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Morphological and Molecular Diversity in *Pterocarpus santalinus* L.f - an Endemic and Endangered Medicinal Plant

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ABSTRACT

The present study is the first report of morphological and molecular variations in accessions of *Pterocarpus santalinus* L.f (endemic, endangered, commercially and medicinally very important) collected from Kerala, Karnataka and mostly from Andhra Pradesh, India. It addresses the determination of genetic variation among accessions using few morphological parameters and RAPD markers. A total of 27 accessions were collected out of which morphological variations were characterized for 14, 15 and 16 and molecular variations for 15 of them. Morphological data opened up lot of variations among the accessions. Molecular investigation revealed that, out of the 40 primers screened, 26 primers selected for the data analysis generated a total of 217 scorable markers, all of which were polymorphic. This high proportion of polymorphism i.e., almost 100%, was found with 53 unique markers. Cluster analysis based on Dice's coefficient showed two major groups indicating that in cross-pollinated plants, high levels of differentiation among accessions exist. The grouping of these accessions was independent of the geographical distance. The significant molecular variations in the accession collected from Tirupathi when compared to other accessions need to be further investigated. Hence the results of the present study can be viewed as a starting point for future research on the population and evolutionary genetics of this species and understanding such variation would facilitate their use in various conservational management practices, rootstock breeding and hybridization programmes.

Keywords: conservation, genetic diversity, morphological markers, RAPD (Randomly Amplified Polymorphic DNA)

INTRODUCTION

Pterocarpus santalinus L.f (Fabaceae), commonly named in trade as "Red sanders", known for its medicinal and commercial value. It is an endangered, endemic tree species that occurs in patches in few regions of Southern Eastern Ghats and has a restricted natural range of 15,540 sq. km (Ahmed and Nayar 1984; Sarma 1993; Jadhav *et al*. 2001). It is a tropical dry deciduous forest species confined to 13°30′-15°N latitude and 78°45′-79°30′E longitude (Kesavaraju and Jagdishwararao 1991) and it grows at an elevation of 100-1000 m above-sea level, mainly on stony or gravelly soil on formations of gneiss, quartzite, shale or laterite and loamy soils, does not tolerate stiff water logged soil, but has been planted with success on rich alluvial grounds (Troup 1921). It is a strong light demander and does not tolerate overhead shade. It is mostly an outcrossing plant species and pollination occurs with the aid of few species of bees (*Apis dorsata*, *A. cerana* var. *indica* and *A. florae*). *A. dorsata* is the main pollinator and shows facultative xenogamy (Purnachandrarao and Solomanraju 2002).

Commercially the plant is known for the wavy grained nature of the wood and medicinally the wood powder is used to cure various diseases i.e., as an astringent, antipyretic, antihelminthic, antiperiodics, diaphoretic, alexeritic and in curing freckles, defects of vision, bone fractures, leprosy, scorpion sting, mental aberrations, hemophilic disorders, inflammation, blood purifier, skin diseases, hemicrania, etc. (Parrotta 2001). The fruit and pod decoction is used as an astringent and tonic and for curing chronic dysentery. Roots and clumps are useful for dyeing cotton, leather and staining wood. Overexploitation poses a severe threat to the existence of this precious timber tree (Ahmed and Nayar 1984). Hence there is a need for assessment of conserved germplasm for future use.

Plant species especially perennials such as trees rely on the availability of genetic diversity for stability and survival under ever-changing environments (National Research Council 1991). Understanding species population genetic structure is essential for their conservation, planning and sustainable management (Sun *et al*. 1998). Hence, a common goal of conservation is to maintain genetic diversity in threatened species, which is crucial for long-term survival and evolutionary response to the changing environment (Hueneke 1991).

Plant populations may show morphological variations as an adaptation to different selection pressures (Morrison and Weston 1985; Nevo *et al*. 1986; Hageman and Fahselt 1990), which may result from phenotypic plasticity, genetic differentiation due to natural selection, evolutionary forces to some extent, environmental conditions, and genetic differentiation, which in turn may be due to genetic divergence or polymorphism. A phenotypic response to environmental conditions may allow a genetically non-adapted population to survive long enough to accumulate variants and then adapt genetically (Baldwin 1896; Osborn 1897). Classical methods like provenance and progeny tests coupled with biometrical analysis of phenotypic identification are not sufficient to solve these problems because of the high cost and slow techniques, instability of the morphological characters i.e., clonal and environmental variability as well as an inability to use such information for identification at juvenile stages or of isolated plant parts and occurrence of lots of developmental changes (National Research Council 1991). Development of molecular markers has complemented the generation of information required to making conservation and management decisions (Virk *et al*. 1995). They offer several advantages over traditional phenotypic markers, as they provide data that can be analyzed objectively (Joshi *et al*. 1999).

RAPD (Randomly Amplified Polymorphic DNA) is one of the techniques used for identification of duplicates, supe-

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Fig. 1 Collection of germplasm of *Pterocarpus santalinus* from various locations in Andhra Pradesh.

rior seeds and to improve the efficiency of seed health tests, besides sensitivity or specificity, assessment of genetic diversity and similarity, phylogenetic studies, monitoring genetic erosion, etc. (Williams *et al.* 1990; Joshi *et al.* 2004; reviewed in Teixeira da Silva *et al.* 2005) and removing duplicates from germplasm collections (Virk *et al.* 1995). The nature of molecular variations uncovered by RAPD is still unclear (Ghany and Zaki 2003). From a conservation point of view it could help us set sampling intervals of areas within populations to optimize the genetic diversity in collections from local populations of rare, endangered, or endemic plant species (Kang and Chung 1997; Maki and Yahara 1997; Chung *et al.* 1998).

Although earlier studies have reported some results of research in *P. santalinus* on phytochemical, pollen morphology and on micropropagation (Ravindranath and Seshadri 1973; Lakshmisita *et al.* 1992; Purnachandrarao and Solomonraju 2002) studies on genetic diversity was unclear. The objective of the present study was to assess the genetic diversity among accessions of *P. santalinus* by morphological and molecular analysis which may help to preserve the biodiversity of *P. santalinus* in Andhra Pradesh (AP), which is under threat, and also will provide a framework for future efforts to incorporate wild germplasm into rootstock breeding and hybridization programmes.

MATERIALS AND METHODS

Plant source

Plant material was collected from Kerala, Karnataka and mostly from AP (**Fig. 1**). The plants were identified based on the flora of AP (Pullaiah and Chennaiah 1998). Out of the 27 accessions collected 14 (leaf characteristics), 15 (shoot length, no. of nodes) and 16 (pod characteristics) of them were used for morphological diversity studies and 15 were used for molecular diversity analysis.

Morphological diversity analysis using phenotypic parameters

Three groups of morphological traits were studied (district names are mentioned in parentheses): pod characteristics (weight, length, width) by using mature pods collected from 16 locations i.e., Tirupathi, Talakona, Seshachalam Hills, Papavinasanam (Chittoor), Balpally, Papireddypally, Cuddapah, Sanipaya, Rajampet, Rayachoti, Gadela, Siddhavotum (Cuddapah), Rapur, Nellore (Nellore), Araku (Visakhapatnam); leaf characteristics (length and width) from 5 months-old germinated seedlings collected from 14 locations i.e., Sorakaipalem, Narsingapuram, Ramachandrapuram, Chittoor (Chittoor), Balpally, Papireddypally, Cuddapah, Rayachoti, Gadela, Siddhavotum, Sanipaya (Cuddapah), Nellore, Tada (Nellore), Visakhapatnam (Visakhapatnam); shoot length and number of axillary buds from 14 locations collected from Rajamundry (E. Godavari), Rajampet, Balpally, Sanipaya, Gadela, Cuddapah, Papireddypally, Rayachoti (Cuddapah), Rapur (Nellore), Cuntrapalem (TP-1) Talakona, Ramachandrapuram, Sorakaipalem, Papavinasanam (Chittoor). Statistical analysis was done by applying ANOVA (Analysis of Variance). The experiments were carried out with 3 replicates of 25 samples each. The means of different morphological characters were differentiated based on LSD (Least significant difference). The level of probability was calculated at the 5% level. CD (Critical Difference) was used to interpret along with the treatment means to compare the data and interpret the significance of the phenotypic data of the accessions (**Tables 2, 5**). In the case of germinated seedlings matured pods of each accession were sown on a square bed with an inter plant and inter row spacing of 10 cm. The bed was irrigated properly and no insecticide was sprayed to reduce the plant damage and to capture the maximum diversity.

Molecular diversity using RAPD markers

For analyzing molecular diversity in 15 accessions collected from different locations i.e., Tirupathi, Narsingapuram, Ramachandrapuram, Talakona, Sorakaipalem (Chittoor), Balpally, Sanipaya, Siddhavotum, Gadela, Kodur (Cuddapah), Tada (Nellore), Rajamundry (E. Godavari), Gachibowli (Hyderabad) and Bangalore (Karnataka State) and Kerala state including the two accessions collected from nurseries as reference to compare with accessions collected from AP (supposed to be naturally grown in forests in Kerala and Karnataka) were assessed using RAPD markers. The details of latitude and longitude regarding accessions used in molecular analysis is given in **Table 1**. The primers used were Operon primers from Kits OPA and OPC (Operon Technologies Inc, Alameda, CA).

Isolation of genomic DNA

DNA isolation from young or mature leaves of *P. santalinus* was very difficult by normal conventional methods like the SDS or CTAB (Sigma Aldrich, USA) protocols (Dellaporta 1983; Doyle and Doyle 1987) and by modifications by using lithium chloride and sorbitol (Sigma Aldrich) separately in the extraction buffer. Due to the presence of secondary metabolites, tannins, polyphenols and polysaccharides, discrete and proper amplification was not observed in RAPDs and thus the DNA was isolated using Plant DNAzol isolation Kit (Invitrogen, Life technologies) according to the maufacturer's instructions. The only modification done was that before extraction, plants were kept in the dark for 3 days (The leaves of *P. santalinus* contain high amounts of polysaccharides, phenols, tannins, and many other secondary metabolites as impurities which might interfere during DNA isolation. When plants are kept in the dark, photosynthesis rate goes down and starch synthesis does not occur, thus polysaccharides will be reduced. Therefore, the interference of polysaccharides with the DNA will be reduced.). From all the accessions DNA was isolated from plants of a similar age group (1 year-old).

Qualitative and quantitative extraction of DNA

To check the purity of DNA samples, the ratio of OD values at 260 and 280 nm were recorded. The value of ratio lies between 1.7 and 1.8 for pure DNA samples. Further purity of DNA was tested by gel electrophoresis, using 0.8% TBE-agarose. Gels were stained with ethidium bromide (Sigma Aldrich) and viewed on a UV trans-illuminator, photographed with the help of a gel documentation system (LTF Labortechnik, Germany). DNA was quantified based on the spectrophotometric measurements of UV absorption at 260 nm, assuming 1 OD at 260 nm is equal to 50 µg of DNA (Sambrook *et al.* 1989). The concentrated DNA was diluted with Milli Q (Milli Q academic) sterile water to 50 ng/µl for RAPD analysis. A total of 40 primers, when screened with OPA and OPC series for appropriate amplification out of which 26 primers, were selected for tests of repeatability of the method.

Table 1 Represents the accessions of *P. santalinus* collected from different locations from Andhra Pradesh used for molecular diversity studies.

Accession №	Location	State/District	Latitude	Longitude
1. PSTI	Tirupathi	Chittoor	12º 08′ N	75º 13′ E
2. PSBY	Balpally	Cuddapah	14º 25′ N	79º 18′ E
3. PSNA	Narsingapuram	Chittoor	14º 08′ N	76º 20′ E
4. PSSA	Sanipaya	Cuddapah	14º 07′ N	78º 58′ E
5. PSRM	Ramachandrapuram	Chittoor	13º 10′ N	77º 20′ E
6. PSSM	Siddhavotum	Cuddapah	04º 25′ N	78º 58′ E
7. PSBE	Bangalore	Karnataka	13º 12′ N	77º 20′ E
8. PSGA	Gadela	Cuddapah	14º 10′ N	79º 30′ E
9. PSTN	Talakona	Chittoor	13º 30′ N	79º 08′ E
10. PSTA	Tada	Nellore	14º 00′ N	80º 05′ E
11. PSKA	Kerala	Kerala	08º 15′ N	77º 04′ E
12. PSRY	Rajamundry	East Godavari	17º 08′ N	81º 20′ E
13. PSSP	Sorakaipalem	Chittor	12º 04′ N	74º 18′ E
14. PSKR	Kodur	Cuddapah	14º 52′ N	78º 01′ E
15. PSHC	Gachibowli	Hyderabad	17º 38′ N	28º 12′ E

RAPD analysis

Forty decamer primers of arbitrary sequence (Kits A and C provided by Operon Technologies Inc, Alameda, CA) were tested for PCR amplification among 15 accessions of *P. santalinus*. The analysis involved 2 steps: a) PCR amplification and annealing of a single arbitrary primer at random on the total genome; b) agarose gel electrophoresis. PCR reactions were carried out in a DNA Thermocycler (MJ Research Inc. USA) with a heated lid. The conditions and the programme for PCR were followed as per the protocol (Padmalatha and Prasad 2006).

PCR products were electrophoresed on 2% (W/V) agarose gels, in 1X TBE buffer at 50 V for 3 hrs and then stained with ethidium bromide (0.5 µg/ml). Gels with amplification fragments were visualized and photographed under gel documentation system.

Data scoring and analysis

For each accession, polymorphism was scored as 1 for the presence and 0 for absence of a band. RAPD data generated with forty primers were used to compile a binary matrix for cluster analysis using the NTSYS-pc (Numerical Taxonomy and Multivariate Analysis System, Biostatistics, New York, USA, software version 2.02j package) (Rohlf 1998). Genetic similarity among accessions was calculated according to the Dice similarity coefficient (Dice 1945). The similarity coefficients were then used to construct a dendrogram using the UPGMA (Unweighted Pair wise-Group Method with Arithmetical averages) through NTSYS package. PCR reactions and electrophoresis were repeated at least thrice to ascertain the reproducibility of the bands.

RESULTS

Morphological diversity analysis in *P. santalinus*

Distinct morphological variations were observed in pod characteristics (weight, width, and length) (**Fig. 2A-H**) and in leaf characteristics (length and width), shoot length and number of nodes in five month-old germinated seedlings.

Table 2 ANOVA table for morphological variations in pod characteristics (length, diameter, weight), leaf characteristics (length, width) and No. of axillary buds and shoot length on 5 months old germinated seedlings in *P. santalinus*.

Source of variation	DF	SS (pod length)	SS (pod diameter)	SS (pod weight)	SS (leaf length)	SS (leaf diameter)	SS (№ of axillary buds)	SS (shoot length)
Treatments	15	38.478	40.394	14.06	105.54	66.452	1577.14	11359.04
Replications	9	4.6775	6.8200	0.670	29.70	26.459	29.9310	152.270
Error	135	31.251	30.730	12.23	94.50	79.543	601.160	1450.19
Total	159	74.407	77.939	26.96	229.83	172.45	2208.24	12961.51
Critical Difference (CD)		0.422	0.419	0.264	0.733	0.673	1.850	2.873

DF: Degrees of freedom; SS: Sum of Squares.

Table 3 LSD table indicating the treatment means and significance for morphological variations in pod characteristics (length, diameter and weight) on five-months old germinated seedlings in *P. santalinus*.

S. №	Accession	Pod length (CD: 0.422)		Pod diameter (CD: 0.419)		Pod weight (CD: 0.264)	
		Mean	Significance*	Mean	Significance*	Mean	Significance*
1	Tirupathi	3.78	0.10 c < CD	3.25	0.37 a < CD	0.70	0.01 d < CD
2	Talakona	3.88	0.12 c < CD	3.62	0.02 e < CD	0.71	0.05 b < CD
3	Balpally	4.00	0.08 c < CD	3.64	0.16 c < CD	0.76	0.01 d < CD
4	Papireddypally	4.08	0.05 d < CD	3.80	0.25 b < CD	0.77	0.09 b < CD
5	Cuddapah	4.13	0.35 a < CD	4.06	0.02 e < CD	0.86	0.16 b < CD
6	Papavinasanam	4.48	0.11 c < CD	4.08	0.10 c < CD	1.02	0.06 b < CD
7	Sanipaya	4.59	0.02 d < CD	4.18	0.09 c < CD	1.08	0.00 d < CD
8	Rajampet	4.61	0.01 d < CD	4.27	0.08 c < CD	1.08	0.05 b < CD
9	Rayachoti	4.62	0.01 d < CD	4.35	0.12 c < CD	1.13	0.01 d < CD
10	Gadela	4.63	0.00 d < CD	4.47	0.05 d < CD	1.14	0.07 b < CD
11	Nellore	4.63	0.11 c < CD	4.52	0.06 d < CD	1.21	0.03 c < CD
12	Rapur	4.74	0.03 d < CD	4.58	0.18 c < CD	1.24	0.03 c < CD
13	Araku	4.77	0.30 a < CD	4.76	0.05 d < CD	1.27	0.07 b < CD
14	Visakapatnam	5.07	0.24 b < CD	4.81	0.19 c < CD	1.34	0.23 a < CD
15	Seshachalam Hills	5.31	0.31 a < CD	5.00	0.05 d < CD	1.57	0.22 a < CD
16	Siddhavotum	5.62		5.05		1.79	

CD: Critical Difference; * Different letters within a column denote statistical similarity among the accessions.

Table 4 LSD table indicating the treatment means and significance for morphological variations in leaf characteristics (length, diameter) on 5 months-old germinated *P. santalinus* seedlings.

S. №	Accession	Leaf length (CD: 0.733)		Leaf diameter (CD: 0.673)	
		Mean	Significance*	Mean	Significance*
1	Sorakaipalem	2.59	0.43 b < CD	2.65	0.27 b < CD
2	Narsingapuram	3.02	0.03 e < CD	2.92	0.03 c < CD
3	Balpally	3.05	0.15 d < CD	2.95	0.05 c < CD
4	Papireddypally	3.20	0.10 d < CD	3.00	0.24 b < CD
5	Cuddapah	3.30	0.00 f < CD	3.24	0.16 b < CD
6	Rayachoti	3.30	0.05 e < CD	3.40	0.00 d < CD
7	Gadela	3.35	0.00 f < CD	3.40	0.25 b < CD
8	Nellore	3.35	0.50 a < CD	3.65	0.20 b < CD
9	Chittoor	3.85	0.13 d < CD	3.85	0.20 b < CD
10	R. puram	3.98	0.12 d < CD	4.05	0.00 d < CD
11	Siddhavotum	4.10	0.27 c < CD	4.05	0.02 c < CD
12	Visakapatnam	4.37	0.23 c < CD	4.07	0.03 c < CD
13	Tada	4.60	0.40 b < CD	4.10	0.20 b < CD
14	Sanipaya	5.00	0.05 e < CD	4.30	0.50 a < CD

CD: Critical Difference; * Different letters within a column denote statistical similarity among the accessions.

The differences in the mean values among the different accessions would be due to the differences due to the effect of edaphic and environmental factors. According to the data analyzed by ANOVA test there is also a statistically significant difference (P = < 0.001) observed, which may be due to external factors (**Table 2**). The mean differences were compared with the critical difference value among the accessions to check the significance of the results obtained. According to the LSD test all the phenotypic characters showed a wide range of variation among the accessions (**Tables 3-5**).

RAPD analysis

Analysis of 15 accessions of *P. santalinus* revealed 100% polymorphism (**Figs. 3A-C, 4A, 4B**) with 26 primers. The number of scorable polymorphic markers generated is 217 equal to that of the total number of markers (**Table 6**). The number of scorable polymorphic markers generated for accessions are shown in **Tables 7** and **8**. Levels of genetic diversity within accessions i.e., the genetic distance ranged from 0.14 to 0.76. The minimum genetic distance of 0.14 exhibited between the accessions collected from Narsingapuram and Raichoti whereas the accessions that exhibited a maximum genetic distance of 0.76 belong to plants collected from Kerala and Raichoti. The mean value of genetic distance among all the accessions is 3.16. The values of genetic distances calculated in plant species which are grouped together in cluster analysis irrespective of the geographical distances are from the accessions collected from Kerala and Hyderabad, exhibited a genetic distance of 0.55, the accessions collected from Kodur and Raichoti exhibited a genetic distance of 0.76, the accessions grouped together i.e., collected from Gadela and Talakona showed a genetic distance of 0.72, those collected from Ramachandrapuram and Siddhavotum exhibited a genetic distance of 0.59 and those

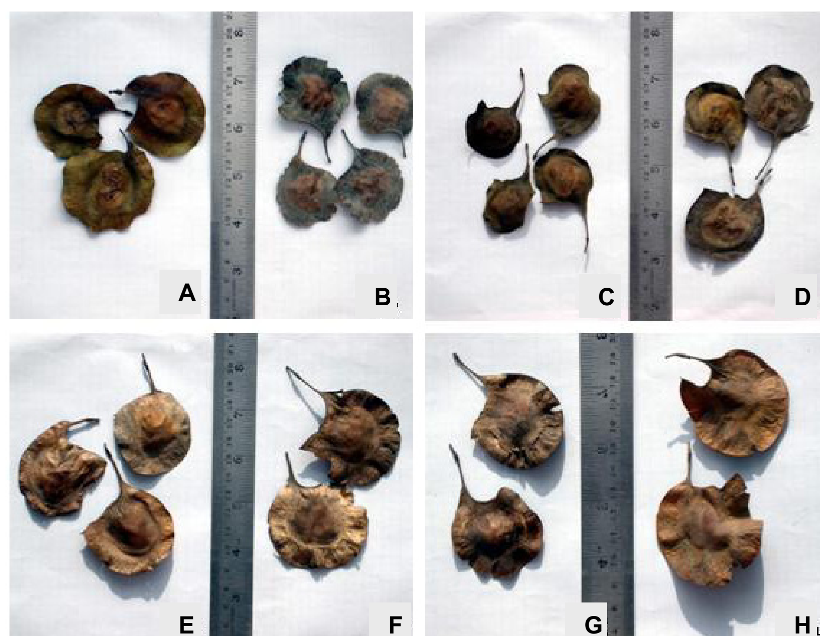

Fig. 2 Morphological variations in pod characteristics (length, width and shape) collected from various locations of Andhra Pradesh (India). (**A**) Papireddypally; (**B**) Raichoti; (**C**) Tirumala Hills; (**D**) Rajampet; (**E**) Cuddapah; (**F**) Visakhapatnam; (**G**) Talakona; (**H**) Venkata Rajampet.

Table 5 LSD table indicating the treatment means and significance for morphological variations in shoot length and No. of axillary buds on 5 months-old germinated *P. santalinus* seedlings.

S. №	Accession	Shoot length (CD: 2.873)		Accession	№ of nodes (CD: 1.850)	
		Mean	Significance		Mean	Significance
1	Rapur	3.33	3.94 b > CD	Rajamundry	4.0	2.6 a > CD
2	Visakapatnam	7.27	1.73 c < CD	Rajampet	6.6	1.4 b < CD
3	Sorakaipalem	9.00	0.52 d < CD	Balpally	8.0	0.0 f < CD
4	Talakona	9.52	0.03 g < CD	Sanipaya	8.0	0.3 d < CD
5	Rajampet	9.55	0.56 d < CD	Gadela	8.3	0.4 c < CD
6	Gadela	10.11	0.29 e < CD	Cuddapah	8.7	0.0 f < CD
7	Rajamundry	10.40	0.55 d < CD	TP-1	8.7	0.1 e < CD
8	Rayachoti	10.95	1.30 c < CD	Papireddypally	8.8	0.2 e < CD
9	Papireddypally	12.25	7.29 a > CD	Papavinasanam	9.0	0.4 c < CD
10	Balpally	19.54	0.18 f < CD	Rayachoti	9.4	1.5 b < CD
11	Papavinasanam	19.72	0.03 g < CD	Visakapatnam	10.9	0.1 e < CD
12	TP-1	20.35	0.05 g < CD	Talakona	11.0	1.6 b < CD
13	Cuddapah	20.40	0.30 e < CD	Rapur	12.6	0.1 e < CD
14	R. puram	20.70	7.70 a > CD	R. puram	12.7	2.2 a > CD
15	Sanipaya	21.00	-	Sorakaipalem	11.5	

collected from Narsingapuram and Sanipaya exhibited a genetic distance of 0.57 (**Figs. 5, 6**). The number of accession specific unique markers belonging to particular location, the primers showing less amount of monomorphism absence of any amplification is shown in **Table 9**. The percentage of polymorphism observed for each accession with all the primers is 100%. The primers with maximum number of polymorphic bands are OPA-18 and OPC-11 with 15 bands and the primers with minimum number of polymorphic bands are OPC-15 and OPC-7 with 3 bands and the number of bands generated with all the primers ranged in between 3 and 15. The GC content of all the primers ranged from 60-70%. The amplified fragments size ranged from 300-3,500 bp. The highest within genetic variation was detected in the accession collected from Tirupathi. In the case of *P. santalinus* (54), an accession specific number of unique markers were generated as mentioned in **Table 9** which may be useful for carrying out further analysis by cloning, sequencing and homology studies. Screening of the entire set of samples was repeated thrice to assess repeatability of the RAPD profiles, and identical RAPD patterns were obtained.

DISCUSSION

Morphological differences between the accessions were highly significant for all the parameters studied, indicating appreciable amount of diversity among the accessions. These variations are not significantly correlated with the geographical distances. Morphological data indicated considerable phenotypic variations among various accessions of *P. santalinus*. The study of natural variations has proved to be useful for analyzing the genetic basis of some developmental processes in the model system *Arabidopsis thaliana* (Perez-Perez *et al*. 2002). The large phenotypic variability obtained for the quantitative traits facilitated a clear distinction among the 14, 15 and 16 accessions collected from different geographical locations indicating the existence of location specific adaptations due to the influence of environmental and edaphic factors (**Fig. 2A-H**). This proved true since large differences were observed in pod weight between Visakhapatnam and Rayachoti, Balpally and Tirupathi pods, pod length between pods of Cuddapah and Gadela, pod width between Tirupathi and Cuddapah pods, and in leaf length between Nellore and Narsingapuram, leaf width between plants from Sanipaya and Siddhavotum, length of shoots between Ramachandrapuram and Balpally plants, and number of nodes between Rajamundry and Rapur, Visakhapatnam and TP-1 plants (**Tables 3-5**). The conventional morphological markers used for characterization of genotypes though readily available suffer from some drawbacks as their expression is influenced by environmental factors and developmental changes leading to errors in scoring.

Lack of knowledge of genetic control of phenotypic traits, insufficient variation, and long time required for appearance of the traits at appropriate growth stage are the

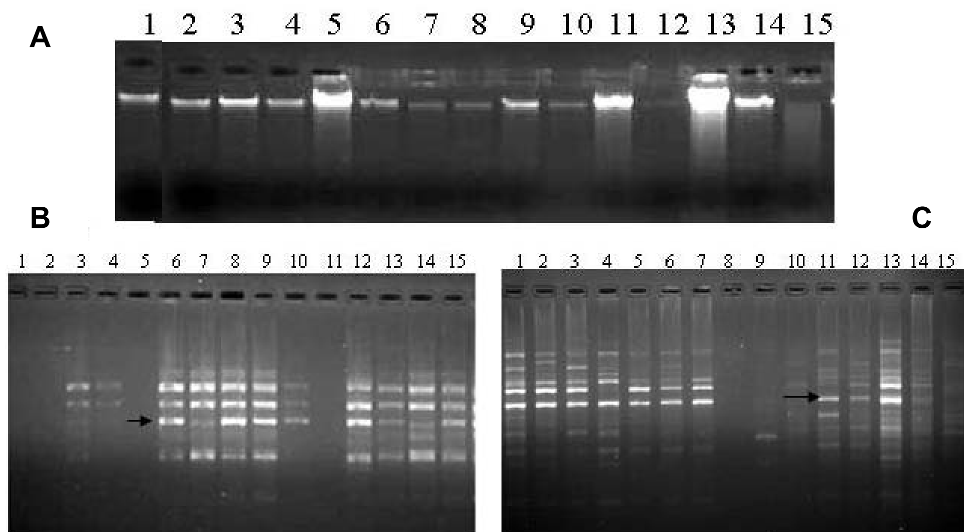

Fig. 3 (**A**) Genomic DNA isolation from fifteen accessions of *Pterocarpus santalinus* L. (**B**) RAPD profile using primer OPA-18 (5′-AGGTGA CCGT-3′). Arrows indicate the presence of polymorphic markers, very faint amplification is observed with accessions in lane "Ra, So and Ti" Maximum number of polymorphic bands are generated with this primer. (C) RAPD profile using primer OPC-2 (5′-GTGAGGCGTC-3′). Arrows indicate the presence of polymorphic markers, very faint amplification is observed with accessions in lane "Ga, Tn and Ta". Maximum polymorphism was observed with this primer.

Table 6 Information regarding primer code, sequence, percentage polymorphism among 15 accessions of *P. santalinus* collected from different locations.

Primer code	Primer sequence 5′–3′	TB (total bands)	PB (polymorphic bands)	Polymorphism (%)
OPA-01	CAGGCCCTTC	8	8	100
OPA-02	TGCCGAGCTG	8	8	100
OPA-03	AGTCAGCCAC	9	9	100
OPA-04	AATCGGGCTG	8	8	100
OPA-05	AGGGGTCTTG	8	8	100
OPA-06	GGTCCCTGAC	13	13	100
OPA-18	AGGTGACCGT	15	15	100
OPA-19	CAAACGTCGG	12	12	100
OPA-20	GTTGCGATCC	6	6	100
OPC-01	TTCGAGCCAG	8	8	100
OPC-02	GTGAGGCGTC	9	9	100
OPC-03	GGGGGTCTTT	14	14	100
OPC-04	CCGCATCTAC	6	6	100
OPC-05	GATGACCGCC	7	7	100
OPC-06	GAACGGACTC	13	13	100
OPC-07	GTCCCGACGA	3	3	100
OPC-08	TGGACCGGTG	5	5	100
OPC-09	CTCACCGTCC	10	10	100
OPC-10	TGTCTGGGTG	6	6	100
OPC-11	AAAGCTGCGG	15	15	100
OPC-13	AAGCCTCGTC	6	6	100
OPC-14	TGCGTGCTTG	6	6	100
OPC-15	GACGGATCAG	3	3	100
OPC-16	CACACTCCAG	9	9	100
OPC-18	TGAGTGGGTG	4	4	100
OPC-20	ACTTCGCCAC	6	6	100

other limitations that are prompting plant breeders towards more reliable and faster methods to score variations. Plant populations under different environmental selection pressures generally show phenotypic differences, may be the result of phenotypic plasticity or genetic diversification as observed in case of accessions of *P. santalinus*.

Phenotypic traits are controlled by genes and affected by environmental effects like landscape, moisture and edaphic conditions but large numbers of accessions can adapt to few environmental factors like rainfall, temperature etc. The phenotypic data also revealed polymorphism i.e., genetic variations indirectly.

RAPD markers have been employed as an alternative for morphological and biochemical markers (Dawson *et al.* 1993; Yoon and Glawe 1993; Pei *et al.* 1995; Wolfe and Liston 1998; Su *et al.* 1999; Esselman *et al.* 2000). Estimating the genetic differentiation coefficient among populations using RAPDs has been problematic due to their dominance, and analytical methods usually rely on knowledge of the selfing rate or assume Hardy-Weinberg equilibrium (Lynch and Milligan 1994). This assumption does not hold when populations exhibit fixed heterozygosity, hence in our studies an alternative method i.e., Dice coefficient was used to partition the genetic diversity which is supposed to be in accordance with the RAPD data and it was found consistent in showing the variation within accessions.

P. santalinus is a woody plant species where the leaves are exceptionally rich in of polysaccharides, polyphenols, tannins, hydrocolloids (sugars and carragenans) and other secondary metabolites such as alkaloids, flavanoids, phenols, terpenes and quinines, which interfered with the DNA isolation and further experiments in molecular technology. Polysaccharides interfere with the PCR by inhibiting *Taq* polymerase activity which, in turn, can inhibit RAPD reactions (Fang *et al.* 1992). Polysaccharides like contaminants which are undetectable by most criteria, can cause anomalous reassociation kinetics but polysaccharide co-precipitation is avoided by adding a selective precipitant like CTAB to keep polysaccharides in solution.

The presence of polyphenols, which are powerful oxidizing agents present in many plant species, can reduce the yield and purity of DNA by binding covalently making it useless for most research applications (Loomis 1974; Katterman and Shattuck 1983; Peterson *et al.* 1997). Additionally tannins, terpenes and resins are difficult to separate from DNA (Doyle and Doyle 1987; Ziegenhagen and Scholz 1998).

Therefore the problems encountered in the isolation and purification of DNA specially from woody medicinal plants include degradation of DNA due to endonucleases, coisolation of highly viscous polysaccharides, inhibitor compounds like polyphenols and other secondary metabolites which directly or indirectly interfere with the enzymatic reactions and moreover the contaminating RNA that precipitates along with DNA causes many problems including suppression of PCR amplification (Pikkart and Villeponteau 1993), interference with DNA amplification involving random primers, e.g. RAPD analysis and improper priming of DNA templates during thermal cycle sequencing (Yoon and Glawe 1993; Mejjad *et al.* 1994).

These factors do not permit optimal DNA yields from one isolation protocol, and perhaps even closely related species may require different isolation protocols (Weishing *et al.* 1995). Hence *P. santalinus* DNA was isolated by using Plant DNAzol isolation Kit where a good quality and quantity of DNA was obtained which was used for RAPD reactions.

The differences among 15 accessions of *P. santalinus* collected from different locations could partly be explained as a result of abiotic (geographical, e.g., hydrographic connections, or climactic differentiation. e.g., annual rainfall

Table 7 Accession wise polymorphic bands in 1-7 *P. santalinus* accessions with both A and C series of Operon primers.

Primer	**1**		**2**		**3**		**4**		**5**		**6**		**7**	
Code	**TB**	**PB**	**TB**	**PB**	**TB**	**PB**	**TB**	**PB**	**TB**	**PB**	**TB**	**PB**	**TB**	**PB**
OPA1	5	5	1	1	2	2	4	4	2	2	1	1	2	2
OPA2	0	0	2	2	5	5	0	0	2	2	6	6	6	6
OPA3	1	1	2	2	0	0	6	6	6	6	0	0	6	6
OPA4	0	0	0	0	2	2	2	2	0	0	5	5	5	5
OPA5	0	0	0	0	3	3	3	3	0	0	5	5	5	5
OPA6	0	0	0	0	0	0	0	0	0	0	5	5	13	13
OPA18	8	8	3	3	2	2	3	3	9	9	9	9	9	9
OPA19	6	6	6	6	2	2	1	1	7	7	9	9	9	9
OPA20	4	4	2	2	0	0	2	2	3	3	1	1	1	1
OPC1	2	2	2	2	3	3	3	3	0	0	4	4	6	6
OPC2	5	5	1	1	1	1	1	1	4	4	3	3	2	2
OPC3	0	0	0	0	6	6	6	6	10	10	11	11	11	11
OPC4	0	0	0	0	6	6	6	6	5	5	5	5	4	4
OPC5	0	0	0	0	0	0	4	4	0	0	1	1	0	0
OPC6	13	13	6	6	5	5	11	11	6	6	4	4	4	4
OPC7	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OPC8	0	0	0	0	0	0	2	2	4	4	3	3	1	1
OPC9	0	0	0	0	3	3	2	2	6	6	2	2	3	3
OPC10	6	6	2	2	4	4	0	0	0	0	4	4	0	0
OPC11	14	14	6	6	6	6	5	5	5	5	6	6	4	4
OPC13	0	0	0	0	0	0	0	0	4	4	4	4	0	0
OPC14	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OPC15	0	0	0	0	0	0	0	0	2	2	2	2	1	1
OPC16	0	0	0	0	1	1	0	0	0	0	3	3	4	4
OPC19	0	0	0	0	0	0	1	1	1	1	1	1	1	1
OPC20	0	0	0	0	2	2	0	0	2	2	0	0	2	2

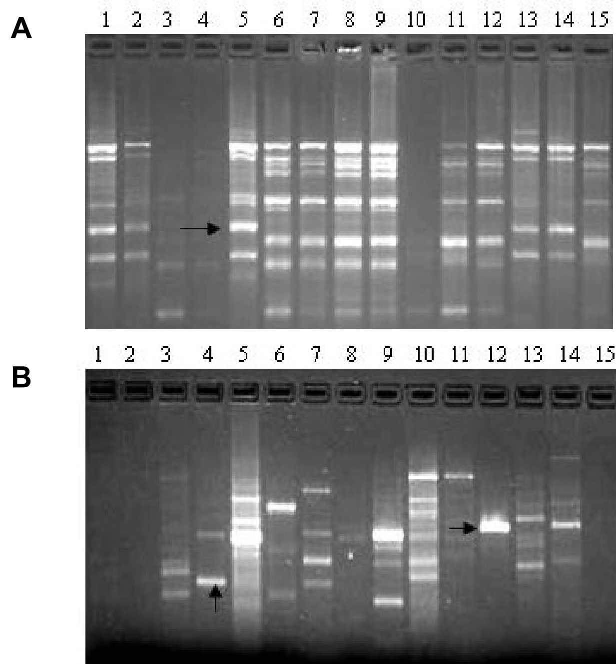

Fig. 4 (**A**) RAPD profile using primer OPA-19 (5′-CAAACGTCGG-3′). Presence of polymorphic bands is observed with most of the accessions represented in the gel. There is no amplification observed in some of the accessions represented in lanes "Na, Sa, Ba and Si" may be due to the absence of priming sites. (**B**) RAPD profile using primer OPC-9 (5′-CTC ACCGTCC-3′). Presence of polymorphic bands is observed with most of the accessions represented in the gel. There is no amplification observed in some of the accessions represented in lanes "Ti and By" may be due to the absence of priming sites.

differences) and biotic (pollination between populations and seed dispersal etc.) factors. It is expected that obligate outcrossing species show more genetic variation at the population level (Apostol *et al*. 1993; Cardoso *et al*. 2000) as observed in *P. santalinus*.

Among the forty primers tested, *P. santalinus* showed 100% polymorphism with 26 primers (**Table 6**) indicating higher genetic diversity within accessions. Distinct polymorphic bands have been observed on 2% agarose gels (**Figs. 3, 4**) (Hamrick *et al*. 1992). RAPD data suggests that in woody legumes most of the variation is maintained within the populations (Schierenbeck *et al*. 1997). Similar results are reported in tropical tree legume, *Gliricidia sepium* that showed more than 60% genetic variation (Chalmers *et al*. 1992).

The high levels of variation found within different accessions suggest that sampling from a few localities for either breeding or conservation could capture a large proportion of the variation within the species. The genetic diversity can be explained by the aid of calculation of polymorphism levels and cluster diagram (**Figs. 5, 6**).

The mean level of genetic diversity within 15 accessions of *P. santalinus* is 3.16. The range of genetic diversity calculated in terms of genetic distance is 0.14-0.76 (**Fig. 5**). From this it is evident that the accessions from different geographical locations exhibited a wide range of genetic distance, which did not show any correlation with geographical distances between the collection sites, negating a simple isolation by physical distance.

In *P. santalinus* cluster analysis based on Dice coefficient showed two major groups (**Fig. 6**) indicating that in cross-pollinated plants, high levels of differentiation among populations and relatively less within-population genetic variation exists. The dendrogram obtained by the aid of similarity matrix revealed that there is a similarity of 76% between the accessions collected from Kerala, Talakona and Raichoti which clearly depicts that genetically they are similar which was confirmed from the owner of the nursery that they were collected from A.P and were grown in Kerala as plantations. There is also a close similarity of 76% observed between the accessions collected from Talakona and Gadela though geographically they are distantly placed in contrary. The accessions collected from Tirupathi and Narsingapuram though closely placed geographically, their genetic similarity is only 32%, which clearly indicates that there is no correlation between genetic make up and geographical distances.

The pattern of genetic diversity in *P. santalinus* may be maintained due to effective gene flow within accessions. Animal drops which aid in seed dispersal may also contribute for inducing variations indirectly within the accessions thus accounting for the high levels of genetic variation

Table 8 Accession wise polymorphic bands in 8-15 *P. santalinus* accessions with both A and C series of Operon primers.

Primer	8		9		10		11		12		13		14		15	
Code	**TB**	**PB**	**TB**	**PB**	**TB**	**PB**	**TB**	**PB**	**TB**	**PB**	**TB**	**PB**	**TB**	**PB**	**TB**	**PB**
OPA1	1	1	0	0	0	0	1	1	2	2	4	4	4	4	4	4
OPA2	7	7	7	7	1	1	2	2	8	8	7	7	7	7	7	7
OPA3	1	1	0	0	0	0	1	1	0	0	7	7	7	7	9	9
OPA4	5	5	5	5	3	3	0	0	5	5	6	6	6	6	6	6
OPA5	5	5	5	5	3	3	1	1	5	5	5	5	7	7	7	7
OPA6	8	8	9	9	1	1	0	0	1	1	6	6	6	6	6	6
OPA18	9	9	9	9	3	3	10	10	8	8	5	5	4	4	7	7
OPA19	9	9	9	9	0	0	8	8	9	9	7	7	6	6	7	7
OPA20	1	1	0	0	0	0	3	3	0	0	5	5	5	5	6	6
OPC1	6	6	5	5	5	5	0	0	0	0	4	4	2	2	2	2
OPC2	0	0	0	0	1	1	1	1	1	1	1	1	0	0	0	0
OPC3	8	8	1	1	0	0	1	1	8	8	9	9	9	9	0	0
OPC4	5	5	6	6	6	6	6	6	0	0	0	0	0	0	0	0
OPC5	0	0	0	0	7	7	1	1	1	1	1	1	1	1	0	0
OPC6	2	2	1	1	6	6	7	7	6	6	4	4	2	2	2	2
OPC7	0	0	3	3	3	3	3	3	0	0	2	2	0	0	0	0
OPC8	0	0	0	0	0	0	0	0	0	0	0	0	2	2	1	1
OPC9	2	2	4	4	4	4	2	2	2	2	3	3	1	1	3	3
OPC10	0	0	0	0	6	6	6	6	3	3	0	0	0	0	0	0
OPC11	0	0	1	1	1	1	6	6	6	6	11	11	8	8	7	7
OPC13	2	2	3	3	0	0	0	0	0	0	4	4	2	2	3	3
OPC14	2	2	0	0	0	0	0	0	0	0	6	6	0	0	2	2
OPC15	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0
OPC16	0	0	1	1	0	0	0	0	0	0	1	1	8	8	2	2
OPC19	1	1	1	1	0	0	0	0	0	0	6	6	6	6	6	6
OPC20	1	1	1	1	0	0	0	0	1	1	2	2	3	3	2	2

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**
PSNR(1)	1.00														
PSSA(2)	0.57	1.00													
PSRM(3)	0.38	0.39	1.00												
PSSM(4)	0.39	0.39	0.59	1.00											
PSBE(5)	0.38	0.48	0.44	0.56	1.00										
PSGA(6)	0.33	0.33	0.55	0.46	0.54	1.00									
PSTN(7)	0.22	0.30	0.42	0.47	0.55	0.72	1.00								
PSKA(8)	0.20	0.33	0.38	0.42	0.46	0.58	0.74	1.00							
PSRY(9)	0.14	0.22	0.34	0.30	0.35	0.62	0.71	0.76	1.00						
PSTI(10)	0.21	0.17	0.39	0.36	0.19	0.42	0.48	0.41	0.55	1.00					
PSSM(11)	0.42	0.46	0.48	0.45	0.47	0.49	0.46	0.50	0.52	0.49	1.00				
PSTA(12)	0.35	0.40	0.54	0.44	0.47	0.66	0.61	0.58	0.62	0.39	0.62	1.00			
PSKR(13)	0.34	0.34	0.40	0.38	0.41	0.55	0.52	0.50	0.42	0.24	0.41	0.60	1.00		
PSHC(14)	0.28	0.33	0.39	0.38	0.43	0.55	0.54	0.43	0.44	0.27	0.34	0.55	0.72	1.00	
PSBI(15)	0.28	0.32	0.32	0.38	0.43	0.50	0.52	0.62	0.45	0.17	0.37	0.53	0.67	0.62	1.00

Fig. 5 Similarity matrix of *P. santalinus* generated from Dice estimate similarity based on the number of shared fragments.

(Loveless and Hamrick 1984; Hamrick and Godt 1989). Distribution range and population size have been identified as the major correlates within population genetic variation in tropical tree species with restricted populations showing significantly less variation than those with broader distribution (Loveless 1992; Travis *et al.* 1996). In case of *P. santalinus*, despite its smaller population size and being endemic in certain districts of A.P i.e., Cuddapah, Chittoor, Nellore and Kurnool high genetic variation is observed, which might be due to highly cross pollinated nature of the plant. It was reported that outcrossed wind pollinated species exhibit vast variation within populations (Loveless and Hamrick 1984; Loveless 1992).

The significant variations in the accessions of *P. santalinus* collected from Tirupathi when compared to other accessions from various locations need to be investigated further. Such observations have been reported previously in *Hordeum spontaneum* populations by Dawson *et al.* 1993. It can be inferred that the accessions clustered in similar groups there is an effective gene flow in those areas but whereas with the accession collected from Tirupathi, the gene flow is less hence they are highly divergent when compared to other accessions. This may be due to highly cross-pollinated nature and occurrence of some mutations and rearrangements in the genome, resulting in variation. In *P. santalinus* some of the morphological parameters like pod weight, pod length and leaf length were in accordance with that of the molecular data but it may not represent the exact trait as there may be many other phenotypic traits which may exhibit variation.

Genetic variation decreases with decrease in population size (Mosseler *et al.* 1992; Baskauf *et al.* 1994; Kappe *et al.* 1995; Frankham 1997; Palacious and Gonzalez-Candelas 1997). One would therefore expect rare and endemic species of small population size often associated with increased inbreeding and genetic drift, processes that lead to loss of genetic variation (Ellstrand and Elam 1993; Gaston and Kunin 1997). Inbreeding is avoided in all the accessions of *P. santalinus* because the plants are dioecious, although within-population gene exchange is unavoidable. This situation may arise in natural populations where there is a possibility of free/random pollen flow and fertilization as in the case of most of the cross pollinated species or may be attributed to the formation of hybrids due to introgressive hybridization. Mutations may also play an important role in causing variations. Sources of polymorphisms in RAPD assay may include base change within priming site sequence, deletions of priming site, insertions that render priming sites too distant to support amplification, and deletions or insertions that change the size of a DNA fragment without preventing its

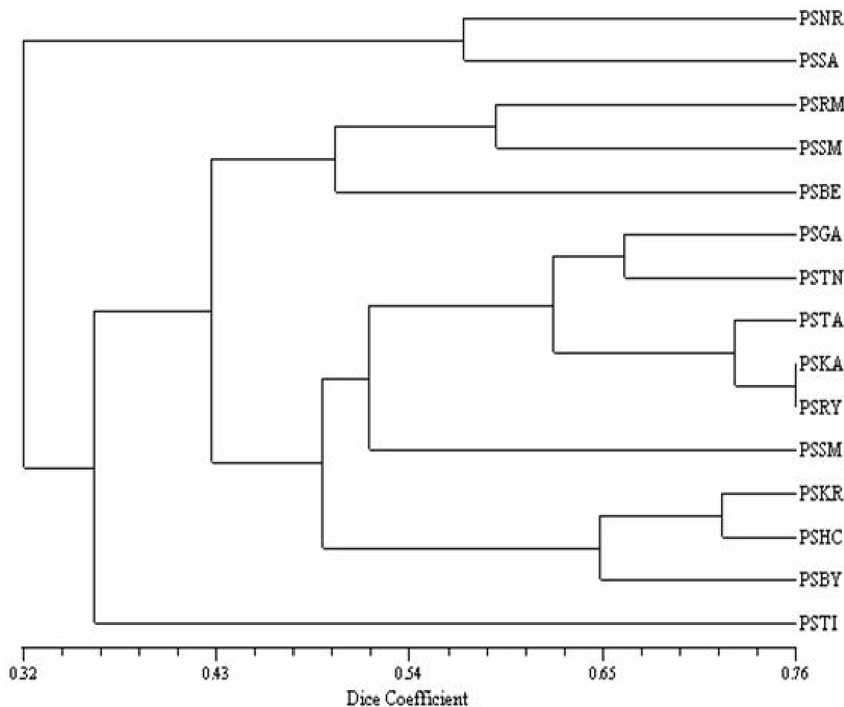

Fig. 6 Cluster analysis of 15 accessions of *P. santalinus* using Dice similarity coefficient.

amplification (Williams *et al*. 1990). In addition the polymorphisms of RAPD markers were observed as different sized DNA fragments from amplification. In *P. santalinus* the strict out crossing, results in higher levels of heterozygosity (Wolff *et al*. 1994).

Another explanation could be low reproducibility of RAPDs (Karp *et al*. 1997). The putatively similar bands originating for RAPDs in different accessions are not necessarily homologous although they share the same size in base pairs. This situation may lead to erroneous results when calculating genetic relationships. Problems of the reliability and repeatability of RAPD markers are well known (Ellsworth *et al*. 1993). However in our experiments, high reproducibility with PCR products for RAPDs was observed.

The gene flow in higher plants is accomplished by dispersal of seeds and pollen as well as by vegetative mobility (Handel 1985; Parker and Hamrick 1992). Gene flow by pollen dispersal is often low in herbaceous plants (Widen and Swenson 1992). In *P. santalinus* none of the accessions collected for our study have less than 25 km distance to each other. Hence, the genetic structure of any of these accessions is stable and free from any gene flow into them. Hence there is a wide range of genetic differentiation. The genetic variation is related to the distances of pollen and seed dispersals. The seeds of *P. santalinus* are winged, which favours the seed dispersal over long distances. *P. santalinus* species is bee pollinated and hence there are more chances of pollen dispersal resulting in a broad range of variations. A detailed on all the aspects related to variations is warranted.

Genetic drift over thousands of generations would lead to significant divergence. This trend may be reinforced for adaptive traits by selection of important ecological differences existing among the areas from where the accessions were sampled. Results from RAPD analysis indicates that genetic drift might have occurred among the studied accessions of *P. santalinus* thereby producing population differentiation. The main reason being overexploitation leading to shrinkage of their habitat. With a larger area of population, the probability of crossing among the individuals increases, which results in the retention of genetic variation. Though many individuals of these species may be existing earlier in due course of time they must have disappeared gradually along with environmental changes in their habitat. For decades much attention has focused on the genetic risks associated with small population size, not only from inbreeding and genetic drift, but also from gene flow. Until now, a precise empirical assessment of how well diversity has been characterized is unavailable.

The wide range of variations may also be due to two evolutionary forces like pollen flow and local selection pressures. Pollen can be dispersed over large distances; this long-term reciprocal movement of pollen must also have contributed to the variation. Recent experiments using pollen traps have shown that oak pollen can migrate at several kilometers (Lahtinen *et al*. 1996). The local selection pressures may be due to the effects of environmental factors and due to struggle for existence in nature. The wide spread occurrence of the wind pollination and breeding systems that promotes outcrossing may lead to higher genetic diversity. Palynological and anthropogenic influences may also be attributed to high levels of genetic variation.

CONCLUSIONS

Distinct morphological variations were observed in *P. santalinus* pod characteristics (weight, width and length), leaf characteristics (length, width), shoot length and number of nodes in five months old germinated seedlings collected from 16 different locations, which could be due to environmental and edaphic factors. The wide variation in genetic distance among the *P. santalinus* revealed by RAPD markers reflected a high level of DNA polymorphism due to outcrossing. *P. santalinus* is endemic to Cuddapah, Nellore, Chittoor and Prakasam of A.P. Endemics are generally reported to have low levels of genetic variation. However in the presently investigated accessions, 100% polymorphism was observed. Therefore, possibly the Cuddapah of A.P might be the center of diversity for *P. santalinus*. The presence of many unique markers may be due to the high rate of mutations in RAPD loci, which are important as they may be diagnostic for particular regions of the genome and are accession specific. Based on the observations of this study and cluster analysis it can be suggested that for *P. santalinus* the *in situ* conservation measures should include Balpally area in Cuddapah of A.P. Seeds of this site need to be conserved (*ex situ*). Further sampling covering wider geographical areas and larger populations and using more number of RAPD primers would be useful for diversity analysis.

Table 9 Depicts the accessions of *P. santalinus* that showed unique markers and absence of amplification.

№ of unique bands	Primer code	Location of collection
1	OPC-1	Talakona
1	OPC-1	Kodur
No amplification	OPC-1	Balpally, Siddhavotum, Talakona
1	OPC-2	Sorakaipalem
1	OPC-2	Siddhavotum
1	OPC-2	Rajampet
No amplification	OPC-2	Kerala
1	OPC-3	Gadela
1	OPC-3	Kodur
No amplification	OPC-3	Sorakaipalem, Bangalore, UH campus, Balpally, Talakona, Rajampet, Kerala
1	OPC4	Tirupathi
No amplification	OPC-4	Narsingapuram, Sanipaya, UH campus, Balpally
2	OPC-5	Sorakaipalem
No amplification	OPC-5	All other accessions
1	OPC-6	Sorakaipalem
No amplification	OPC-6	Kerala, UH campus
1	OPC-6	Tirupathi
3	OPC-7	Tirupathi, Siddhavotum, Talakona
No amplification	OPC-7	Narsingapuram, Sanipaya, Rajampet, Sorakaipalem, Balpally, Gadela, Talakona, Kerala, Kodur, UH campus, Balpally
2	OPC-8	Balpally, Gadela, Bangalore
2	OPC-8	Talakona, Kerala
No amplification	OPC-8	Narsingapuram, Sanipaya, Rajampet, Sorakaipalem, Rajampet, Tirupathi, Siddhavotum, Kodur, UH campus
2	OPC-9	Tirupathi, Siddhavotum
2	OPC-9	Gadela, Tirupathi
2	OPC-9	Bangalore, Talakona
1	OPC-9	Talakona
2	OPC-9	Rajampet, Gadela
1	OPC-9	Rajampet
No amplification	OPC-9	Narsingapuram, Sanipaya, Balpally
1	OPC-10	Siddhavotum
No amplification	OPC-10	Narsingapuram, Sanipaya, Rajampet, Kerala, Talakona, Balpally
No amplification	OPC-13	Narsingapuram, Sanipaya, Rajampet, Sorakaipalem, Rajampet, Tirupathi, UH campus
No amplification	OPC-14	Except in Talakona, amplification not seen in other locations
2	OPC-15	Kodur, Balpally
2	OPC-15	Balpallly, Gadela
No amplification	OPC-15	No amplification in remaining accessions
2	OPC-16	Gadela, Talakona
2	OPC-16	Rajampet, Talakona
3	OPC-16	Rajampet, Talakona, UH campus
3	OPC-16	Gadela, Talakona, Balpally
No amplification	OPC-19	Narsingapuram, Sanipaya, Sorakaipalem, Rajampet, Tirupathi, Siddhavotum
No amplification	OPA-1	Rajampet, Gadela, Rajamundry, Tirupathi, Siddhavotum, Talakona
No amplification	OPA-2	Sanipaya, Rajampet, Sorakaipalem, Gadela, Kerala, Rajampet, Tirupathi, Talakona, Kodur, UH campus
No amplification	OPA-3	Narsingapuram, Sanipaya, Rajampet, Sorakaipalem, Balpally, Siddhavotum, Banglore
No amplification	OPA-4	Narsingapuram, Sanipaya, Balpally, Siddhavotum
1	OPA-5	Rajampet, Rajamundry
1	OPA-5	Talakona
1	OPA-5	UH campus
No amplification	OPA -5	Narsingapuram, Sanipaya, Rajampet, Sorakaipalem, Balpally, Gadela, Talakona
2	OPA-18	Rajampet, Sorakaipalem
6	OPA-18	Kodur, UH campus
No amplification	OPA-18	Tirupathi
No amplification	OPA-19	Narsingapuram, Sanipaya, Bangalore, Siddhavotum

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