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Genetic diversity analysis of teak in South Gujarat by RAPD marker

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Abstract
Teak is a deciduous tree species belonging to the Lamiaceae family producing good quality timber. Its wood is rightly famous for furniture making due to fine grain, mellow color and durability. The present investigation was carried out to assess the genetic diversity in teak in south Gujarat by RAPD molecular marker. Out of 30 RAPD primers tested, 20 primers produced clear and reproducible band. The number of loci amplified by these RAPD primers ranged between 4 (OPD-02 and OPF-09) to 15 (OPF-01). A total of 192 loci were amplified out of which 150 (78.13%) loci were polymorphic. The percentage of polymorphism ranged from 25% for OPD-02 to 100% for OPF-06. Highest PIC value (0.5), effective multiplex ratio (6.34), Marker Index (2.99) and resolving power (8.60) observed with OPD-03, OPF-05, OPD-03 and OPF-01, respectively. Maximum Jacard's similarity coefficient (0.81) and Nei& Li's coefficient (0.89) observed between Vansada and Dharampur, indicating a close relationship between these populations. However, the smallest Jacard's similarity value (0.47) and Nei& Li's coefficient (0.64) were observed between Vyara and Bardipada-B. Dendrograms of the accessions were constructed based on the Jacard's coefficient and Nei& Li's coefficient gave exact similar clustering of the ten populations. All the populations from Valsad Forest division tend to fall in same cluster. Populations from Dang forest division stayed in same cluster except Bardipada-B population. The PCoA results corresponded well with the cluster analysis obtained through UPGMA. The first three coordinates accounted for 20.53, 14.90 and 13.31 percent of the total variance, respectively. Thus, the total cumulative variance accounted by these three coordinates was 48.73 percent. Overall, the Vyara population was quite distinct.

Keywords: *Tectona grandis*, populations, RAPD, diversity

Introduction
Teak (*Tectona grandis* Linn. f.) belonging to the Lamiaceae family is important tropical timber species of world and known as "King of Timber" (White1991) [41]. Its wood is excellent for furniture making due to excellent grain color and quality (Tsoumis1991) [36]. The timber is suitable for various uses including house construction, shipbuilding, furniture making, poles, veneer, carvings etc. Teak is native to India, Myanmar, northern Thailand, Laos and Indonesia (Troup1921) [35] however it is also extensive planted throughout the tropics as exotics due to high demands and price on the international market (Fofana *et al*. 2008) [9].

The information on the genetic variation within and between populations plays an important role in conservation and sustainable management of genetic resources and further tree improvement (Sharma *et al*. 2015; Dhaka 2016; Dhaka and Jha 2017a; Dhaka and Jha2018) [31, 7, 6, 4]. Existence of large amount of genetic variation in teak has been revealed by molecular markers studies (Isoda *et al*. 2000; Watanabe *et al*. 2004; Nicodemus *et al*. 2005; Shrestha *et al*. 2005; Narayanan *et al*. 2007; Fofana *et al*. 2008; Fofana *et al*. 2009; Verhaegen *et al*. 2010; Ansari *et al*. 2012; Sreekanth *et al*. 2012; Alcântara and Veasey 2013; Fofana *et al*. 2013; Sreekanth *et al*. 2014; Hansen *et al*. 2015; Murukan and Murugan 2015; Vaishnaw *et al*. 2015; Giustina *et al*. 2017) [19, 40, 26, 32, 24, 9, 10, 39, 33, 2, 1, 11, 34, 16, 23, 37, 12]. However, most of the population variability study in teak is confined to southern or central India except few study related to population variation in seed character and nursery traits of teak populations in south Gujarat (Dhaka and Jha 2017a; Dhaka and Jha 2017b; Dhaka and Jha 2018) [6, 5, 4]. Dhaka (2016) [7] found the existence of large inter and intra population variation within teak of south Gujarat on the basis of seed character variation. However no report on molecular studies in teak of south Gujarat is available so far despite Gujarat stands on fourth position for area under teak forest in India. This is the first report on molecular study to access molecular diversity in teak populations from south Gujarat using RAPD (random amplified polymorphic DNA) marker.

Material and Methods

Plant Material

Ten Natural populations (Vyara, Bhenskatri, Kalibel, Bardipada-A, Bardipada-B, Tapti, Vansda NP, Dharampur, Chikhli and Kaparada) of teak located in three forest division of Gujarat viz. Dang, Valsad and Vyara marked with the help of GPS and represented in Fig. 1. Five trees were selected from each natural population and the leaves were collected. Fresh and disease free leaves were collected from the selected trees in the natural population and carried to the laboratory in thermocol box with ice bags within 2-3 hours of collection and kept in deep freezer (-20 °C) for future DNA extraction.

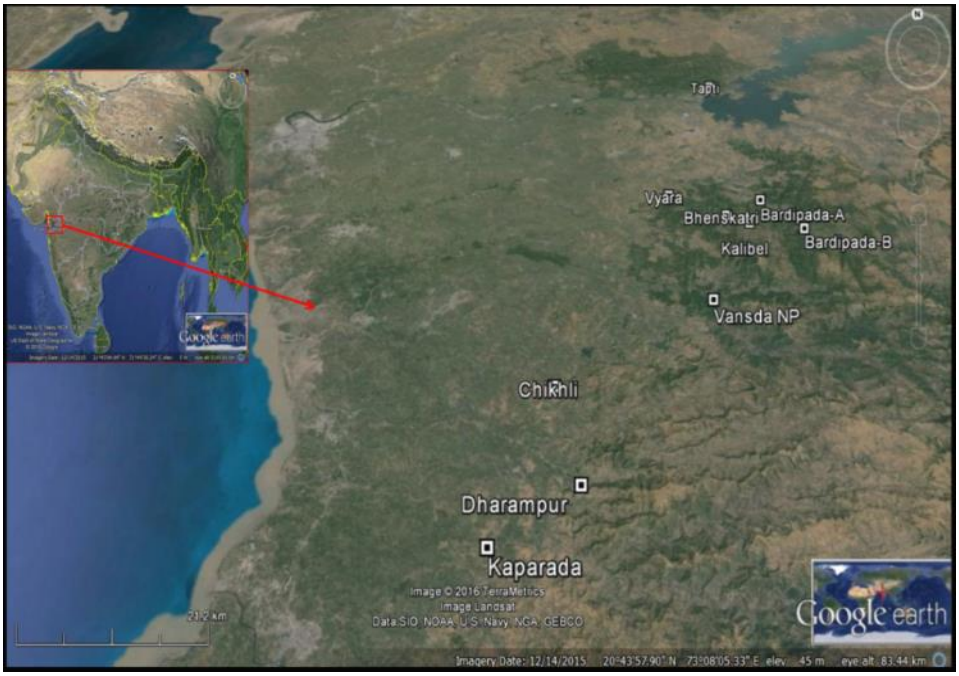

Fig 1: Geographic locations of the different populations of teak

DNA Extraction and Quantification

The total genomic DNA was extracted from young leaf tissue following the method of as described by Doyle and Doyle (1990) [8] with some modifications. Quantification of DNA was done by Nanodrop (at 260 nm) and the quality of DNA was checked on agarose gel electrophoresis.

Primer Selection and RAPD-PCR analysis

The RAPD assays were performed using random 10-mer oligonucleotide primers from Operon technology Inc., USA. PCR amplifications were carried out with 2 specimens. The primers that gave clear and polymorphic amplification patterns were chosen for further analysis. Out of 30 RAPD primers tested, 20 primers (Table 1.) produced clear and reproducible band. For each primer, the PCR amplification was carried out in 25 µl reaction volume mixture (2 µl Genomic DNA (50-60ng), 2.5 µlTaq Buffer (10X) with 1.5 mM $MgCl_2$, 0.5 µl dNTP pre-mix (2.5 mM), 0.3 µl Taq polymerase (3 U/µl), 1.0 µl Primer (10 pmoles/ml) and 19.2 µl Sterile Millipore water) as described by Williams *et al.* (1990)[42] in an Applied Biosystems, Verity 96 well, thermal cycler. The genomic DNA per population was pooled DNA from five randomly selected tree per population. The thermal cycler was programmed for an initial denaturation of 10 minutes at 94 °C, followed by 35 cycles of denaturation of 1 minute at 94 °C, 30 seconds of annealing at 40°C. The extension was carried out at 72 for 2 minutes and final extension at 72 °C for 10 minutes and a hold temperature of 4 °C at the end. The amplified product was collected from the thermal cycler and loaded on to 1.8 percent (w/v) agarose gel containing 4 µl ethidium bromide in 100 ml (0.4 µg/ml electrophoresis buffer) prepared in 1x TBE (pH 8.0). The gel was viewed under UV light and photographed using GeNei UVITEC, Gel Documentation system Cambridge.

Table 1: Sequence details of screened primer used for RAPD amplification

Sl. No.	Primers Name	Sequence Details
1	OPD-02	GGACCCAACC
2	OPD-03	GTCGCCGTCA
3	OPD-04	TCTGGTGAGG
4	OPD-05	TGAGCGGACA
5	OPF-01	ACGGATCCTG
6	OPF-02	GAGGATCCCT
7	OPF-03	CCTGATCACC
8	OPF-05	CCGAATTCCC
9	OPF-06	GGGAATTCGG
10	OPF-07	CCGATATCCC
11	OPF-09	CCAAGCTTCC
12	OPF-10	GGAAGCTTGG
13	OPG-02	GGCACTGAGG
14	OPG-03	GAGCCCTCCA
15	OPG-04	AGCGTGTCTG
16	OPG-10	AGGGCCGTCT
17	OPG-12	CAGCTCACGA
18	OPG-13	CTCTCCGCCA
19	OPG-16	AGCGTCCTCC
20	OPG-18	GGCTCATGTG

Statistical analysis

Reproducible RAPD products were manually scored for band presence (1) or absence (0) for each accession and a binary qualitative data matrix was constructed. Initially, the potential of the marker for estimating genetic variability of *T. grandis* was examined by measuring the marker informativeness through the counting of bands. To analyze the suitability of the marker to evaluate genetic profiles of teak, the performance of the marker was measured using three parameters: polymorphic information content (PIC), marker index (MI) and resolving power (RP). The PIC value for each

locus was calculated using formula (Roldàn-Ruiz *et al*. 2000) [30]. The frequency was calculated as the ratio between the number of amplified fragments at each locus and the total number of accessions. The PIC of each primer was calculated using the average PIC value from all loci of each primer. Effective multiplex ratio (EMR) was calculated using formula = n*β, where n is the average number of fragments amplified by accession to a specific system marker (multiplex ratio) and β is estimated from the number of polymorphic loci (PB) and the number of nonpolymorphic loci (MB); β = PB/(PB + MB). Marker index for the marker was calculated to characterize the capacity of each primer to detect polymorphic loci among the genotypes. Marker index for each primers was calculated as a product of polymorphic information content and effective multiplex ratio (Varshney *et al*. 2007) [38]. The resolving power (RP) of each primer was calculated as (Prevost and Wilkinson 1999) [28]. The data matrix of marker was then converted into genetic similarity matrix using Jaccard's coefficient (Jaccard, 1908)[20] and Nei& Li's coefficient (Nei and Li 1973) [25] in NTSYS-PC 2.02j (Rohlf 1998)[29]. Dendrogram was constructed using unweighted pair group method with arithmetic average (UPGMA). Principal coordinate analysis (PCoA) was performed using the DCENTER and EIGEN programs described by Gower (1966) [14] in the NTSYSpc. This multivariate approach was chosen to complement the cluster analysis as PCoA is more informative regarding distances among major groups (Hauser and Crovello 1982) [17] while cluster analysis is more sensitive to closely related individuals.

Results and Discussion

20 primers produced clear and reproducible band out of 30 RAPD primers tested (Table 2.). The number of loci amplified by these RAPD primers ranged between 4 (OPD-02 and OPF-09) to 15 (OPF-01) (Fig. 2. and Table 2.). A total of 192 loci amplified of different size ranging from 175 to 4023 bp out of which 150 (78.13%) loci were polymorphic and 42 (21.88%) loci were monomorphic. The percentage of polymorphism ranged from 25% for OPD-02 to 100% for OPF-06. High PIC value of 0.5 (OPD-03) and low PIC value of 0.12 (OPD-02), with an average value of PIC per primer 0.27 were obtained (Table 2.). The effective multiplex ratio depends on the fraction of polymorphic fragments. In this study, the highest effective multiplex ratio (EMR) 6.34 was observed with the primer OPF-05 and the lowest effective multiplex ratio (EMR) 2.13 was observed with the primer OPD-02 with an average EMR of 4.63 per primer (Table 2.). To determine the general usefulness of the system of markers used, the MI (marker index) for each RAPD primer was calculated. The highest MI was observed with the primer OPD-03 (2.99) and lowest in the primer OPD-02 (0.26), with an average MI of 1.33 per primer was obtained. The resolving power (RP) is a parameter that indicates the discriminatory potential of the primers chosen. The highest RP value was observed with the primer OPF-01 (8.60) and the lowest with the primer OPD-02 (0.80) with an average RP of 4.06 per primer (Table 2.). Overall, RAPD showed higher resolving power and polymorphism hence it can be successfully utilized for diversity analysis of teak. In present study, significant level of polymorphism was detected in ten natural population of teak of south Gujarat by RAPD (78.12%). Random dominant markers has been successfully used for genetic diversity analysis of teak (Nicodemus *et al*. 2003; Watanabe *et al*. 2004; Nicodemus *et al*. 2005; Narayanan *et al*. 2007) [27, 40, 26, 24] and other trees species (Goulão *et al*. 2001; Dasgupta *et al*. 2015; Goyal *et al*. 2015; Kulhari *et al*. 2015; Long *et al*. 2015) [13, 3, 15, 21, 22].

Table 2: Primer-wise analysis of banding patterns generated by RAPD marker assays for ten populations of *T. grandis*

Primer	NB	Nm	Np	PP	PIC	EMR	MI	Rp
OPD-02	4	3	1	25.00	0.12	2.13	0.26	0.80
OPD-03	6	1	5	83.33	0.50	5.98	2.99	3.40
OPD-04	14	1	13	92.86	0.30	4.28	1.28	6.20
OPD-05	8	1	7	87.50	0.23	5.98	1.38	2.20
OPF-01	15	3	12	80.00	0.34	5.36	1.82	8.60
OPF-02	13	2	11	84.62	0.36	4.93	1.77	7.80
OPF-03	7	3	4	57.14	0.21	4.05	0.85	2.40
OPF-05	8	1	7	87.50	0.29	6.34	1.84	3.20
OPF-06	10	0	10	100.00	0.34	5.60	1.90	5.20
OPF-07	10	1	9	90.00	0.36	4.68	1.68	5.60
OPF-09	4	1	3	75.00	0.25	4.73	1.18	1.40
OPF-10	9	3	6	66.67	0.15	3.48	0.52	1.80
OPG-02	14	3	11	78.57	0.33	4.35	1.44	7.40
OPG-03	13	3	10	76.92	0.25	4.77	1.19	5.40
OPG-04	8	2	6	75.00	0.22	4.05	0.89	2.20
OPG-10	10	2	8	80.00	0.34	4.48	1.52	5.20
OPG-12	10	3	7	70.00	0.23	3.99	0.92	3.40
OPG-13	11	4	7	63.64	0.20	4.16	0.83	3.20
OPG-16	11	4	7	63.64	0.18	3.78	0.68	2.60
OPG-18	7	1	6	85.71	0.31	5.42	1.68	3.20
Overall	192	42	150	78.12	0.27	6.2	4.63	1.33

Note: Number of bands (NB); Number of monomorphic bands (Nm); number of polymorphic bands (Np), percentage of polymorphism (PP); polymorphism information content (PIC); effective multiplex ratio (EMR); marker index (MI) and resolving power (Rp).

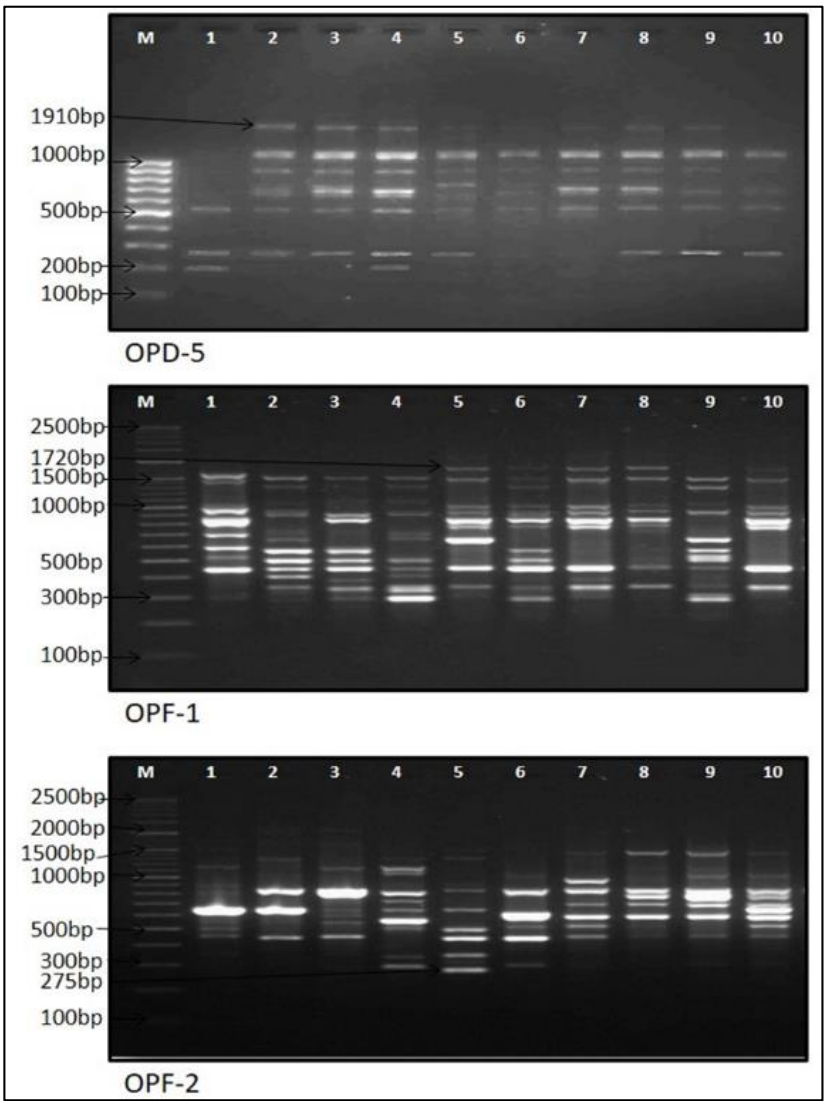

Fig 2: RAPD fingerprints of 10 populations generated by OPD-5, OPF-1 and OPF-2

The Jacard's and Nei and Li's similarity matrices of the RAPD data are presented in Tables 3. and Table 4. Both matrices showed high similarity trend. Highest Jacard's similarity value (0.81) and Nei& Li's coefficient (0.89) observed between Vansada and Dharampur, indicating a close relationship between these populations. High Jacard's coefficient (0.75) and Nei's coefficient (0.85) values were also found between Dharampur and Chikhali. The smallest Jacard's similarity values (0.47) and Nei& Li's coefficient (0.64) were observed between Vyara and Bardipada-B. Two dendrograms of the accessions were constructed based on the Jacard's and Nei& Li's coefficient gave exact similar clustering of the ten populations (Fig. 3. and Fig. 4.). All the population could be divided into three major groups. The first group was further subdivided into three subgroups: Ia) all the populations from Valsad forest division (Vansada, Dharampur, Chikhali, Kaparada), Ib) Tapti population from Vyara forest division, and Ic) Bardipada-B population from the Dang forest divisions. The second group (II) included populations from the dang forest division (Bhenskatri, Kalibel, Bardipada-A) and the third group included single Vyara population from the Vyara forest division.

Table 3: Jaccard's similarity matrix for ten populations of *T. grandis* as generated by RAPD marker

Populations	Vyara	Bhenskatri	Kalibel	Bardi-A	Bardi-B	Tapti	Vansada	Dharampur	Chikhali	Kaprada
Vyara	1.00									
Bhenskatri	0.56	1.00								
Kalibel	0.55	0.68	1.00							
Bardi-A	0.51	0.62	0.66	1.00						
Bardi-B	0.47	0.54	0.58	0.58	1.00					
Tapti	0.56	0.61	0.63	0.55	0.60	1.00				
Vansada	0.51	0.58	0.61	0.62	0.61	0.67	1.00			
Dharampur	0.49	0.56	0.61	0.57	0.62	0.61	0.81	1.00		
Chikhali	0.51	0.63	0.62	0.60	0.57	0.59	0.72	0.75	1.00	
Kaprada	0.50	0.50	0.52	0.53	0.59	0.58	0.66	0.71	0.64	1.00

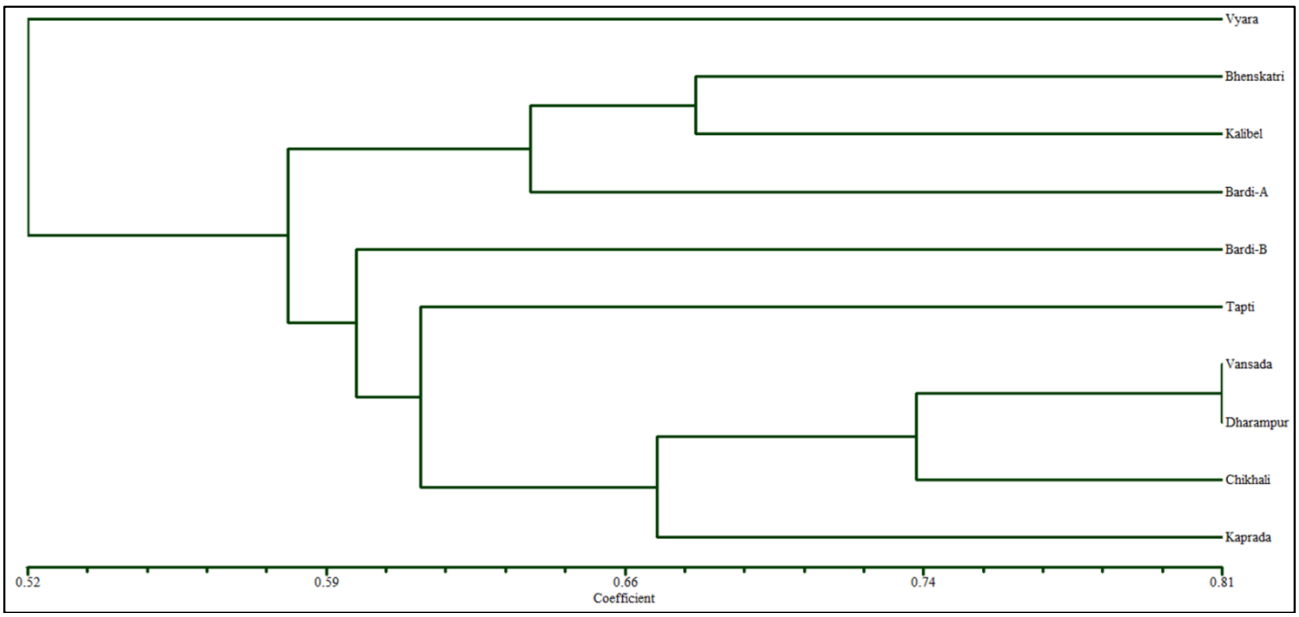

Fig 3: UPGMA dendrograms of ten populations of *T. grandis* based on Jaccard's genetic indices and Coefficient, as revealed by RAPD marker

Table 4: Nei& Li's coefficient matrix for ten populations of *T. grandis* as generated by RAPD marker

Populations	Vyara	Bhenskatri	Kalibel	Bardi-A	Bardi-B	Tapti	Vansada	Dharampur	Chikhali	Kaprada
Vyara	1.00									
Bhenskatri	0.72	1.00								
Kalibel	0.71	0.81	1.00							
Bardi-A	0.68	0.77	0.79	1.00						
Bardi-B	0.64	0.70	0.74	0.73	1.00					
Tapti	0.71	0.76	0.77	0.71	0.75	1.00				
Vansada	0.68	0.74	0.76	0.77	0.76	0.81	1.00			
Dharampur	0.66	0.72	0.75	0.73	0.76	0.76	0.89	1.00		
Chikhali	0.68	0.77	0.77	0.75	0.72	0.74	0.84	0.85	1.00	
Kaprada	0.66	0.67	0.69	0.70	0.74	0.74	0.79	0.83	0.78	1.00

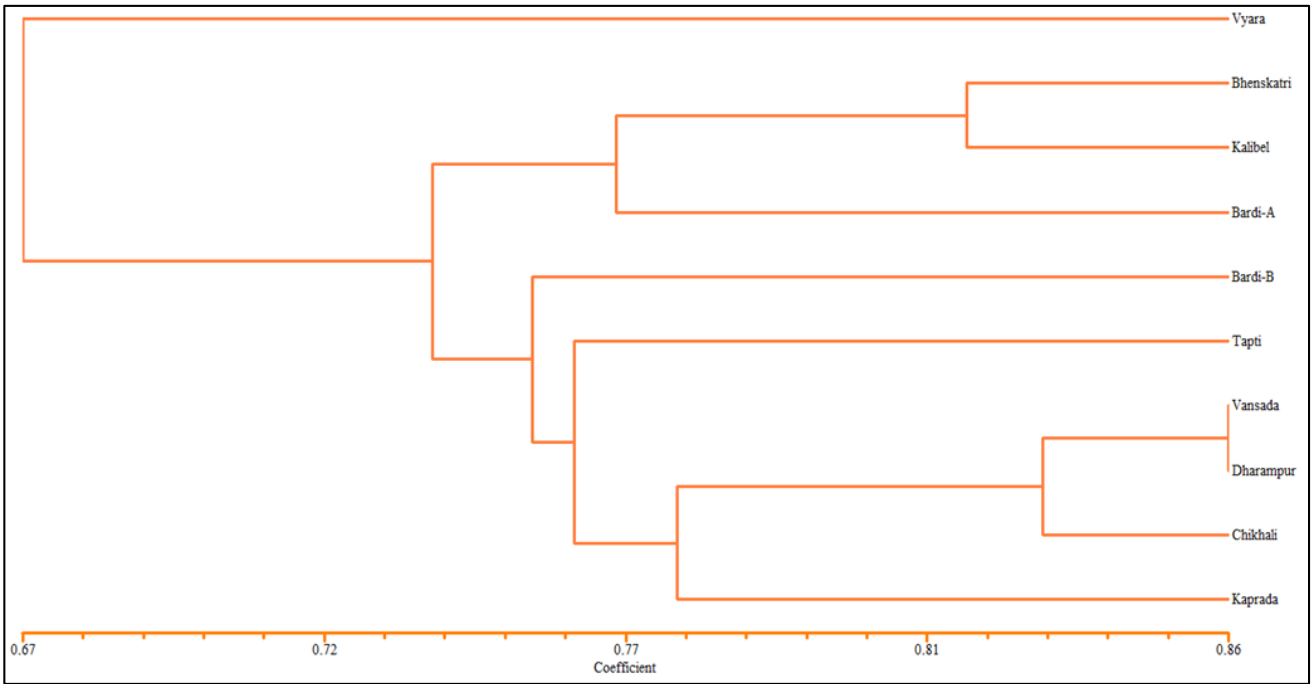

Fig 4: UPGMA Dendrogram of ten populations of *T. grandis* based on Nei& Li's Coefficient, as revealed by RAPD marker

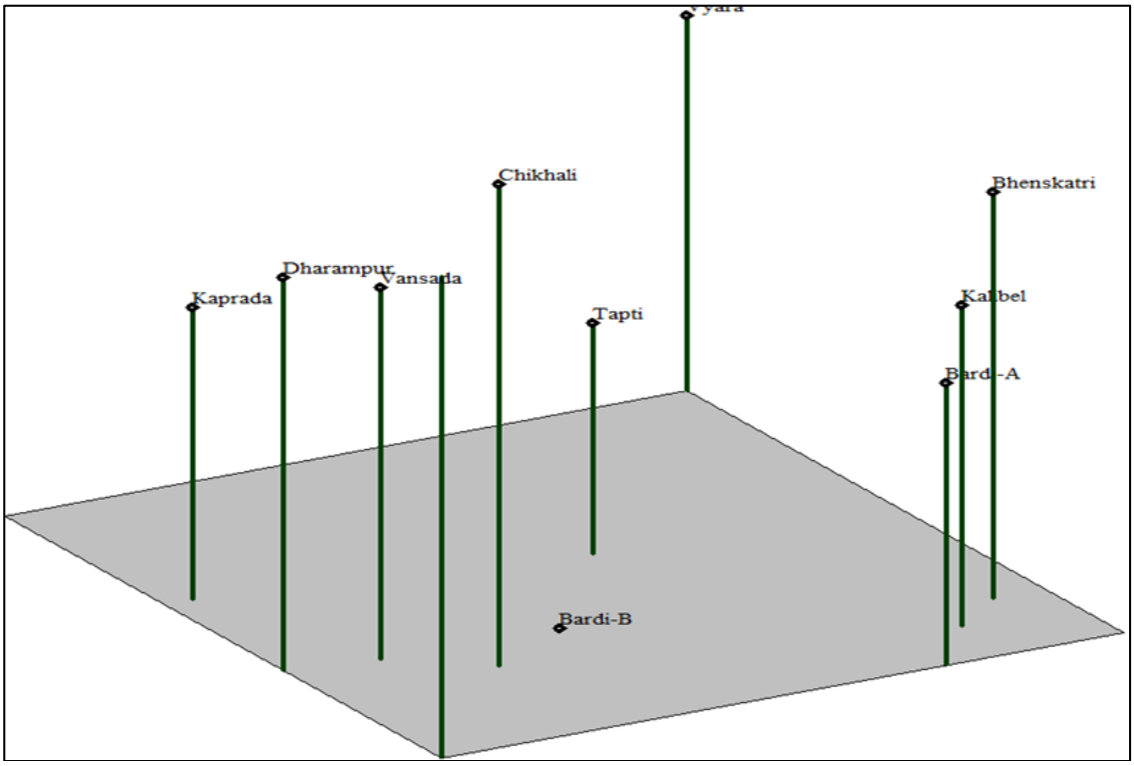

Fig 5: Principal Coordinate Analysis using RAPD markers and the Jaccard's similarity index

To affirm the genetic relationships among 10 teak populations, PCoA was generated from the DCENTER and EIGEN with the program NTSYSpc v 2.1. The results corresponded well with the cluster analysis obtained through UPGMA (Fig. 5.). The first three coordinates accounted for 20.53, 14.90 and 13.31 percent of the total variance, respectively. Thus, the total cumulative variance accounted by these three coordinates was 48.73 percent. In this analysis, the majority of the groupings followed the same pattern as depicted in the dendrogram with minor differences. For example, the populations belonging to I group and its sub-groups in the dendrogram were also clustered together in the PCoA. The Vyara population was distantly placed in both the analyses. Overall, large variation among teak of south Gujarat observed is present study. Teak is a perennial, woody, cross pollinated species and in such species the majority of the genetic variation is expected within populations. The present study is in agreement with finding of other researchers in teak (Nicodemus *et al*. 2005; Narayanan *et al*. 2007; Fofana *et al*. 2009; Verhaegen *et al*. 2010; Ansari *et al*. 2012; Sreekanth *et al*. 2012; Alcântara and Veasey 2013; Hansen *et al*. 2014; Sreekanth *et al*. 2014; Vaishnaw *et al*. 2014; Hirao *et al*. 2016) [26, 24, 10, 39, 2, 33, 1, 16, 34, 37, 18].

Conclusion

The present study was intended to assess genetic diversity in teak in south Gujarat by RAPD molecular marker. RAPD fingerprinting showed higher resolving power and polymorphism for genetic analysis which provides a powerful tool for successfully utility for diversity analysis of teak. Furthermore, genetic diversity was established through dendrogram construction based on the Jacard's coefficient and Nei& Li's coefficient for clustering of the ten teak populations. All the population was divided into three major groups viz., Valsad, Vyara and Dang forest divisions with the cluster analysis obtained through UPGMA. Overall, the Vyara population was altogether different among teak populations corresponded to the PCoA results as well as the clustering through UPGMA. Captured genetic variation within and between populations should be utilized for conservation and sustainable management of teak genetic resources in south Gujarat.

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