International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
IJCS 2018; 6(5): 259-264

Received: 21-07-2018
Accepted: 25-08-2018

**Modi JS**
Department of Silviculture and Agroforestry, ASPEE College of Horticulture and Forestry, Navsari Agricultural University, Navsari, Gujarat, India

**Tandel MB**
Department of Silviculture and Agroforestry, ASPEE College of Horticulture and Forestry, Navsari Agricultural University, Navsari, Gujarat, India

**Prajapati VM**
Department of Silviculture and Agroforestry, ASPEE College of Horticulture and Forestry, Navsari Agricultural University, Navsari, Gujarat, India

**Parekh VB**
Department of Silviculture and Agroforestry, ASPEE College of Horticulture and Forestry, Navsari Agricultural University, Navsari, Gujarat, India

**Ahir BR**
Department of Silviculture and Agroforestry, ASPEE College of Horticulture and Forestry, Navsari Agricultural University, Navsari, Gujarat, India

**Correspondence**
**Modi JS**
Department of Silviculture and Agroforestry, ASPEE College of Horticulture and Forestry, Navsari Agricultural University, Navsari, Gujarat, India

# Molecular variations in teak (*Tectona grandis* L. f.) clones

**Modi JS, Tandel MB, Prajapati VM, Parekh VB and Ahir BR**

**Abstract**
The investigation entitled "Molecular variations in Teak (*Tectona grandis* L. f.) clones" was carried out at Rajpipla Silviculture Forest Division, Rajpipla and Biotechnology Laboratory, ASPEE College of Horticulture and Forestry, Navsari Agricultural University, Navsari (Gujarat). The experiment comprised of 15 clones *viz.*, $C_1$-Bandhpada (North Dangs), $C_2$-Chikhli (South Dangs), $C_3$-Nilambo (Dungarda), $C_4$-African (Dungarda), $C_5$-Satkhasi (Vyara), $C_6$-Khalta (Bariya), $C_7$-Achhala (Godhra), $C_8$-Kevadi (Chotaudepur), $C_9$-Raighadh (Sabarkantha North), $C_{10}$-Danta (Banaskantha), $C_{11}$-Vanaj (South Sabarkantha), $C_{12}$-Dankiwadu (Gir West), $C_{13}$-Bhavnath (Junaghadh), $C_{14}$-Develvel (Gir East) and $C_{15}$-Khatam (Rajpipla East). This experiment was laid out in Randomized Block Design (RBD) comprising of fifteen clones and three replications. The seven decamer primers produced a total of 50 scorable bands in the fifteen clones of *Tectona grandis* L. f. out of them 20 were polymorphic and 30 were monomorphic. The percentage of polymorphism ranged from a maximum 50.00 % by OPE-18 to a minimum of 16.67 % by OPE-10. The lowest genetic similarity (0.7209) was between the clones 4 & 7 whereas the highest genetic similarity (0.9761) was between the clones 9 & 10. The dendrogram of fifteen clones reflected that the clones were divided in two main clusters named A and B. Further Cluster-B was divided in to two sub clusters $B_1$ and $B_2$. The clones $C_7$ and $C_8$ reported in Cluster-A which were found to be more diverse as compared to other clones. The clones $C_1$, $C_3$, $C_5$, $C_2$, $C_6$ and $C_4$ were observed in sub-cluster-$B_1$. Whereas, clones $C_9$, $C_{10}$, $C_{15}$, $C_{11}$, $C_{12}$, $C_{13}$ and $C_{14}$ were recorded in sub-cluster-$B_2$. The clones $C_4$ and $C_{14}$ found in $B_1$ and $B_2$ sub-cluster, respectively which having minimum similarity with other clones.

**Keywords:** molecular variations, teak (*Tectona grandis* L. f.) and clones

## Introduction
The forests of India are ancient in nature with high diversity. They are not only rich in tree species composition but also provide shelter to a wide range of fauna, avian-fauna and insects. The teak tree is native to South-East Asia, more specifically to India, Myanmar, Thailand and Laos. Over the past 150 years, it has been planted extensively both within its native range and in other tropical and sub-tropical region of Asia, Africa and America. It is naturalized in the Indonesian island of Java and some of the smaller islands east of Java, where it is believed to have been introduced some 400-600 years ago.

Teak (*Tectona grandis*) is a tall and handsome deciduous tree representing Lamiaceae family in plant kingdom. Locally, it is also known as Sagon, Saigon, Saj, Taku, Kayum, etc. in various Indian languages. The teak tree is well known for its versatile timber. Its heartwood combines several qualities like termite and decay resistance, lightness and strength, drying without warping and splitting, easy workability and attractive appearance, making it one of the world's finest timbers.

Teak genetic improvement was started in India in the year 1954. Teak improvement was limited to establishing seed production areas (SPAs) and clonal seed orchards (CSO). CSOs are established with grafted plants of superior phenotypes (plus trees) selected from natural forests and plantations. These "plus trees" were chosen at a very high intensity (often one in several hectares of forests) with rigorous selection criteria. CSOs are the only source of ex-situ conservation for Teak in India. More than 1,000 ha of CSOs have been established in India with 450 ha in Maharashtra, 240 ha in Madhya Pradesh, 120 ha in Karnataka, 92 ha in Andhra Pradesh, 35 ha in Kerala, 30 ha in Orissa and Tamil Nadu, and 25 ha in Arunachal Pradesh (Katwal, 2005) [6].

Molecular markers have been looked upon as tools for a large number of applications ranging from localization of a gene to improvement of plant varieties by marker-assisted selection.

They have also become extremely popular for phylogenetic analysis adding new dimensions to the evolutionary theories. If we look at the history of the development of these markers, it is evident that they have been improved over the last two decades to provide easy, fast and automated assistance to scientists and breeders. Genome analysis based on molecular markers has generated a vast amount of information and a number of databases are being generated to preserve and popularize it (Joshi *et al.* 1999) [5].

**Materials and Methods**

Clonal Teak Seed Orchard, Rajpipla (TSO) was established in 2000-2001 at Rajpipla in Narmada District for getting good quality seed material. Clonal seed orchard, Rajpipla is located at 21º 53' N Latitude, 73º 31' E Longitude at 45 meters above the mean sea level in Narmada district of South Gujarat in India. The climate of Rajpipla is tropical characterized by fairly hot weather, moderately cold winter with humid and warm monsoon coupled with moderately heavy rainfall. The monsoon commenced from second week of June and lasts up to first week of October. Most of the precipitation received from the South-West monsoon during July and August. The average annual rainfall is 1055 mm. Molecular Profile to be subjected to Jaccard's similarity coefficient analysis using NTSYSpc ver 2.2 software. The genomic DNA extraction protocols was standardized and subjected for analyzing existing molecular variations among Teak clones. Number of monomorphic band, Number of polymorphic band and Genetic similarity were recorded. The leaf samples of *Tectona grandis* L. f. from field plants were collected and stored at -20ºC. The leaf samples of *Tectona grandis* L. f. from field plants were collected and stored at -20ºC. The genomic DNA samples extracted from *Tectona grandis* L. f. leaves were subjected to PCR amplification. Amplification was carried out in a 200 µl thin walled PCR tube containing a 25 µl reaction mix volume.

The reaction volume of 25 µl was subjected to amplification through PCR in a thermal cycler (Eppendorf) along with a control (without genomic DNA). Prior to amplification, reaction mixture was gently tapped and spun briefly.

The genomic DNA amplified using random primers of OPC, OPE, OPF, OPG and OPJ series (Operon Tech., California, USA). The PCR reactions for RAPD were carried out in a 25 µl of reaction mixture as described by William *et al.* (1990) [12].

**The PCR amplification was carried out under following thermal cycling regime**

| | | | | |
|---|---|---|---|---|
| 1) | Initial Denaturation: | | 94 °C for 5 minutes | |
| 2) | Annealing | : | 94 °C for 1 minute | 30 times |
| | | | 35 °C for 45 seconds | |
| | | | 72 °C for 1 minute | |
| | | | 94 °C for 1 minute | 30 times |
| | | | 36 °C for 50 seconds | |
| | | | 72 °C for 1.30 minute | |
| 3) | Final Extension | : | 72 °C for 7 minutes | |
| 4) | Hold | : | at 4 °C | |

The amplified product was collected from the thermal cycler and loaded on to 1.5 percent (w/v) agarose gel containing 5 µl ethidium bromide prepared in 0.5X TBE (pH 8.0). The required volume of 0.5X TBE (pH 8.0) was used as running buffer. Whole of the 25 µl PCR amplified product was mixed with 6X gel loading dye of which 15 µl was loaded in well. Along with the samples 'O' Range Ruler 500bp ladder ready to use molecular weight premix DNA ladder was also loaded. A potential difference of 5-6 V/cm was provided till the bands resolved properly.

The band profiles were visualized and documented using gel documentation system (Syngene). For each locus the presence and absence of the polymorphic band was recorded as 0 and 1, respectively.

Band positions for each Teak clones and primer combination were scored as either present (1) or absent (0). The scores were entered into a database programme (Microsoft Excel) and compiled in a binary matrix for phylogenetic analysis using NTSYS-pc (Numerical Taxonomy and Multivariate analysis) system version 2.2 by Exeter Software (Rohlf, 2004) [7]. The SIMQUALK programme was used to calculate Jaccard's similarity coefficient and a graphical phenogram (dendrogram) of the genetic relatedness among the 15 clones was produced by means of the un-weighted pair group method with arithmetic average (UPGMA) analysis (Sneath and Sokal, 1973) [9].

**Results and Discussion**

The genomic DNA extracted from each clone was subjected to polymerase chain reaction using random decamers. Initially, a total of 200 primers belonging to OPC, OPE, OPF, OPG and OPJ series of universal primers set, each consisting of 20 decamers were screened. However, primers from OPA and OPL and OPM showed no amplification may be due to absence of complementary sequence in the genome. Finally, 7 primers *viz.*, OPC-17, OPE-10, OPE-18, OPF-20, OPG-3, OPG-4 and OPJ-1 were selected for evaluating molecular differences. The nucleotide sequences of each primer are given in Table - 1.

It can be seen from Table – 1 that seven decamer primers produced a total of 50 scorable bands in the 15 clones of *Tectona grandis* L. f. out of which 20 were polymorphic and 30 were monomorphic. The percentage of polymorphism ranged from a maximum 50.00 % by OPE-18 to a minimum of 16.67 % by OPE-10.

**Genetic similarity**

From Table - 2 it is evident that the lowest genetic similarity (0.7209) was between the clones 4 & 7. The highest genetic similarity (0.9761) was between the clones 9 & 10.

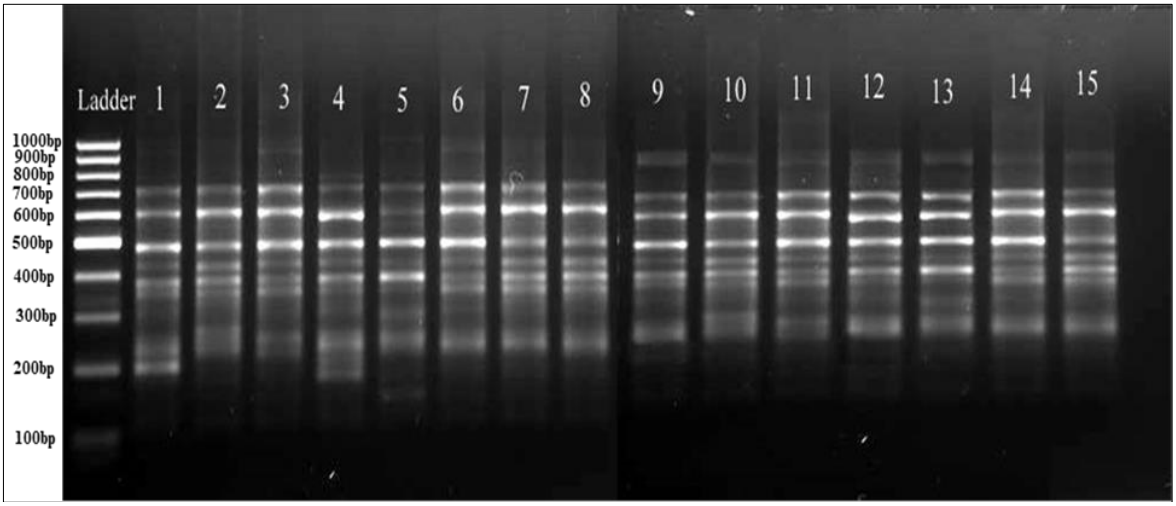
According to dendrogram (Fig. - 1), at a similarity level of 70.00 % the clones were divided in two main clusters named A and B. Further Cluster-B was divided in to two sub clusters $B_1$ and $B_2$. The clones $C_7$ and $C_8$ reported in Cluster-A which were found to be more diverse as compared to other clones. The clones $C_1$, $C_3$, $C_5$, $C_2$, $C_6$ and $C_4$were observed in sub-cluster-$B_1$. Whereas, clones $C_9$, $C_{10}$, $C_{15}$, $C_{11}$, $C_{12}$, $C_{13}$ and $C_{14}$ were registered in sub-cluster-$B_2$. The clones $C_4$ and $C_{14}$ found in $B_1$ and $B_2$ sub-cluster, respectively were having minimum similarity with other clones. Still a screening of more number of primers is recommended to evaluate the present set of clones. Moreover, screening of more clones may also give some diverse clones. Several workers have used RAPD markers to detect genetic variation *viz.*, in willow clones, in 29 populations of teak (*Tectona grandis*) which were collected from central and peninsular India, Ginwal *et al.* (2010) [4] in 55 geographically distinct populations of *Pinus roxburghii* of the Himalayan region, Fofana *et al.* (2008) [3] in *Tectona grandis* grown in Cote d'Ivoire, Behera

*et al*. (2008) [1] in 38 diverse Indian bitter gourd (*Mormordica charantia* var. *charantia* and var. *muricata*) accessions, Shiran *et al*. (2007) [8] in 39 cultivars and species of almond, Syamkumar and Sasikumar (2007) [11] in 15 curcuma species, Faseela and Joseph (2007) [2] in amaranth landraces belonging to three species *viz*., *Amranthus tricolor*, *A. dubius* and *A. hypochondriacus*, Zoghlami *et al*. (2007) [13] in 36 Tunisian *Opuntia ficus indica* L. Mill. ecotypes and Surkhosh *et al*. (2006) [10] in 24 genotypes of Iranian pomegranate (*Punica granatum* L.).

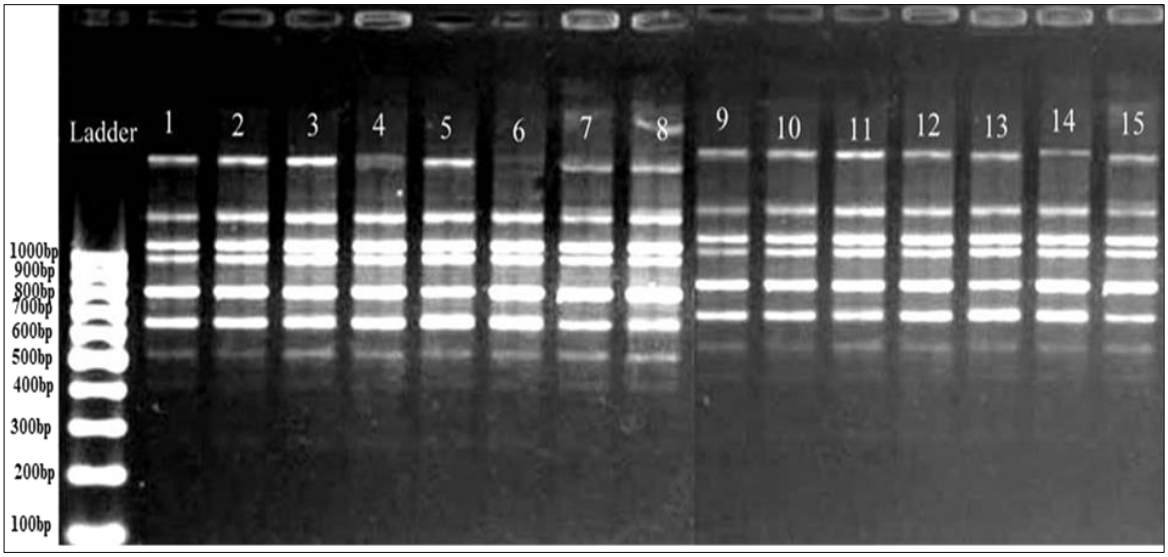
**Table 1:** Details of amplification obtained with different RAPD primers in different clones of *Tectona grandis* L. f.

| Primer | Number of monomorphic Band | Number of polymorphic Band | Total number of bands | Percentage of polymorphism |
|---|---|---|---|---|
| OPC-17 | 6 | 5 | 11 | 45.45 |
| OPE-10 | 5 | 1 | 6 | 16.67 |
| OPE-18 | 2 | 2 | 4 | 50.00 |
| OPF-20 | 4 | 3 | 7 | 42.86 |
| OPG-03 | 5 | 3 | 8 | 37.50 |
| OPG-04 | 4 | 1 | 5 | 20.00 |
| OPJ-01 | 4 | 5 | 9 | 55.56 |
| Total | 30 | 20 | 50 | |

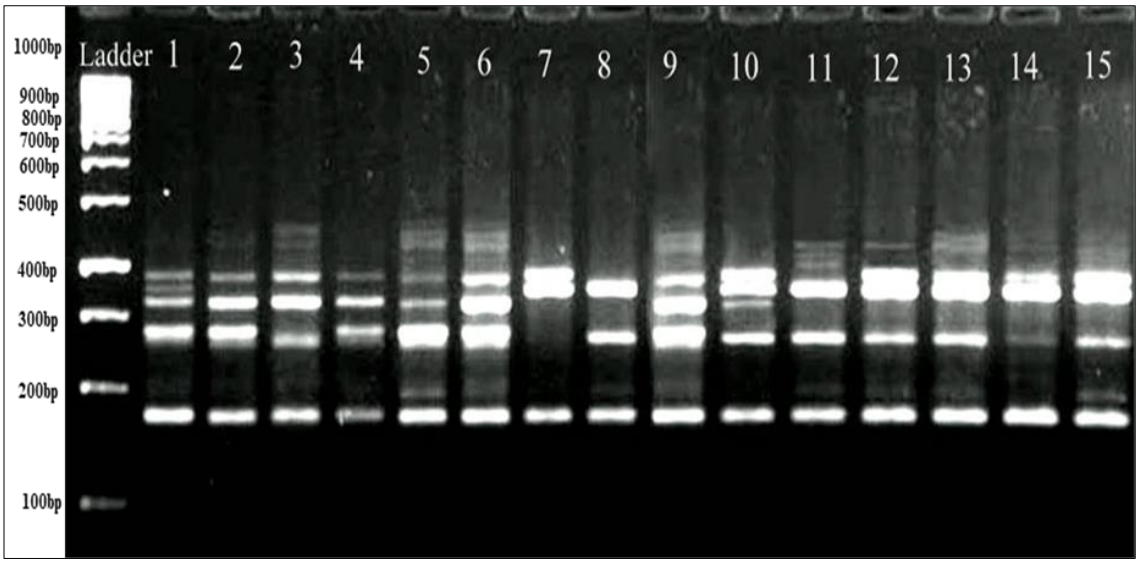
**(A) OPC-17**



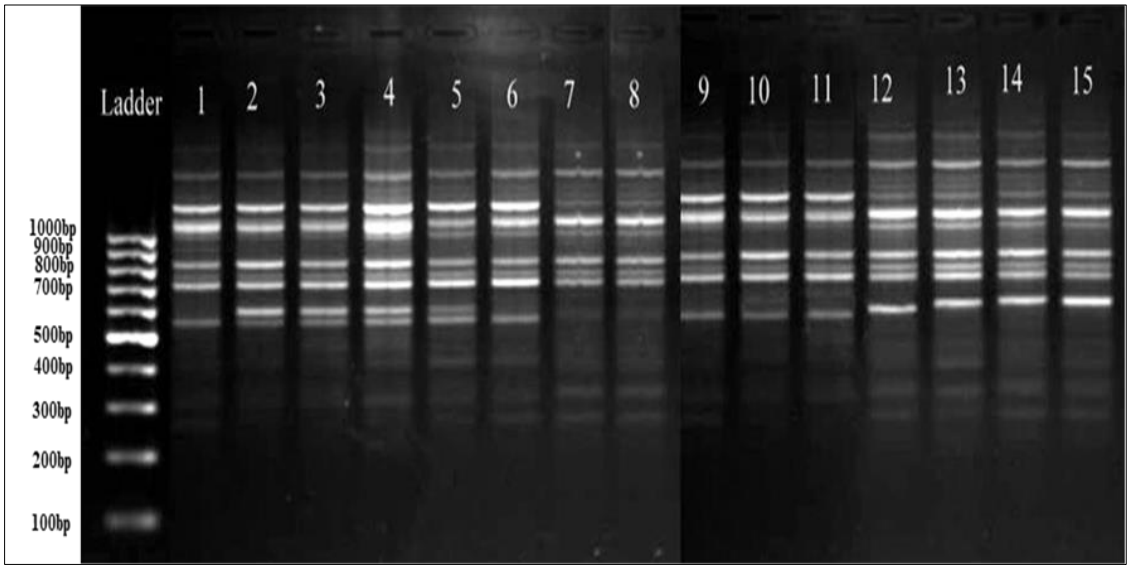

**(B) OPE-10**



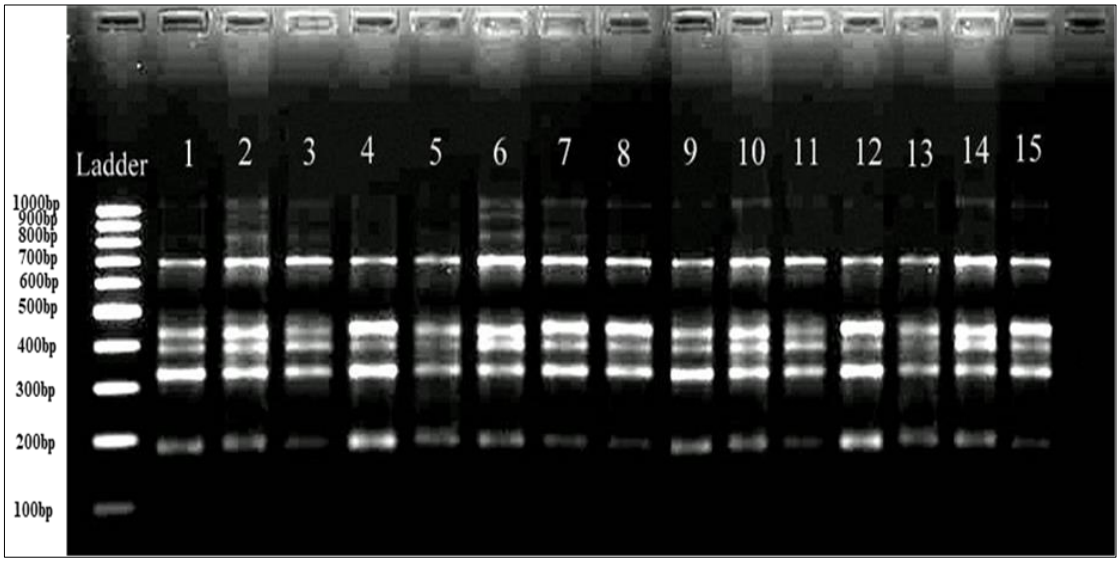
**(C) OPE-18**



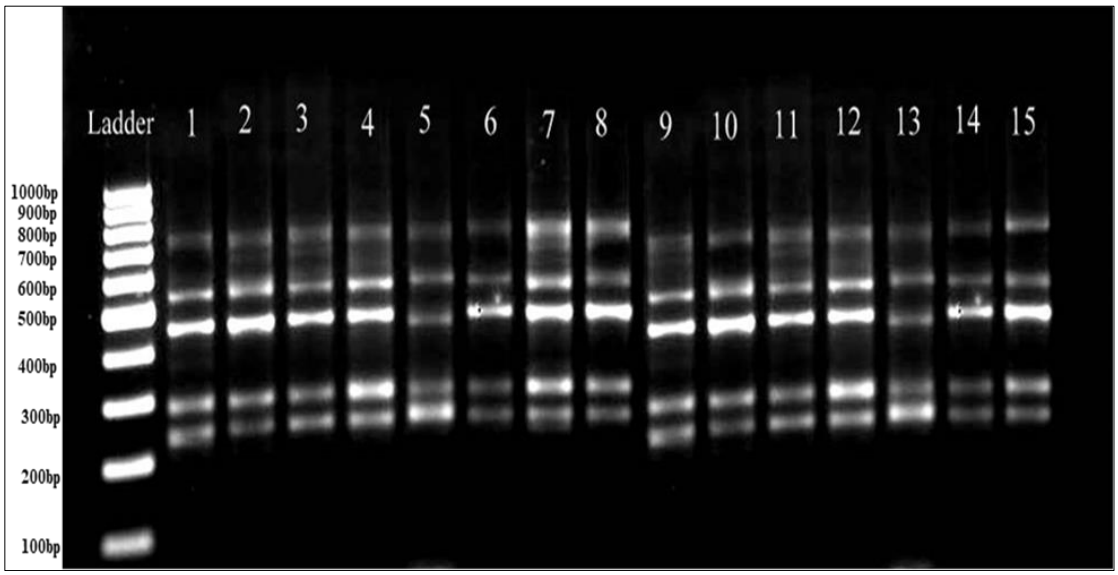

**(D) OPF-20**




**(F) OPG-3**




(G) OPG-4




(H) OPJ-1

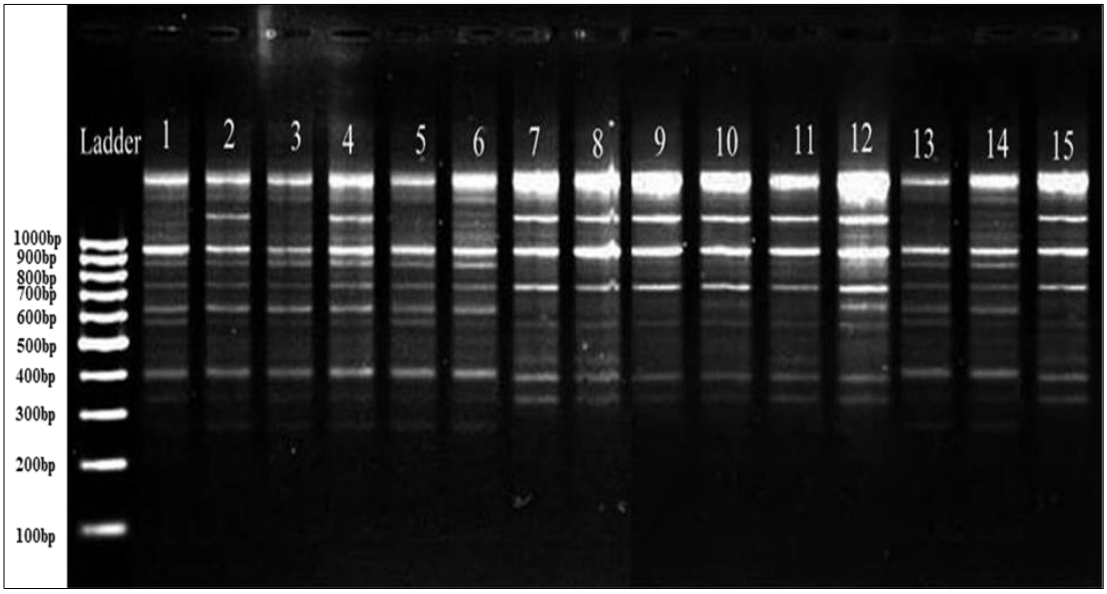



**Plate 1:** RAPD amplification pattern of different clones of *Tectona grandis* L. f. by using various series of primer

**Table 2:** Jaccard's similarity coefficient among different clones of *Tectona grandis* L. f. based on the RAPD data

| | **$C_1$** | **$C_2$** | **$C_3$** | **$C_4$** | **$C_5$** | **$C_6$** | **$C_7$** | **$C_8$** | **$C_9$** | **$C_{10}$** | **$C_{11}$** | **$C_{12}$** | **$C_{13}$** | **$C_{14}$** | **$C_{15}$** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $C_1$ | 1 | | | | | | | | | | | | | | |
| $C_2$ | 0.8409 | 1 | | | | | | | | | | | | | |
| $C_3$ | 0.8809 | 0.8636 | 1 | | | | | | | | | | | | |
| $C_4$ | 0.8139 | 0.8409 | 0.8372 | 1 | | | | | | | | | | | |
| $C_5$ | 0.9024 | 0.8409 | 0.9268 | 0.8571 | 1 | | | | | | | | | | |
| $C_6$ | 0.8181 | 0.8863 | 0.8837 | 0.7777 | 0.8181 | 1 | | | | | | | | | |
| $C_7$ | 0.8048 | 0.7500 | 0.7441 | 0.7209 | 0.7619 | 0.6888 | 1 | | | | | | | | |
| $C_8$ | 0.8048 | 0.7500 | 0.7441 | 0.7619 | 0.8048 | 0.6888 | 0.9444 | 1 | | | | | | | |
| $C_9$ | 0.7777 | 0.8043 | 0.8837 | 0.8181 | 0.8181 | 0.7826 | 0.7674 | 0.7674 | 1 | | | | | | |
| $C_{10}$ | 0.7608 | 0.8260 | 0.8636 | 0.8000 | 0.8000 | 0.8043 | 0.7500 | 0.7500 | 0.9761 | 1 | | | | | |
| $C_{11}$ | 0.7391 | 0.7659 | 0.8409 | 0.8181 | 0.8181 | 0.7446 | 0.7674 | 0.8095 | 0.9523 | 0.9302 | 1 | | | | |
| $C_{12}$ | 0.7826 | 0.7708 | 0.8444 | 0.7826 | 0.8222 | 0.7500 | 0.8139 | 0.8139 | 0.9090 | 0.8888 | 0.9090 | 1 | | | |
| $C_{13}$ | 0.8409 | 0.7872 | 0.9069 | 0.8000 | 0.8837 | 0.8043 | 0.7500 | 0.7500 | 0.8863 | 0.8666 | 0.8444 | 0.9318 | 1 | | |
| $C_{14}$ | 0.7777 | 0.8043 | 0.8837 | 0.7777 | 0.8181 | 0.8222 | 0.7272 | 0.6888 | 0.8636 | 0.8863 | 0.8222 | 0.8666 | 0.9302 | 1 | |
| $C_{15}$ | 0.7234 | 0.7872 | 0.8222 | 0.7608 | 0.7608 | 0.7659 | 0.7906 | 0.7906 | 0.9302 | 0.9534 | 0.9302 | 0.9318 | 0.8666 | 0.8863 | 1 |

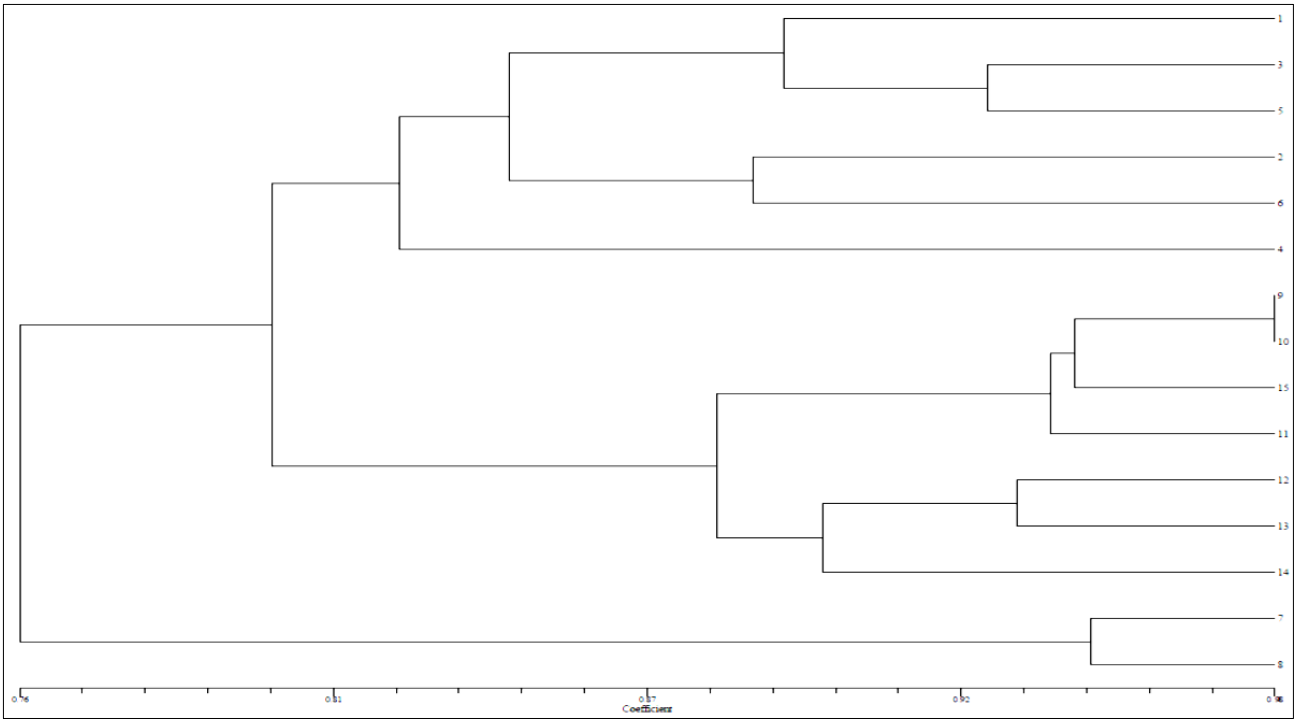


**Fig 1:** Dendrogram depicting the genetic relationship among different clones of *Tectona grandis* L. f. based on RAPD data

## Conclusion

From this molecular variation study it is concluded that, the seven decamer primers produced a total of 50 scorable bands in the fifteen clones of *Tectona grandis* L. f. Out of them 20 were polymorphic and 30 were monomorphic. The percentage of polymorphism ranged from a maximum 50.00 % by OPE-18 to a minimum of 16.67 % by OPE-10. The lowest genetic similarity (0.7209) was between the clones 4 & 7 whereas the highest genetic similarity (0.9761) was between the clones 9 & 10. The dendrogram of fifteen clones reflected that the clones were divided in two main clusters named A and B. Further Cluster-B was divided in to two sub clusters $B_1$ and $B_2$. The clones $C_7$ and $C_8$ reported in Cluster-A which were found to be more diverse as compared to other clones. The clones $C_1$, $C_3$, $C_5$, $C_2$, $C_6$ and $C_4$ were observed in sub-cluster-$B_1$. Whereas, clones $C_9$, $C_{10}$, $C_{15}$, $C_{11}$, $C_{12}$, $C_{13}$ and $C_{14}$ were recorded in sub-cluster-$B_2$. The clones $C_4$ and $C_{14}$ found in $B_1$ and $B_2$ sub-cluster, respectively were having minimum similarity with other clones.

## References


1. Behera TK, Singh AK, Staub JE. Comparative analysis of genetic diversity in Indian bitter gourd (*Momordica charantia* L.) using RAPD and ISSR markers for developing crop improvement strategies. Scientia Horticulture. 2008; 115:209-217.
2. Faseela KV, Joseph S. Molecular characterization of amaranth land races and assessment of interspecific relationships among *Amaranthus* spp. (L.) using RAPD markers. Indian J Genet. 2007; 67(1):12-17.
3. Fofana IJ, Ofori E, Poitel M, Verhaegen D. Diversity and genetic structure of Teak (*Tectona grandis*) in its natural range using DNA microsatellite markers. Tropical Conservation Science. 2008; 1(3):279-292.
4. Ginwal HS, Chauhan Priti, Singh Shalini Maurya, Jadon Vikas S. Genetic variability in *Pinus roxburghii* by RAPD markers. Bioremediation, Biodiversity and Bioavailability, Global Science Books, Ltd., Special, 2010, 28-34.
5. Joshi SP, Prabhakar KR, Gupta VS. Molecular markers in plant genome analysis. Plant Molecular Biology Group, Division of Biochemical Sciences, National Chemical Laboratory, Pune, India, 1999.
6. Katwal RPS. Teak in India: Status prospects and perspectives. In: Bhat K.M., Nair K.K.N., Bhat K.V., Muralidharan E.M., Sharma J.K., eds., Quality timber products of teak from sustainable Forest Management Proceedings of International Conference, Kerala Forest Research Institute, India and International Tropical Timber Organization, Japan, 2005, 1-17.
7. Rohlf FJ. NTSYS-pc Numerical Taxonomy and Multivariate Analysis System version 2.2, Exeter Software, Applied Biostatics, New York, 2004.
8. Shiran B, Amirbakhtiar N, Kiani S, Mohammadi, Sayed-Tabatabaei BE, Moradi H. Molecular characterization and genetic relationship among almond cultivars assessed by RAPD and SSR markers. Scientia Horticulture. 2007; 111:280-292.
9. Sneath PHA, Sokal RR. Numerical Taxonomy. The principle and practice of Numerical Classification. W.H. Freemon and Company, San Francisco, 1973.
10. Surkhosh A, Zamani Z, Fatahi R, Ebadi A. RAPD marker reveal polymorphism among some Iranian pomegranate (*Punica granatum* L.) genotypes. Scientia Horticulture. 2006; 111:24-29.
11. Syamkumar S, Sasikumar B. Molecular marker based genetic diversity analysis of Curcuma species from India. Scientia Horticulture. 2007; 112:235-241.
12. William JGK, Kubelik KJ, Livak KJ, Rafalski JA, Tingey SV. DNA polymorphisms amplified by arbitrary primers are useful genetic markers. Nucleic Acid Res. 1990; 18:6531-6535.
13. Zoghlami N, Chrita I, Bouamana B, Gargouri M, Zemni H, Ghorbel A *et al*. Molecular based assessment of genetic diversity within Barbary fig (*Opuntia ficus indica* (L.) Mill.) in Tunisia. Scientia Horticulture, 2007; 113:134-141.