RAPD AND ISSR MARKERS FOR MOLECULAR CHARACTERIZATION OF TEAK (*TECTONA GRANDIS*) PLUS TREES

C. Narayanan, S. A. Wali, N. Shukla, R. Kumar, A. K. Mandal & S. A. Ansari*

Genetics and Plant Propagation Division, Post Office – RFRC, Mandla Road, Tropical Forest Research Institute, Jabalpur 482 021, India

Received November 2006

NARAYANAN, C., WALI, S. A., SHUKLA, N., KUMAR, R., MANDAL, A. K. & ANSARI, S. A. 2007. RAPD and ISSR markers for molecular characterization of teak (*Tectona grandis*) plus trees. RAPD (random amplified polymorphic DNA) and ISSR (inter simple sequence repeats) markers were used to study DNA polymorphism of 48 assorted accessions (plus trees) of *Tectona grandis* at the National Teak Germplasm Bank, Chandrapur, India. In general, the germplasm exhibited a very high level of molecular diversity and DNA polymorphism. Molecular diversity based on Jaccard's similarity coefficients among 48 plus trees ranged from 0.31–0.85 in RAPD assay and 0.27–0.88 in ISSR assay. RAPD primers amplified more loci than ISSR primers. However, RAPD and ISSR primers amplified 93.2 and 95.9% polymorphic loci respectively. Jaccard's similarity coefficient matrices for both molecular marker systems exhibited a poor correlation (r = 0.36) among themselves and clustering pattern of plus trees did not strictly follow their territorial distribution, possibly due to frequent movement of seeds/planting stocks across localities/regions. From application point of view, RAPD primers amplified several loci specific to 16 plus trees while ISSR primers, few loci specific to three plus trees. These loci can be sequenced and developed into SCAR (sequence characterized amplification region) markers for genotype fingerprinting or development of specific DNA probes for identification of clones or ramets of teak.

Keywords: Germplasm, polymorphism, primers, genetic diversity

NARAYANAN, C., WALI, S. A., SHUKLA, N., KUMAR, R., MANDAL, A. K. & ANSARI, S. A. 2007. Penanda RAPD dan ISSR untuk pencirian molekul bagi pokok terbaik jati (*Tectona grandis*). Penanda RAPD dan ISSR diguna untuk mengkaji polimorfisme DNA bagi 48 pokok terbaik *Tectona grandis* di Bank Germplasma Pokok Jati Kebangsaan, Chandrapur, India. Secara amnya, germplasma menunjukkan aras kepelbagaian molekul dan polimorfisme DNA yang sangat tinggi. Kepelbagaian molekul berdasarkan pekali keserupaan Jaccard di kalangan 48 pokok terbaik itu berjulat antara 0.31–0.85 dalam cerakinan RAPD dan 0.27–0.88 dalam cerakinan ISSR. Primer RAPD mengamplifikasi lebih banyak lokus berbanding primer ISSR. Bagaimanapun, primer RAPD dan ISSR masing-masing mengamplifikasi 93.2% dan 95.9% lokus polimorf. Matriks pekali keserupaan Jaccard bagi kedua-dua sistem penanda molekul ini menunjukkan korelasi yang lemah (r = 0.36) antara kedua-duanya. Tambahan lagi, corak pengelompokan pokok terbaik ini tidak mengikut taburan geografinya mungkin kerana pergerakan kerap biji benih/stok tanaman merentas ketempatan/wilayah. Dari segi aplikasi, primer RAPD mengamplifikasi beberapa lokus yang spesifik kepada 16 pokok terbaik sementara primer ISSR, sekadar beberapa lokus yang spesifik kepada tiga pokok terbaik. Semua lokus ini boleh dijujuk dan dibangunkan menjadi penanda SCAR untuk penentuan cap jari genotip atau pembangunan prob DNA spesifik untuk pengecaman klon atau ramet pokok jati.

INTRODUCTION

Teak (*Tectona grandis*), a entomophilous outcrossing forest tree, is naturally distributed in the moist and dry monsoon forests of India, Myanmar, Laos and Thailand. It is widely planted outside of its natural distribution in tropical Asia as well as in Africa and Latin America mainly due to outstanding durability and very high commercial value of its timber.

In India, the teak plus trees were selected from natural forests on the basis of their superior phenotypes, namely, (1) vigour (height and girth), (2) straight and cylindrical woody stem devoid of basal fluting or buttressing, (3) narrow or compact crown with light branching, (4) reasonable amount of seed production, and (5) free from pest and disease. The selected plus

**Author for correspondence. E-mail address: shamimansari_1@yahoo.co.uk*

trees serve as source material for production of improved planting stock by clonal propagation for large scale plantation programmes or are used as parents in breeding programme for superior traits (Mandal & Rambabu 2001). In the National Teak Germplasm Bank in Chandrapur, India, a large number of plus trees of *T. grandis* has been established and maintained for their sustainable use in tree improvement programme. However, *T. grandis* plus trees do not possess distinct morphological characters and hence need to be characterized at molecular level for maintenance of genetic identity of their clones or ramets and, thus, preventing errors in identification due to visual inspection (Wilhelmina & McNicol 1995).

DNA markers precisely characterize cultivars, provenances or genotypes and measure their genetic relationships. The markers are highly heritable, environmentally stable and exhibit sufficient polymorphism to discriminate very closely related genotypes (Narayanan *et al.* 2007). Both RAPD and ISSR markers are rapid and economical and are extensively used for diversity analysis, mapping and genotype identification of plant species including forest trees (Karp *et al.* 1998). RAPD (random amplified polymorphic DNA) markers involving PCR (polymerase chain reaction) amplification of genomic DNA with random decamer primers are fast, simple and most effective tools for clone management (Takata & Shiraishi 1996, Watanabe *et al.* 2004). They make precise distinction among clones, varieties and cultivars of many forest trees (Goto *et al.* 1999, Tessier *et al.* 1999). On the other hand, ISSR (inter simple sequence repeats) markers use short microsatellite motif containing primers anchored at the 3' or 5' end by two to four arbitrary degenerate nucleotides to amplify the DNA sequence lying between two microsatellite regions (Zietkiewicz *et al.* 1994). ISSR markers have been successfully used for varietal identification and assessment of genetic relationships in many plant species (Ajibade *et al.* 2000).

The present study reports the use of RAPD and ISSR markers for assessment of genetic diversity and DNA polymorphism among 48 plus trees of *T. grandis*. Results will be useful for the efficient conservation of these plus trees and their sustainable use as suitable diverse parents in breeding strategy or as donors in clonal propagation of superior stock for teak improvement programmes.

MATERIALS AND METHODS

Plant material and DNA extraction

A total of 48 assorted plus tress of *T. grandis* from the National Teak Germplasm Bank, Chandrapur were chosen for this study (Table 1). The trees represented the 11 teak-growing states in the country. Genomic DNA was extracted from fresh leaves using slightly modified methods of Doyle and Doyle (1990). To remove large amounts of polyphenols and polysaccharides, 3% PVP was added to the extraction buffer. An additional washing step with cold ethanol was included for removal of remaining impurities. Integrity and quantity of the extracted DNA were estimated spectrophotometrically and visually verified on 1% agarose gel.

Table 1 Geographical distribution of teak plus trees of India

Plus trees	State (geographical location)
APKEA-23, APKEC-2, APJNB-1, APNPL-1, APT-7, SBL-1	Andhra Pradesh (12° 39'–19° 50' N; 76° 41'–84° 44' E; 0–600 m)
AC-I, AC-II	Arunachal Pradesh (26° 32'–29° 31' N; 91° 41'–97° 41' E; 3000–4500 m)
G+10, G+13	Gujarat (20° 06'–24° 43' N; 68° 10'–74° 20' E; 150–300 m)
MYHD-3, MYSA-1, ST-15, ST-17	Karnataka (11° 40'– 18° 27' N; 74° 11'–78° 39' E; 420–1350 m)
KEN-4, KLK-1, KLK-2, KLS-4	Kerala (81° 2'–12° 49' N; 73° 17'–77° 28' E; 0–900 m)
BLC-10, CSC-9, PT-1, PT-3, PT-41, PT-46	Madhya Pradesh (17° 47'–02-6° 48' N; 73° 53'–84° 27' E; 150–840 m)
MHALA-4, MHALA-5, MHALA-7, MHAL-8, MHALP-4, MHALP-9, MHEMR-2, MHWYK-3	Maharashtra (15° 43'–21° 52' N; 72° 40'–80° 54' E; 100–1110 m)
ORANR-4, ORANR-7, ORJEK-1, ORPB-18, ORPB-27, ORPLM-1	Orissa (17° 47'–22° 37' N; 81°17'–87° 29' E; 0–600 m)
TNT-4, TNT-5, TNT-6, TNT-7	Tamil Nadu (8° 06'–13° 42' N; 76° 08'–80° 08' E; 0–375 m)
UP-D, UP-H, UP-I, UP-K	Uttar Pradesh (23° 52'–31° 27' N; 77° 05'–84° 37' E; 0–350 m)
WB, WB+4	West Bengal (21° 31'–27° 27' N; 85° 49'–89° 17' E; 0–300 m)

RAPD assay

DNA amplification (Williams *et al.* 1990) was carried out in a 25 µl reaction volume containing 2× Taq polymerase buffer, 200 µmoles of each nucleotide in dNTPs (i.e. ATP, TTP, GTP and CTP), 2 mM $MgCl_2$, 1 unit Taq polymerase (Genie, Bangalore, India), 20 pmoles of decamer primer (Operon Technologies, Alameda, USA) and 20 ng genomic DNA in a programmable thermal cycler (PalmCycler; Corbett Research, Australia). Amplification reactions were cycled 35 times for 1 min at 92 °C (denaturation), 1 min at 37 °C (annealing) and 2 min at 72 °C (extension) with a final extension step for 5 min. Amplification products were mixed with loading buffer (2 µl 1× TBE containing 40% glycerol and 0.025% bromophenol blue) and fractionated on 3% agarose-1× Tris-borate-EDTA-ethidium bromide gel electrophoresis in 1× TBE buffer at 200 mA. RAPD bands were visualized and photographed on a UV transilluminator (Figure 1a). All experiments were duplicated. Preliminary screening with 200 RAPD primers identified 26 RAPD primers showing amplification of teak genomic DNA, of which 22 primers showed maximum polymorphic bands. For further study, 10 RAPD primers (Table 2) which consistently produced reproducible, scorable and highly polymorphic bands were used.

ISSR assay

DNA amplification (Zietkiewicz *et al.* 1994) was carried out in 10 µl reaction volume containing 20 ng genomic DNA, 1× Taq polymerase buffer, 0.1 mM of each dNTPs, 2.5 mM $MgCl_2$, 1 unit Taq polymerase, and 0.8 µM of primer (University of British Colombia, Canada; UBC Kit #9). Amplification cycle consisted of an initial 3 min denaturation at 94 °C, 35 cycles for 30 sec at 94 °C, 30 sec at 50 °C, 1 min at 72 °C, and final extension step for 10 min at 72 °C. The amplification products of each reaction mixture along with 1× TBE loading buffer were size-fractionated by electrophoresis on a 3% agarose gel with 0.5 µg/ml ethidium bromide and visualized on UV transilluminator (Figure 1b). After a preliminary screening with one hundred ISSR primers, 10 primers were selected based on more number of polymorphic bands. Finally, five ISSR primers (Table 2) were selected on the basis of clarity and their polymorphic nature of amplified products.

Data analysis

Banding profiles generated by RAPD and ISSR assays were separately compiled into a data matrix on the basis of presence (1) or absence (0) of bands. The binary matrices were used to estimate

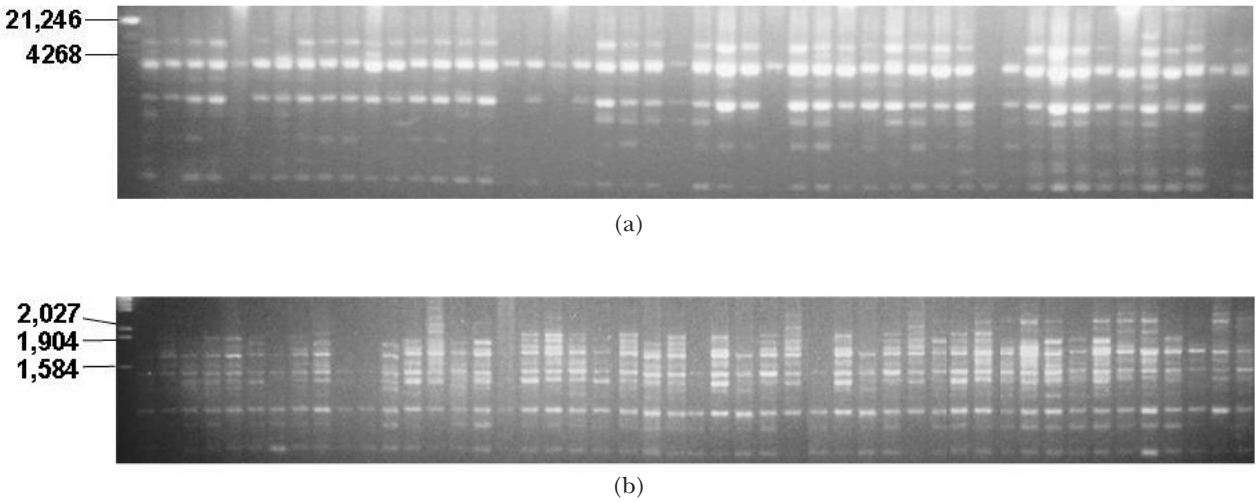


Figure 1 Gel electrophoresis patterns of amplified loci among teak (*Tectona grandis*) plus trees using (a) RAPD primer OPE-06 and (b) ISSR primer UBC-834. First lane exhibits molecular ladder fragments with molecular weights (bp); lanes 2-49 represent teak plus trees: APKEA-23, APKEC-2, APJNB-1, APNPL-1, APT-7, SBL-1 , AC-I, AC-II, G+10, G+13, MYHD-3, MYSA-1, ST-15, ST-17, KEN-4, KLK-1, KLK-2, KLS-4, BLC-10, CSC-9, PT-1, PT-3, PT-41, PT-46, MHALA-4, MHALA-5, MHALA-7, MHAL-8, MHALP-4, MHALP-9, MHEMR-2, MHWYK-3, ORANR-4, ORANR-7, ORJEK-1, ORPB-18, ORPB-27, ORPLM-1, TNT-4, TNT-5, TNT-6, TNT-7, UP-D, UP-H, UP-I, UP-K, WB, WB+4.

Table 2 Details of RAPD and ISSR primers and amplified loci specific to teak plus trees

No.	Primer*	Sequence	Size (bp) of specific loci (plus tree)
1	OPAP-07	ACCACCCGCT	1611 (PT-3)
2	OPAP-13	TGAAGCCCCT	1493 (MHWYK-3), 3063 (MHAL-8)
3	OPR-15	GGACAACGAG	-
4	OPM-05	GGGAACGTGT	1869 (MHALP-9)
5	OPM-06	CTGGGCAACT	1770 (MHWYK-3), 2923 (MHAL-8), 5947 (MHALP-9); 2014 (TNT-4)
6	OPM-13	GGTGGTCAAG	-
7	OPO-20	ACACACGCTG	9845 (MHWYK-3); 5678 (TNT-5); 5807 (PT-1)
8	OPB-18	CCACAGCAGT	-
9	OPE-06	AAGACCCCTC	8656 (UP-I), 9206 (UP-K); 591.2, 2483 (TNT-5)
10	OPE-15	ACGCACAACC	1857 (MHALA-5)
11	UBC-801	ATATATATATATATATT	6191 (MHALP-4)
12	UBC-834	AGAGAGAGAGAGAGAGYT	-
13	UBC-880	GGAGAGGAGAGGAGA	3255 (WB)
14	UBC-899	CATGGTGTTGGTCATTGTTCCA	-
15	UBC-900	ACTTCCCCACAGGTTAACACA	881 (WB)

*No. 1-10: RAPD primers (Operon, USA); 11-15: ISSR primers (University of British Columbia, Canada)

DNA polymorphisms and genetic relatedness of teak genotypes. Data analyses were performed using the software NTSYS-pc (Numerical Taxonomy System for Windows, Version 2.20e). Both monomorphic and polymorphic bands were used to calculate pair-wise genetic similarity among teak plus trees using Jaccard's coefficient (Jaccard 1908). Similarity matrices were utilized to compute descriptive statistics of central tendency as well as to construct the UPGMA (unweighted pair-group arithmetic average method) dendrograms (Sneath & Sokal 1973). For estimating congruence among dendrograms, cophenetic matrices for each marker and index type were computed and compared using the Mantel test (Mantel 1967). Statistical stability of the branches in the clusters was estimated by bootstrap analysis with 2000 replicates using WINBOOT software program (Yap & Nelson 1996).

RESULTS AND DISCUSSION

Molecular diversity among teak plus trees

Molecular diversity based on Jaccard's similarity coefficients among 48 plus trees ranged from 0.31–0.85 in RAPD assay and from 0.27–0.88 in ISSR assay. The ranges indicated very diverse nature of plus trees, which was expected due to rigorous selections made from various teak forest types thriving on different geo-climates of the country. The agreement among values obtained from both molecular marker systems confirmed the broad genetic base of sampled plus trees in the present analysis. Descriptive statistics also revealed normal distribution of Jaccard's similarity coefficients obtained for RAPD and ISSR markers (Figure 2). It is interesting to note that both molecular marker systems, despite targeting different regions of the genome, yielded almost similar results for diversity indices. The finding implies that the molecular variability is evenly dispersed on the genomes of selected plus trees. Several researchers have compared two or more molecular systems for testing the diversity of trees, e.g. RAPD and ISSR in *Haloxylon ammodendron* (Yan *et al.* 2004), ISSR, RAPD and cytological markers in *Picea mariana, P. glauca* and *P. engelmannii* (Nkongolo *et al.* 2005), and ISSR, RAPD and SSR in *Juglans regia* and *J. nigra* (Pollegioni *et al.* 2005).

DNA polymorphism among teak plus trees

RAPD primers amplified more loci than ISSR primers (Table 3). The difference in resolution of RAPD and ISSR markers could possibly be explained by the fact that the two marker systems

Table 3 Number of loci amplified in RAPD and ISSR assay in teak plus trees

Variables	Molecular markers	
	RAPD	ISSR
Total loci	133	49
Polymorphic loci	124	47
Polymorphic loci/primer	12.4	9.4

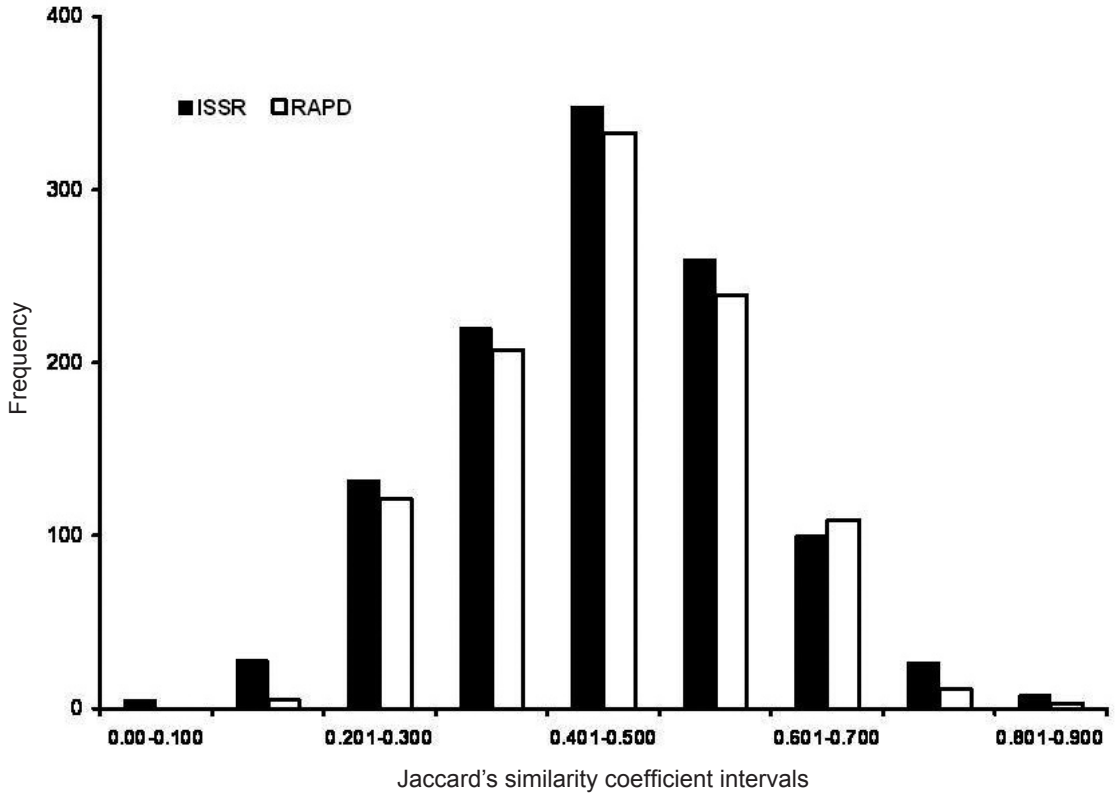


Figure 2 Distribution of pair-wise Jaccard's similarity coefficients obtained for RAPD (kurtosis = -0.404, skewness = 0.252) and ISSR (kurtosis = 0.121, skewness = -0.030) molecular markers

target different regions of the genome. In RAPD, the imperfect random priming at many sites results in amplification of more loci (Williams *et al.* 1990). On the other hand, ISSR markers target a small segment between two microsatellites of the genome which possibly make few loci available for amplification by ISSR primers (Zietkiewicz *et al.* 1994). However, RAPD and ISSR primers amplified 93.2 and 95.9% polymorphic loci respectively which were commensurate with the observations on molecular diversity cited above. We observed that five ISSR primers detected almost the same or even more polymorphism than 10 RAPD primers. ISSR primers are able to amplify highly variable but small segments, i.e. regions between two microsatellites of the genome (McGregor *et al.* 2000). That is why a few ISSR primers can capture much more variability from a genomic segment than several RAPD primers with random coverage of entire genome (Moreno *et al.* 1998). Expectedly, RAPD amplified loci of large size, i.e. 500 to 11 000 bp and ISSR, of small size, i.e. 70 to 8900 bp. From application point of view, our study showed that RAPD primers amplified several loci specific to 16 plus trees while ISSR primers, few loci specific to three plus trees (Table 2). These loci may be sequenced and developed into SCAR (sequence characterized amplification region) markers for genotype fingerprinting or development of specific DNA probes for identification of clones or ramets of teak plus trees (Roy *et al.* 1992, Albani *et al.* 2004).

Genetic relationships among plus trees of teak

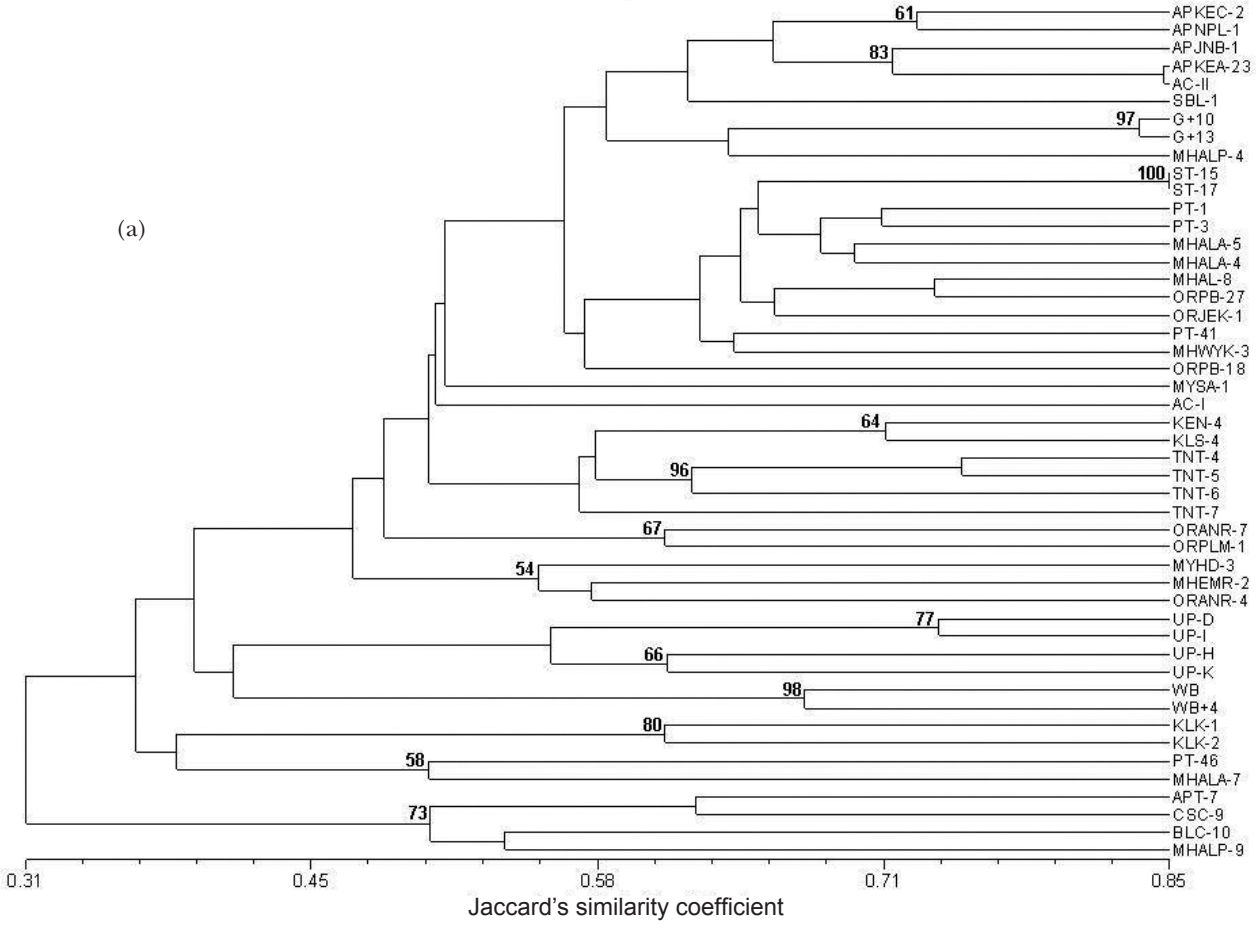
Jaccard's similarity coefficient matrices exhibited a poor correlation (r = 0.36) among themselves. This an indication that they target different regions of the genome. Similar low correlation value (r = 0.32) between RAPD and ISSR matrices have been reported in blackgram (Souframanien & Gopalakrishna 2004). However, comparison between RAPD and ISSR matrices of cross-pollinated tropical broad-leaved trees has not much been investigated. Nevertheless, data obtained with various molecular markers have been compared in several horticultural trees, e.g. RAPD, ISSR and AFLP in cashew nut (Archak *et al.* 2003) and RAPD and ISSR in mulberry (Awasthi *et al.* 2004). The variable clustering patterns

obtained in UPGMA analysis-based dendrograms for both molecular marker systems attest to the poor correlation mentioned above.

The stable cluster nodes supported by > 50% bootstrap values were 14 in RAPD (Figure 3a) and 10 in ISSR (Figure 3b). This indicated that the RAPD dendrogram was statistically robust and reproducible as well as biologically sound for clustering plus trees according to their geographical distribution. We observed that plus trees from Andhra Pradesh (APT-7) grouped with Madhya Pradesh (CSC-9) in RAPD but with Orissa (ORJEK-1) and Maharashtra (MHALP-9) in ISSR; Arunachal Pradesh (AC-II) with Andhra Pradesh (APKEA-23) in RAPD but with Karnataka (MYSA-1) in ISSR; Kerala (KLK-1 & KLK-2) with Madhya Pradesh (PT-46) and Maharashtra (MHALA-7) in RAPD but with Orissa (ORANR-4) in ISSR. In contrast, both molecular marker systems also resulted in similar clusterings for some plus trees of different states e.g. Karnataka (ST15 and ST17), Madhya Pradesh (PT1 and PT3), Uttar Pradesh (UP-D and UP-I) and West Bengal (WB and WB+4) (Figures 3a and b). These plus trees appeared to be selected from populations that share maximum common alleles which may have resulted from frequent movement of seeds/planting stocks across these localities/regions. Findings on other plants including Azuki bean (Yee *et al.* 1999) and groundnut (Dwivedi *et al.* 2001) are also in agreement with our results.

CONCLUSIONS

Both molecular marker systems generated enough polymorphisms for the distinction of teak plus trees, and some of them even at individual levels. Accessions of plus trees included in this investigation were highly diverse but did not group according to their territorial distribution, thus, suggesting either a common genetic base or frequent natural or anthropogenic gene flow among various populations of teak from where the plus tree selections were made. The putative plus tree specific RAPD and ISSR loci may be converted into much stringent SCAR markers for the preparation of precise passport database of entire accessions of plus trees established in the National Germplasm Bank or elsewhere in



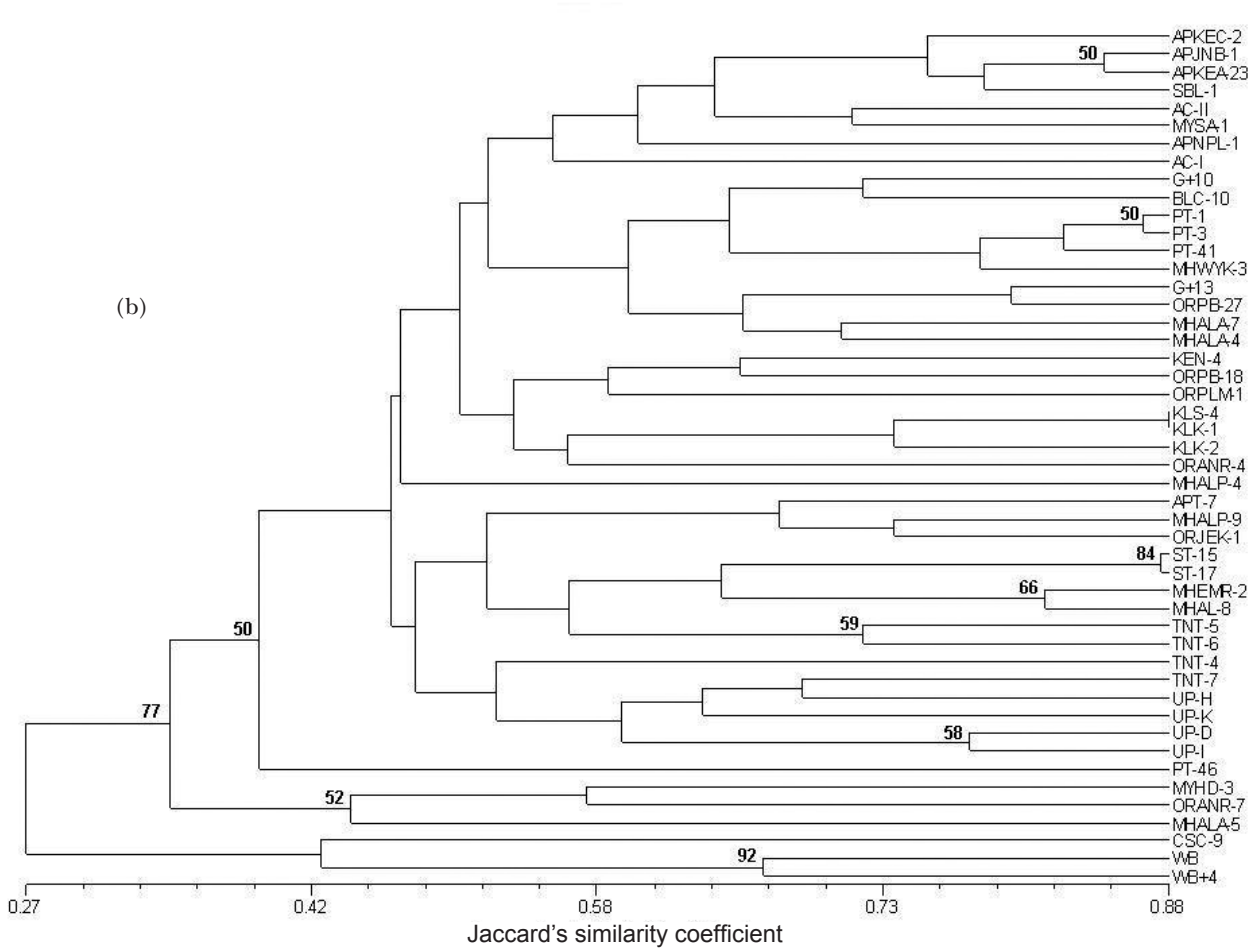


Figure 3 UPGMA dendrograms showing relationships among teak (*Tectona grandis*) plus trees: (a) RAPD and (b) ISSR data. Numbers denoted at cluster nodes are bootstrap values (%).

the country. The genetic diversity of plus trees may be effectively employed for making choices for selection of parental material for breeding programme aimed at improving productivity and wood quality of teak.

ACKNOWLEDGEMENTS

The authors are thankful to the Department of Biotechnology, Ministry of Science and Technology, India for funds to carry out the research work and FDCKM, Chandrapur for permission to collect teak plus tree samples for molecular analysis.

REFERENCES

Ajibade, S. R., Weeden, N. F. & Chite, S. M. 2000. Inter simple sequence repeat analysis of genetic relationships in the genus *Vigna Euphytica* 111: 47–55.

Albani, M. C., Battey, N. H. & Wilkinson, M. J. 2004. The development of ISSR-derived SCAR markers around the seasonal flowering locus (SFL) in *Fragaria vesca*. *Theoretical and Applied Genetics* 109: 571–579.

Archak, S., Gaikwad, A. B., Gautam, D., Rao, E. V., Swamy, K. R. & Karihaloo, J. L. 2003. Comparative assessment of DNA fingerprinting techniques (RAPD, ISSR and AFLP) for genetic analysis of cashew (*Anacardium occidentale* L.) accessions of India. *Genome* 46: 362–369.

Awasthi, A. K., Nagaraja, G. M., Naik, G. V., Kanginakudru, S., Thangavelu, K. & Nagaraju, J. 2004. Genetic diversity and relationships in mulberry (genus *Morus*) as revealed by RAPD and ISSR marker assays. *BMC Genetics* 5: 1. Doi:10.1186/1471-2156-5-1.

Doyle, J. J. & Doyle, J. L. 1990. Isolation of plant DNA from fresh tissue. *Focus* 12: 13–15.

Dwivedi, S. L., Gurtu, S., Chandra, S., Yuejin, W. & Nigam, S. N. 2001. Assessment of genetic diversity among selected groundnut germplasm. *Plant Breeding* 120: 345–349.

Goto, S., Ieiri, R. & Miyahara, F. 1999. RAPD analysis of cutting cultivars and plus tree clones of sugi (*Cryptomeria japonica* D. Don) in Fukuoka Prefecture. *Journal of Japanese Forest Society* 81: 187–193.

Jaccard P. 1908. Nouvelles reserches sur la distribution florale. *Bulletin Societe Vaudoise Des Sciences Naturelles* 44 : 223–270.

Karp, A., Issar, P. G. & Ingram, D. S. 1998. *Molecular Tools for Screening Biodiversity.* Chapman and Hall, London.

Mandal, A. K. & Rambabu, N. 2001. Quantitative genetic aspects of teak improvement. Pp. 135–145 in Mandal, A. K. & Ansari, S. A. (Eds.) *Genetics and Silviculture of Teak.* International Book Distributors, Dehra Dun.

Mantel, N. 1967. The detection of disease clustering and a generalized regression approach. *Cancer Research* 27: 209–220.

McGregor, C. E., Lambert, C. A., Greyling, M. M., Louw, J. H. & Warnich, L. 2000. A comparative assessment of DNA fingerprinting techniques (RAPD, ISSR, AFLP and SSR) in tetraploid potato (*Solanum tuberosam* L.) germplasm. *Euphytica* 113: 135–144.

Moreno, S., Martin, J. & Ortiz, J. 1998. Inter simple sequence repeats PCR for characterization of closely related grapevine germplasm. *Euphytica* 101: 117–125.

Narayanan, C., Mandal, A. K. & Ansari, S. A. 2007. Molecular markers and their applications in forest trees. Pp. 31–56 in Trivedi, P. C. (Ed.) *Biotechnology Current Perspectives and Potential Applications.* Aavishkar Publishers & Distributors, Jaipur.

Nkongolo, K. K., Michael, P. & Demers, T. 2005. Application of ISSR, RAPD, and cytological markers to the certification of *Picea mariana, P. glauca,* and *P. engelmannii* trees, and their putative hybrids. *Genome* 48: 302–311.

Pollegioni, P., Major, A., Bartoli, S., Ducci, F., Proietti, R. & Malvolti, M. E. 2005. Application of microsatellite and dominant molecular markers for the discrimination of species and interspecific hybrids in genus *Juglans. Acta Horticulturae* 705: 191–197.

Roy, A., Frascaria, N., MacKay, J. & Bousquet, J. 1992. Segregating random amplified polymorphic DNAs (RAPDs) in *Betula alleghaniensis. Theoretical and Applied Genetics* 85: 173–180.

Sneath, P. H. A. & Sokal, R. R. 1973. *Numerical Taxonomy.* W. H. Freeman, San Fransisco.

Souframanien, J. & Gopalakrishna, T. 2004. A comparative analysis of genetic diversity in blackgram genotypes using RAPD and ISSR markers. *Theoretical and Applied Genetics* 109: 1687–1693.

Takata, K. & Shiraishi, S. 1996. Discrimination of Japanese cedar (*Cryptomeria japonica* D. Don) cutting cultivars in Kyushu region with RAPD markers. *Bulletin of Kyushu University of Forestry* 75: 1–14.

Tessier, C., David, J., This, P., Boursiquot, J. M. & Charrier, A. 1999. Optimization of the choice of molecular markers for varietal identification in *Vitis vinifera. Theoretical and Applied Genetics* 98: 171–177.

Watanabe, A., Widyatmoko, A., Rimbawanto, A. & Shiraishi, S. 2004. Discrimination of teak (*Tectona grandis*) plus trees using selected random amplified polymorphic DNA (RAPD) markers. *Journal of Tropical Forest Science* 16(1): 17–24.

Wilhelmina, T. G. V. & McNicol, R. J. 1995. The use of RAPD markers for the identification of Sitka spruce (*Picea sitchensis*) clones. *Heredity* 75: 126–132.

Williams, J. G. K., Kubelik, A. R., Livak, K. J., Rafalski, J. A. & Tingey, S. V. 1990. DNA polymorphims, amplified by arbitrary primers are useful on genetic markers. *Nucleic Acids Research* 18: 6531–6535.

Yan, S., Wei-Hong, Z, Ke-Quan, P. & Ke-Ping, M. 2004. Population genetic structure of a dominant desert tree, *Haloxylon ammodendron* (Amaranthaceae), in the southeast Gurbantunggut desert detected by RAPD and ISSR markers. *Acta Botanica Sinica* 46: 675–681.

Yap, I. V. & Nelson, R. J. 1996. *WINBOOT: A Program for Performing Bootstrap Analysis of Binary Data to Determine the Confidence Limits of UPGMA-Dendrograms.* IRRI Discussion Paper Series No.14. International Rice Research Institute, Manila.

Yee, E., Kidwell, K. K., Sills, G. R. & Lumpkin, T. A. 1999. Diversity among selected *Vigna angularis* (Azuki) accessions on the basis of RAPD and AFLP markers. *Crop Science* 39: 268–275.

Zietkiewicz, E., Rafalski, A. & Labuda, D. 1994. Genome fingerprinting by simple sequence repeat (SSR)-anchored polymerase chain reaction amplification. *Genomics* 20: 176–183.