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Assessment of ISSR based molecular variability in teak of South Gujarat

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Abstract
In the present investigation, the molecular variability of teak in south Gujarat has been studied using ISSR marker. A total 12 primers produced clear and reproducible band out of 15 ISSR tested primers. The number of loci was ranged between 6 (UBC-888) to 14 (UBC-884) through amplification of these ISSR primers. Total 91 loci were polymorphic (80.53 %) and remaining 22 loci were monomorphic (19.47 %). Polymorphism percentage (PP) was ranged from 60 % for UBC-889 to 92.31 % for UBC-886. Maximum polymorphic information content value (0.36) EMI (5.68), MI (1.38) and resolving power (5.60) were observed with UBC-878, UBC-887, UBC-876 and UBC-886, respectively. Highest Jacard's similarity value (0.68) was observed between Tapti and Vansada and Dharampur and Chikhali. However highest Nei & Li's coefficient (0.84) observed between Bhenskatri and Kalibel. Two dendrograms of the genotypes constructed based on the Jacard's and Nei & Li's coefficient for clustering of the ten populations. All the genotypes could be divided in three major groups. The first group was further subdivided in to three subgroups: Ia) Tapti, Vansada and Bardipada-B population, Ib) Bardipada-A Ic) Chikhali and Dharampur, Bhenskatri and Kalibel. The second group (II) included Vyara and Kaprada populations. The results of PCoA corresponded well with the cluster analysis obtained through UPGMA. The first three coordinates accounted for 19.55, 16.07 and 13.12 per cent of the total variance, respectively. Thus, total cumulative variance was 48.75 per cent accounted by these three coordinates. Overall, the findings from the study is very useful for survival and conservation of teak genetic resources in threat of climatic change.

Keywords: population, teak, ISSR, diversity, conservation, climate change

Introduction
Teak is one of the most important and valuable hardwoods of the world. Its wood is extraordinary for furniture making and suitable for various uses including house construction, shipbuilding, furniture making, poles, veneer, carvings etc. Teak has a wide but discontinuous distribution in India. It grows well from sea level to an elevation of 1200 m and annual rainfall range from 800 to 2500 mm. The natural teak forests in India are occupying an area of 8.9 mha (Tewari 1992) [35]. Globally natural teak forests declined between 1992 and 2010 by 1.3 per cent. India alone planted 38 per cent of the world's teak forests after declining natural teak due to overharvesting and deterioration of the quality of teak wood. It is essential in the near future to implement a programme for the genetic conservation of native teak resources in the four countries viz. India, Laos, Myanmar and Thailand (Anonymous 2012) [2]. Gopalakrishnan *et al*. (2010) [15] projected 30% of teak grids in India are vulnerable to climate change because the unfavorable future climate.

Variability measurement is the pioneer step of any tree improvement and genetic resource management programme. Inter and intra specific variation among teak populations plays an important role for sustainable management of teak genetic resources and further tree improvement (Dhaka 2016; Dhaka and Jha 2017a; Dhaka and Jha 2017b; Dhaka and Jha 2018) [10, 9, 8, 7]. The genetic diversity among teak population has been studied from the natural and introduced populations with the help of molecular markers. Various methods have been used to unfold these tree improvement and conservation programme such as isozyme systems (Kertadikara and Prat 1995) [22], Random Amplification of Polymorphic DNA (Watanabe *et al*. 2004; Nicodemus *et al*. 2005; Narayanan *et al*. 2007) [40, 29, 27], Amplified Fragment Length Polymorphism (Shrestha *et al*. 2005)[34], Sequence Characterized Amplified Regions (Isoda *et al*. 2000) [20], ISSR (Narayanan *et al*. 2007; Ansari *et al*. 2012) [27, 3] and nuclear SSR (Fofana *et al*. 2009; Verhaegen *et al*. 2010; Alcântara and Veasey 2013) [12, 39, 1]. ISSR (Inter simple sequence repeat) is a PCR-based molecular marker and highly useful for studying genetic diversity (Zietkiewicz *et al*. 1994) [41] among natural populations and this technique is reliable, reproducible and cost effective.

However, most of the population variability study in teak is limited to southern or central India except few relevant study on population variation for seed and seedling traits in south Gujarat teak populations (Dhaka and Jha 2017a; Dhaka and Jha 2017b; Dhaka and Jha 2018) [9, 8, 7]. This is the pioneer work using ISSR technique to access molecular diversity in teak populations from south Gujarat for future conservation measures.

Material and Methods

Plant Material

Vyara, Bhenskatri, Kalibel, Bardipada-A, Bardipada-B, Tapti, Vansda NP, Dharampur, Chikhli and Kaparada natural populations of teak used in the present study and marked with the help of GPS (Table 1.) and the leaves were collected in each natural population for future DNA extraction.

Table 1: Geographic locations of the different populations of teak presented in the study

Population	Latitude (N)	Longitude (E)	Altitude (m)
Vyara	20'59"	73'28"	114.2
Bhenskatri	20'56"	73'33"	161.1
Kalibel	20'55"	73'35"	204.6
Bardipada-A	20'58"	73'37"	221.8
Bardipada-B	20'54"	73'40"	209.2
Tapti	21'17"	73'37"	186.1
Vansda NP	20'46"	73'29"	148.1
Dharampur	20'30"	73'15"	132.9
Chikhli	20'38"	73'14"	134.6
Kaparada	20'26"	73'08"	94.9

DNA extraction and quality check

The total genomic DNA was extracted from young leaf tissue as described by Doyle and Doyle (1990) [11], with some modifications. The quality of DNA was checked on agarose gel electrophoresis. The quality of DNA was done by Nanodrop (at 260 nm). For PCR amplification, the DNA from five trees from each population were pooled together to represent one population.

ISSR Assays

ISSR analysis was carried out with 15 ISSR primers of UBC (University of British Columbia, Canada). After initial screening of these 15 primers, 12 primers were selected for further analysis (Table 2.) produced clear and reproducible bands. For each primer, the PCR amplification was carried out in 25 µl reaction volume mixture (Zietkiewicz *et al*. 1994) [41] (Table 3.).

Table 2: Sequence details of screened primer used for ISSR amplification.

S. No.	Primers name	Sequence Details
1	UBC-827	ACACACACACACACACG
2	UBC-876	GATAGATAGACAGACA
3	UBC-878	GGATGGATGGATGGAT
4	UBC-880	GGAGAGGAGAGGAGA
5	UBC-884	HBHAGAGAGAGAGAGAG
6	UBC-885	BHBGAGAGAGAGAGAGA
7	UBC-886	AAACTCTCTCTCTCTCT
8	UBC-887	DVDTCTCTCTCTCTCTC
9	UBC-888	BDBCAC ACA CAC ACA CA
10	UBC-889	DBDACACACACACACAC
11	UBC-892	TAGATCTGATATCTGAATTCCC
12	UBC-900	ACTTCCCCACAGGTTAACACA

The thermal cycler was programmed as per following steps: (i) Initial denaturation at 95°C for 10 minutes, (ii) Denaturation at 95°C for 1 minute, (iii) Primer annealing at 53°C for 45 seconds, (iv) Primer extension at 72°C for 2 minutes, (v) Final extension at 72°C for 10 minutes and a hold temperature of 4°C at the end. Cycles were set by reapting steps (ii) to (iv) for 35 cycles. The amplified product was collected from the thermal cycler stored at -20°C till further use. PCR products were loaded on to 1.8 percent (w/v) agarose gel containing 4 µl ethidium bromide in 100 ml prepared in 1x TBE buffer. The gel was photographed using GeNei UVITEC, Gel Documentation system Cambridge.

Table 3: List of different components of PCR master mix used for amplification reaction in ISSR

S. No.	PCR master mix Component	Volume
1	Sterile Millipore water	19.2 µl
2	Taq Buffer (10X) with 1.5mM MgCl2 (KAPA)	02.5 µl
3	dNTP pre-mix (2.5 mM) (KAPA)	00.5 µl
4	Taq polymerase (3 U/µl) (KAPA)	00.3 µl
5	Primer (10 pmoles/ml)	01.0 µl
6	Genomic DNA (50-60ng)	02.0 µl
	Total	25.0 µl

Statistical analysis

ISSR products were manually scored for band absence (0) or presence (1) for each genotype and binary data matrix was constructed. Primer banding characteristics such as number of total bands (NB), number of monomorphic band (Nm), number of polymorphic bands (NP) and percentage of polymorphic bands (PPB) were obtained by counting of bands. Three parameters viz., polymorphic information content, marker index and resolving power was calculated to analyze the suitability of the marker to evaluate genetic profiles of *T. grandis*. The polymorphic information content (PIC) value calculated using formula (Roldàn-Ruiz *et al*. 2000) [33]; PICi = 2fi (1 - fi), Where PICi is the polymorphic information content of the locus i, fi is the frequency of the amplified fragments and 1 - fi is the frequency of non-amplified fragments. 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 was calculated using formula; EMR (effective multiplex ratio) = 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 non-polymorphic loci (MB); β = PB/(PB + MB). Marker index for each primers was calculated as a product of polymorphic information content and effective multiplex ratio (Varshney *et al*. 2007) [37]; MI = EMR X PIC. The resolving power (RP) of each primer was calculated as (Prevost and Wilkinson 1999) [31]; RP = ∑ Ib, Where Ib represents the informative fragments. The Ib can be represented on a scale of 0/1 by the following formula; Ib = 1 - (2 *|0.5 - pi|), where pi is the proportion of accessions containing the ith band. The data matrix of marker was then converted into genetic similarity matrix using Jaccard's coefficient (Jaccard 1908) [21] and Nei & Li's coefficient (Nei and Li 1973) [28] in NTSYS-PC 2.02j (Rohlf 1998) [32]. Dendrogram was constructed using un weighted pair group method with arithmetic average (UPGMA). Principal coordinate analysis (PCoA) was performed using the DCENTER and EIGEN programs described by Gower (1966) [17] in the NTSYSpc.

Cluster analysis as PCoA is more informative regarding distances among major groups (Hauser and Crovello 1982) [19] while cluster analysis is more sensitive to closely related individuals.

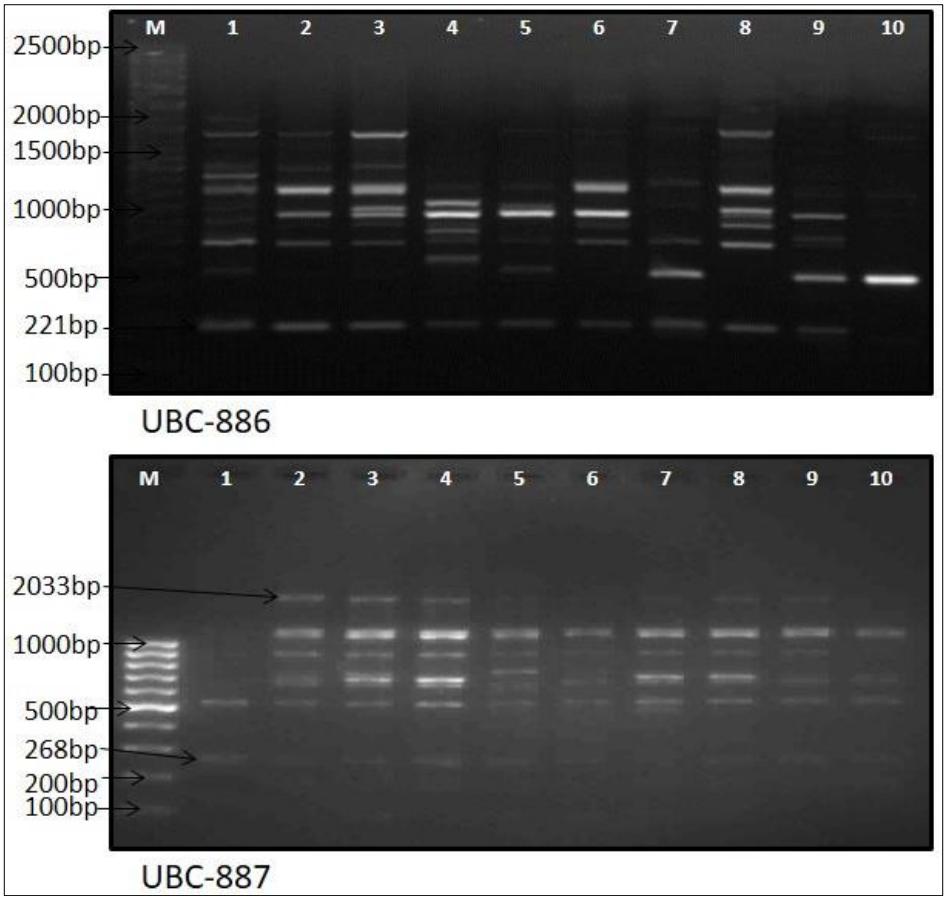
Results and Discussion

Recent development of molecular markers has complemented and drastically reduced the time taken for generating information required in making conservation and management decisions. Inter-Simple Sequence Repeat (ISSR) techniques is considered as highly informative fingerprinting tools as no previous knowledge of the genome is required, a large number of markers are easily generated, they are reliable, reproducible and cost effective, and require only micrograms of DNA. The ISSR marker generate a large number of polymorphic loci without prior information of genome (Ansari *et al*. 2012) [3] and microsatellite regions encoding specific proteins (Vaishnav and Ansari 2018) [36]. Out of 15 ISSR primers tested, 12 primers (Table 4.) produced clear and reproducible band. The number of loci amplified by ISSR primers ranged between 6 (UBC-888) to 14 (UBC-884). A total of 113 loci amplified of different size ranging from 181 to 3989 bp out of which 91 (80.53 %) loci were polymorphic and 22 (19.47 %) loci were monomorphic. The polymorphism percentage ranged from 60 % for UBC-889 to 92.31 % for UBC-886 (Fig. 1.). Maximum polymorphic information content value of 0.36 (UB-C878) and low PIC value of 0.19 (UBC-887 and UBC-889), with an average value of PIC per primer 0.27 were obtained (Table 4.). The highest effective multiplex ratio (EMR) 5.68 was observed with the primer UBC-887 and the lowest EMR 3.32 was observed with the primer UBC-885 with an average EMR of 4.49 per primer (Table 4.). The highest marker index (MI) was observed with the primer UBC-876 (1.38) and lowest in the primer UBC-880 (0.80), with an average MI of 1.19 was obtained. The resolving power is a parameter that indicates the discriminatory potential of the primers chosen. The highest RP value was observed with the primer UBC-886 (5.60) and the lowest with the primer UBC-887 (1.60) with an average RP of 3.75 per primer. Overall, ISSR molecular marker revealed higher polymorphism (80.53 %) and resolving power. ISSR has been successfully utilized for diversity analysis of teak (Narayanan *et al*. 2007; Ansari *et al*. 2012; Lyngdoh *et al*. 2013; Chimello *et al*. 2017; Giustina *et al*. 2017; Mohammad *et al*. 2017; Vaishnav and Ansari 2018) [27, 3, 25, 5, 14, 26, 36] and other trees species (Goulão *et al*. 2001; Arif *et al*. 2009; Gajera *et al*. 2011; Vashishtha *et al*. 2013; Dasgupta *et al*. 2015; Goyal *et al*. 2015; Kulhari *et al*. 2015; Long *et al*. 2015; Patel *et al*. 2016) [16, 4, 13, 38, 6, 18, 23, 24, 30].

Table 4: Primer-wise analysis of banding patterns generated by ISSR marker assays for ten populations of *T. grandis*

Primer	NB	Nm	Np	PP	PIC	EMR	MI	Rp
UBC-827	8	2	6	75.00	0.26	4.95	1.29	3.00
UBC-876	8	1	7	87.50	0.34	4.05	1.38	4.60
UBC-878	8	1	7	87.50	0.36	4.49	1.62	4.60
UBC-880	7	2	5	71.43	0.24	3.34	0.80	2.60
UBC-884	14	2	12	85.71	0.28	4.21	1.18	5.40
UBC-885	12	2	10	83.33	0.27	3.32	0.90	4.40
UBC-886	13	1	12	92.31	0.29	4.69	1.36	5.60
UBC-887	7	2	5	71.43	0.19	5.68	1.08	1.60
UBC-888	6	2	4	66.67	0.24	5.23	1.26	2.20
UBC-889	10	4	6	60.00	0.19	5.16	0.98	2.80
UBC-892	8	2	6	75.00	0.27	3.75	1.01	3.20
UBC-900	12	1	11	91.67	0.29	4.97	1.44	5.00
Overall	113	22	91	80.53	0.27	5.82	4.49	1.19

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)



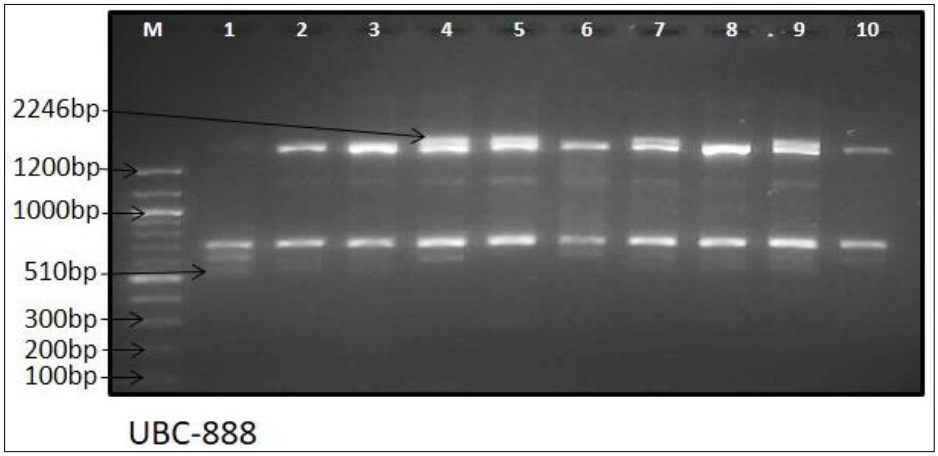


Fig 1: ISSR fingerprints of 10 populations generated by UBC-886, UBC-887 and UBC-888

The Jacard's similarity matrices (Tables 5.) and Nei & Li's matrices (Table 6.) of the ISSR data were showed high similarity between populations. Highest Jacard's similarity (0.68) was observed between Tapti and Vansada and Dharampur and Chikhali. Nei & Li's matrices (0.84) observed between Bhenskatri and Kalibel. The smallest Jacard's (0.49) and Nei & Li's (0.59) coefficients were observed between Bardipada-A and Vyara. On the basis of Jacard's and Nei & Li's coefficient, two dendrograms were constructed to gave similar clustering of the ten populations (Figs. 2. and Figs. 3.). Ten populations divided into three major groups. The first group was subdivided into three subgroups: Ia) Tapti, Vansada and Bardipada-B population, Ib) Bardipada-A population from the Dangs forest division and Ic) Chikhali and Dharampur population from the Valsad forest divisions and Bhenskatri and Kalibel from the Dangs forest division. The second group (II) included Vyara population from the Vyara forest division and Kaprada population from the Valsad forest division.

Table 5: Jaccard's similarity matrix for ten teak populations as revealed by ISSR marker

Population	Vyara	Bhenskatri	Kalibel	Bardi-A	Bardi-B	Tapti	Vansada	Dharampur	Chikhali	Kaprada
Vyara	1.00									
Bhenskatri	0.53	1.00								
Kalibel	0.47	0.72	1.00							
Bardi-A	0.42	0.56	0.62	1.00						
Bardi-B	0.47	0.54	0.57	0.59	1.00					
Tapti	0.55	0.60	0.56	0.56	0.63	1.00				
Vansada	0.52	0.57	0.61	0.55	0.71	0.68	1.00			
Dharampur	0.48	0.63	0.68	0.63	0.65	0.64	0.67	1.00		
Chikhali	0.43	0.62	0.64	0.64	0.61	0.58	0.67	0.68	1.00	
Kaprada	0.56	0.58	0.56	0.52	0.50	0.56	0.61	0.49	0.60	1.00

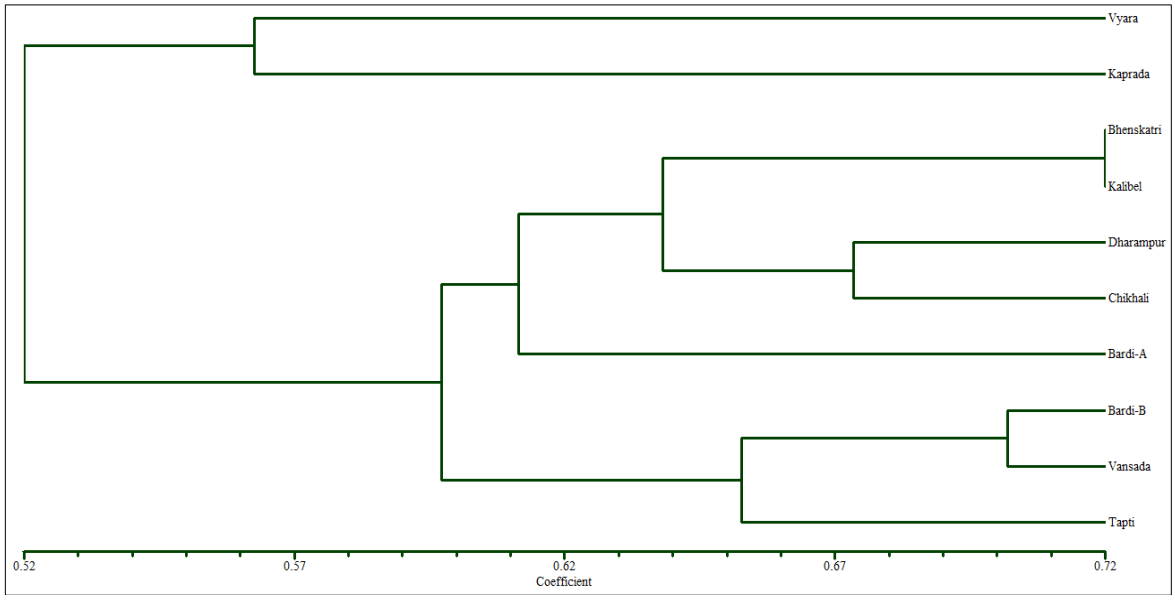


Fig 2: UPGMA dendrogram of ten teak populations as revealed by ISSR marker based on Jaccard's Coefficient

Table 6: Nei & Li's coefficient matrix for ten teak populations as revealed by ISSR marker

Population	Vyara	Bhenskatri	Kalibel	Bardi-A	Bardi-B	Tapti	Vansada	Dharampur	Chikhali	Kaprada
Vyara	1.00									
Bhenskatri	0.69	1.00								
Kalibel	0.64	0.84	1.00							
Bardi-A	0.59	0.72	0.76	1.00						
Bardi-B	0.64	0.70	0.73	0.74	1.00					
Tapti	0.71	0.75	0.72	0.72	0.78	1.00				
Vansada	0.68	0.73	0.76	0.71	0.83	0.81	1.00			
Dharampur	0.65	0.78	0.81	0.78	0.78	0.78	0.80	1.00		
Chikhali	0.60	0.76	0.78	0.78	0.76	0.73	0.80	0.81	1.00	
Kaprada	0.72	0.74	0.72	0.69	0.67	0.72	0.76	0.66	0.75	1.00

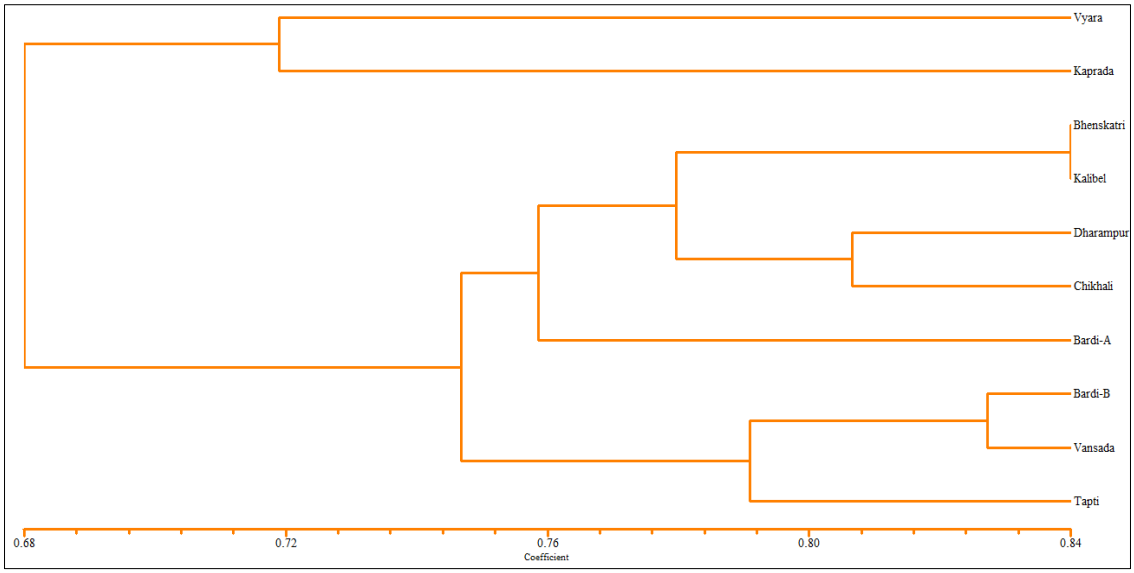


Fig 3: UPGMA dendrogram of ten teak populations as revealed by ISSR marker based on Nei & Li's Coefficient.

PCoA was generated from the DCENTER and EIGEN with the program NTSYSpc v 2.1. To confirm the genetic relationships among 10 teak populations from south Gujarat. The results of PCoA tallied with the cluster analysis prevailed through UPGMA (Fig. 4.). The first three coordinates contributed 19.55, 16.07 and 13.12 per cent of the total variance, respectively. Hence total cumulative variance from these three coordinates was 48.75 per cent. Vyara population was seemed to be totally different in both the analyses. Significant level of polymorphism detected in ten natural population of teak from south Gujarat by ISSR (80.53 %).

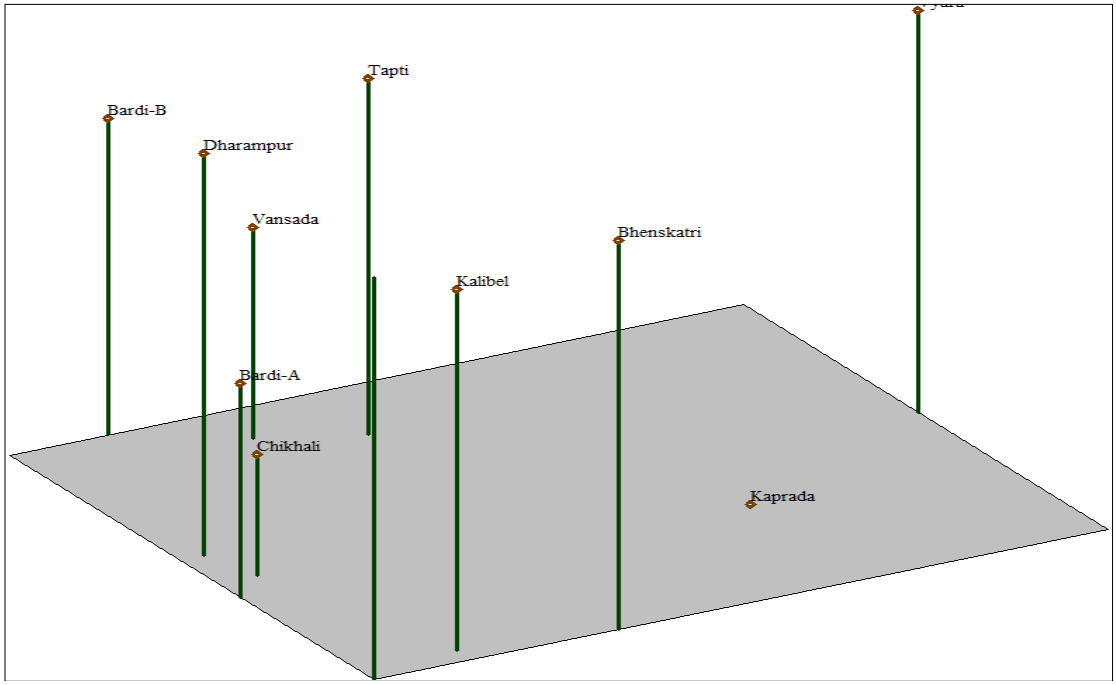


Fig 4: Principal Coordinate Analysis as revealed by ISSR markers based on the Jaccard similarity index

Teak is a perennial woody and cross pollinated tropical tree species which have a majority of the genetic variation to be expected within populations. The results obtained in this study is in agreement with finding of other researchers in teak (Narayanan *et al*. 2007; Ansari *et al*. 2012; Lyngdoh *et al*. 2013; Chimello *et al*. 2017; Giustina *et al*. 2017; Mohammad *et al*. 2017; Vaishnav and Ansari 2018) [27, 3, 25, 5, 14, 26, 36]. The genetic differentiation of teak meta-population in India was investigated by Vaishnav and Ansari (2018) [36] employing dominant ISSR markers to understand adaptability of the species. Meta-population represents 290 teak genotypes from 29 locations of its natural distribution. Meta-population clustered in three sub-population clusters on the basis of the genetic and geographical variables, where geographical variables played a significant role in gene flow. Overall, the findings from the study can assist for teak survival in threat of changing climatic situations throughout India.

Conclusion

Our results indicate the presence of higher amount of great genetic variability among teak ten populations from three forest divisions of Gujarat. Over all higher diversity within forest division indicates cautious selection should be implemented while selecting plus tree for establishing of breeding population or seed orchard. More number of trees per population should be selected to capture maximum diversity and realizing better gain since the genetic gain is directly proportional to existing variability.

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