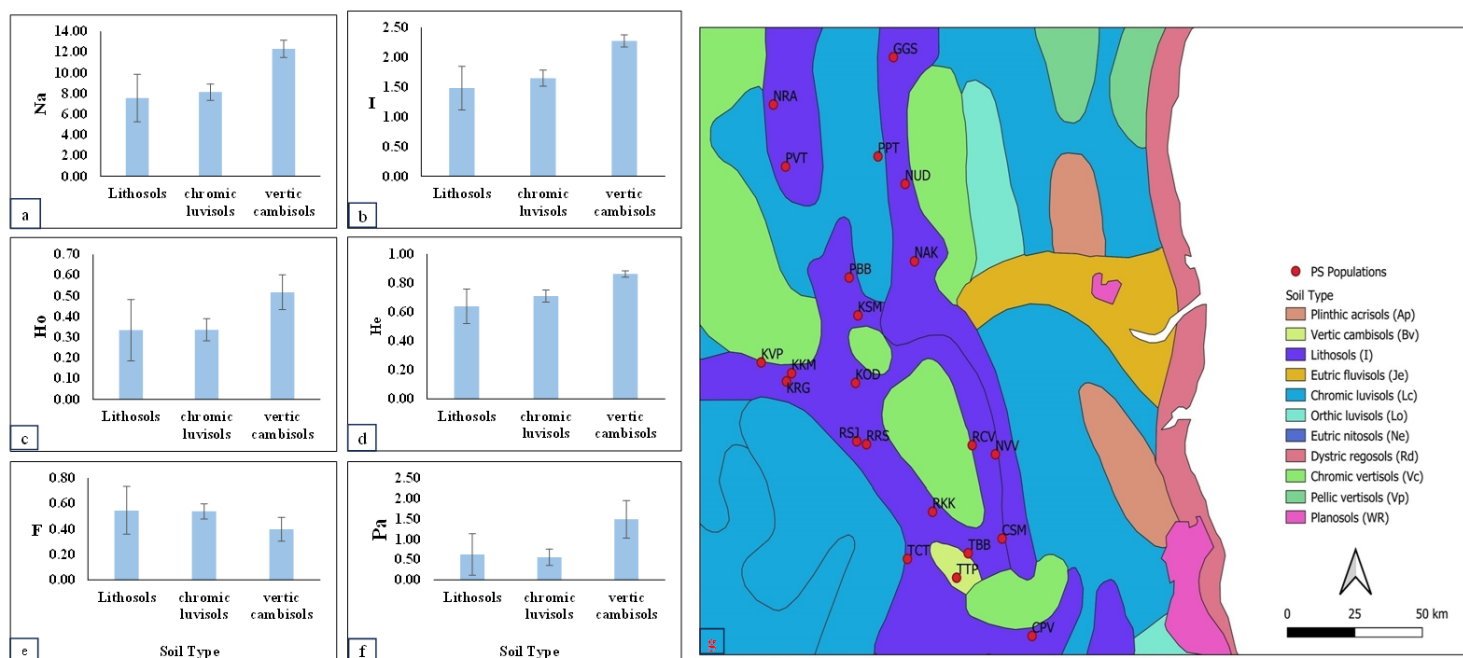


Figure 7

Genetic diversity indices across the different soil type (a to f) distributions of *Pterocarpus santalinus* populations. g. Distribution of *Pterocarpus santalinus* populations with respect to soil type in Eastern Ghats, Andhra Pradesh, India.

Supplementary Files

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