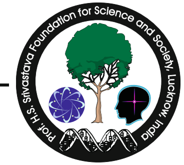
Physiol Mol Biol Plants (July–September 2013) 19(3):389–397
DOI 10.1007/s12298-013-0170-x



RESEARCH ARTICLE

Genetic diversity and population structure of *Butea monosperma* (Lam.) Taub.- a potential medicinal legume tree

Amit Vashishtha · Tabassum Jehan · Suman Lakhanpaul

Published online: 14 March 2013
© Prof. H.S. Srivastava Foundation for Science and Society 2013

Abstract Three molecular marker systems, Random Amplified Polymorphic DNA (RAPD), Inter-Simple Sequence Repeats (ISSR) and Sequence-Related Amplified Polymorphism (SRAP) were employed to investigate the genetic structure and diversity among the 14 natural populations of *Butea monosperma* collected from different geographical regions of India. Detected by 17 RAPD, 15 ISSR and 11 SRAP primer combinations, the proportions of polymorphic bands were 84.2 %, 77.2 % and 91.9 %, respectively, and the mean Nei's genetic distances among the populations were 0.13, 0.10 and 0.13, respectively. Partitioning of genetic variability by Analysis of molecular variance (AMOVA) revealed that the high genetic diversity was distributed within the populations. AMOVA also revealed that the coefficient of gene differentiation among populations based on F_{ST} was very high irrespective of markers used. The overall gene flow among populations (Nm) was very low. Cophenetic correlation coefficients of Nei's distance values and clustering pattern by Mental test were statistically significant for all three marker systems used but poor fit for ISSR data than for RAPD, SRAP and combined data set of all three markers. For all markers, a high similarity in dendrogram topologies was obtained, although some differences were observed with ISSR. The dendrogram obtained by RAPD, SRAP and combined data set of all three markers reflect relationship of most of the populations according to their geographic distribution.

Keywords *Butea monosperma* · Genetic diversity · Genetic structure · Molecular markers · RAPD · ISSR and SRAP

Introduction

Butea monosperma (Lam.) Taubert, a medicinal legume tree, commonly known as 'Flame of forest' or Palas, belongs to the family Fabaceae. It is distributed in greater part of India, Himalayas up to 900 m and in peninsular India up to 1,200 m height. It is a multipurpose tree species that grows naturally in the Indian subcontinent (Mitra 1988). Its medicinal properties are enshrined in ancient Indian scriptures with almost each part of the plant namely roots, stem, bark, leaves, flowers, fruits, seeds and gum are used in Ayurvedic and Unani medicine (Mazumder et al. 2011). Recently, number of studies on biological activities, pharmacological actions of *Butea* compounds and extract, clinical study and plausible medicinal applications are supporting important medicinal or therapeutic properties of *B. monosperma*. Bioactive compounds and extract isolated from various parts of *B. monosperma* showed potential hepatoprotective, anti-tumorigenic properties especially in breast, hepatic carcinogenesis, anti-osteoporotic, thyroid inhibitory, anti-inflamatory, anti-diabetic, anti-fungal, antioxidant, anti-diarrhoeal, dermal wound healing, anti-viral, anti-microbial anti-convulsive, and anthelmintic activities (Burli and Khade 2007; Mazumder et al. 2011; Pal and Bose 2011). Moreover, the flowers of *B. monosperma* are boiled in water to extract natural orange or yellow dye, which is eco-friendly and being used as substitute for synthetic dye for coloring garments (Burli and Khade 2007). It also has a long history of use by tribals for lac cultivation in India. Lac, a natural resin secreted by females of *Kerria lacca* (Kerr), is economically important and commercially being used in

A. Vashishtha · T. Jehan · S. Lakhanpaul (✉)
Department of Botany, University of Delhi, Delhi 110007, India
e-mail: sumanlp2001@yahoo.com

various industries (Sharma and Ramani 1999). Therefore, an understanding of the level of genetic diversity; ascertaining the relationships and genetic structure among the populations of *B. monosperma* is extremely important for genetic improvement and conservation. No detailed study is available regarding genetic structure using different molecular markers among *B. monosperma* populations.

A number of different approaches namely, morphological, biochemical and molecular markers are applied for the assessment of genetic diversity. Conventionally, morphological characters like growth habit, leaf type, floral morphology and fruit characters; and biochemical markers such as isozymes (Schnabel and Hamrick 1990) and allozymes (Hamrick and Godt 1989) have been used for identification and study of genetic relationships among the tree species. The advantages of isozyme and allozyme studies include low cost, ability to detect heterozygous individuals and reveal functional polymorphism. However, the targeted region of genome is small and represents underestimation of genetic diversity (Storfar 1996). Moreover, in case of forest tree species, the major disadvantage of enzyme based markers are the difficulties encountered for keeping the sampled material fresh, while transporting it from the natural forest to the laboratory. Instead, several types of molecular markers have conquered such disadvantages and thus, have been successfully developed and utilized variously in the last two decades, *viz.* Restriction Fragment Length Polymorphisms (RFLP), a non-PCR based technique and PCR-based techniques, such as Simple Sequence Repeats (SSR), Random Amplified Polymorphic DNA (RAPD), Inter-Simple Sequence Repeats (ISSR), Amplified Fragment Length Polymorphisms (AFLP), Sequence-Tagged Sites (STS), Expressed Sequence Tag (EST), Sequence Characterized Amplified Region (SCAR), Diversity Array Technology (DArT) and Sequence-Related Amplified Polymorphism (SRAP) (Semagn et al. 2006; Li and Quiros 2001)

Since 1990s, RAPD and ISSR markers have been perhaps the most frequently used markers for assessing genetic diversity in trees (Song and Bao 2006; Juchum et al. 2007; Xue et al. 2007). Li and Quiros (2001) developed and tested the Sequence-Related Amplified Polymorphism (SRAP) marker technique in a series of recombinant inbred and doubled-haploid lines of *Brassica oleracea* L. SRAP aims at the amplification of open reading frames (ORFs) and is based on two-primer amplification. Therefore, these PCR-based techniques of random multi-locus analysis (RAPD, ISSR and SRAP) were selected for the present study; firstly, because no prior sequence information is available in *B. monosperma* and secondly, each marker targets different parts of the genome.

In the present study, RAPD, ISSR and SRAP markers were used to investigate the genetic structure, relationship, extent and distribution of genetic diversity among the 14 natural populations of *Butea monosperma* collected from different regions of India.

Materials and methods

Plant materials

Young and actively growing leaves from 14 different populations of *Butea monosperma* were collected from different regions of India (Table 1). One population of *Butea superba* collected from Aurangabad was included in the analysis as outgroup. The harvested leaf material was immediately frozen in liquid nitrogen and transferred to - 20 °C till further use.

DNA isolation and quantification

Genomic DNA from young leaves of *B. monosperma* and *B. superba* was isolated using the CTAB extraction procedure developed by Saghai-Maroof et al. (1984) with minor modifications. The DNA was quantified using bisbenzimide (Hoechst Dye-H33258) by fluorometer (Amersham Biosciences, USA).

RAPD, ISSR and SRAP analysis

Seventeen random decamer primers [OPE5, OPE7, OPE8, OPE9, OPE11, OPE12, OPE14, OPE17, OPE18, OPE19, OPB1, OPB4, OPB10, OPB11, OPB17, OPB18, OPB20 (Operon Technologies, U.S.A.)] were selected from 50 tested primers, based on the reproducible and polymorphic banding pattern. Each PCR amplification reaction contained 200 μM each of dATP, dCTP, dGTP and dTTP; 3.0 mM of $MgCl_2$; 1 Unit of Taq DNA polymerase (Bangalore Genei, Bangalore); 10 ng of genomic DNA; 10 mM of PCR buffer and 0.4 μM of 10- mer random primer (Operon Technologies Inc.) in a volume of 25 μl. Amplification was carried out in a thermocycler (PTC100, MJ Research) using the following programme: 4 min at 94 °C for 1 cycle; for 39 cycles 1 min at 94 °C, 1 min at 35 °C, 2 min at 72 °C and 1 cycle of 5 min at 72 °C.

Fifteen ISSR primers [$(AC)_8C$, $(AC)_8G$, $(AC)_8T$, $(TG)_8G$, $(AG)_8YA$, $(GA)_8YT$, $(GA)_8YC$, $(TC)_8RT$, $(AC)_8RT$, $(AC)_8YT$, $(TG)_8RC$, $(TG)_8RC$, $(TG)_8RA$, $(CTC)_6$, $(GACA)_4$] were selected for amplification. ISSR-PCR reaction mixture contained 200 μM each of dATP, dCTP, dGTP and dTTP; 3.0 mM of $MgCl_2$; 1 Unit of Taq DNA polymerase; 15 ng of genomic DNA; 10 mM of PCR buffer and 0.4 μM of ISSR primer in a volume of 25 μl. Initial denaturation was for 4 min at 94 °C for 1 cycle; followed by 30 s at 94 °C, 45 s at 35 °C, 1.30 min at 72 °C for 39 cycles and a 5 min final extension at 72 °C.

Table 1 Populations of *Butea monosperma* sampled from different geographical regions of India

S.No	Site of collection	Site code	Species	Latitude (°N)	Longitude (°E)
1.	Indian Institute of Natural Resin and Gum, Ranchi, Jharkhand	BMR	*Butea monosperma*	23.23	85.23
2.	Shivaji University Campus, Kolhapur, Maharashtra	BMK1; BMK2 BMK3; BMK4	*Butea monosperma*	16.42	74.16
3.	Gautala Autram Ghat Wildlife Sanctuary, Hiverkheda, Aurangabad, Maharashtra	BMA; BSA	*Butea monosperma* *Butea superba*	19.53	75.23
4.	Kumbhalgarh Wildlife Sanctuary, Udaipur, Rajasthan	BMU1; BMU2	*Butea monosperma*	25.12	74.32
5.	Coimbatore, Tamil Nadu	BMC1; BMC2 BMC3	*Butea monosperma*	11.00	77.00
6.	Railway Station Colony, Jhansi, U.P.	BMJ	*Butea monosperma*	25.27	78.37
7.	Bhatti Mines, Delhi	BMD	*Butea monosperma*	28.38	77.12
8.	Ladwa, Haryana	BMH	*Butea monosperma*	29.59	77.05

Eleven SRAP primer pairs gave good reproducible and polymorphic patterns and therefore, selected from 25 combinations of five forward (Me1, Me2, Me3, Me4 and Me5) and five reverse (Em1, Em2, Em3, Em4 and Em5) primers for PCR amplification (Li and Quiros 2001). The final 25 μl reaction mix contained 200 μM each of dATP, dCTP, dGTP and dTTP; 3.0 mM of $MgCl_2$; 1 Unit of Taq DNA polymerase; 20 ng of genomic DNA; 10 mM of PCR buffer, 0.3 μM of forward primers and 0.3 μM of reverse primers. DNA amplification was carried out with an initial step at 94 °C for 4 min and five cycles of 1 min at 94 °C, 1 min at 38.5 °C, 2 min at 72 °C; followed 35 cycles of 1 min at 94 °C, 1 min at 49 °C, 2 min at 72 °C with a final extension at 72 °C for 10 min.

PCR products, thus obtained were mixed with 2.5 μl of 10X loading dye and spun in a microcentrifuge before loading. The amplification products were resolved on 1.8 % agarose gel (w/v) and stained with ethidium bromide (0.5 μg/ml) and photographed under UV light for RAPD, ISSR and SRAP.

Data analysis

Molecular weight of the bands was estimated by using 100 bp DNA ladder (100 bp Generular Plus, MBI Fermentas) as standard. Each amplification product (band) was scored manually for presence (1) or absence (0) across all the lanes. To compare the efficiency of the three markers (RAPD, ISSR and SRAP), we estimated the following for each assay unit (U) (the product of PCR amplification obtained with one set of primers) used: Number of Assay units (U); Number of polymorphic bands (n_p); Number of monomorphic bands (n_m); Percentage Polymorphism ($\%_P$); Average number of polymorphic bands per assay unit (n_p/U); Number of loci (L): The theoretical maximum number of loci is equal to total number of bands (n_p+n_m) obtained for each marker type; Number of loci per assay unit: n_u=L/U and Average Nei's genetic distance.

The two-way data matrix was calculated by pair-wise genetic distance values (Nei and Li 1979). This matrix was subjected to Unweighted Pair Group Method with Arithmetic Averages (UPGMA) (Sneath and Sokal 1973) procedure of SAHN (sequential agglomerative hierarchical nested clustering), clustering to generate a dendrogram using average linkage procedure. For principal component analysis (PCA), first three components extracted were plotted in three possible combinations to produce the biplots. The level of correlation between taxonomic distance and cophenetic matrices derived from the different data sets were determined using Mantel test (Mantel 1967). All the numerical taxonomic analysis was conducted using the computer program NTSYS-pc, version 1.7 (Rohlf 1992). Bootstrap analysis was done with 1,000 replications using FREE TREE.

Wright's *F*-statistic (fixation index) was computed using the ARLEQUIN version 3.01. An estimate of gene flow among populations (*N*m) was calculated using Wright's (1951) formula, $Nm=(1–F_{st})/4F_{st}$. The Analysis of Molecular Variance procedure was used (AMOVA; Excoffier et al. 1992) to describe genetic structure and variability among *B. monosperma* populations, where the variation component partitioned among groups, among populations within groups and within populations. In order to carry out this analysis, 14 populations of *B. monosperma* were placed in three groups depending upon their geographical distribution. Significance levels for variance-component estimates were computed by permutational procedures (500 permutations). The AMOVA was performed using ARLEQUIN version 3.01 (Schneider et al. 2000).

Results

All the three markers, namely RAPD, ISSR and SRAP showed high polymorphism and were effective in

discriminating *B. monosperma* populations. The results obtained are recapitulated in Table 2. Specific numbers of bands were obtained for each primer ranging from four to ten, giving a total of 114, 92 and 62 amplification products ranging from 200 bp to 2,000 bp with an average of 6.7, 6.1 and 5.6 bands per primer of RAPD, ISSR and SRAP, respectively (Table 2). The percentage of polymorphism was maximum (91.9 %) for SRAP while 84.2 and 77.2 for RAPD and ISSR, respectively.

A wide range of values of Nei's genetic distance (0.008–0.207), (0.0–0.22) and (0.016–0.29) with an average value 0.13, 0.10 and 0.13 was obtained among the 14 *B. monosperma* populations by RAPD, ISSR and SRAP, respectively. The populations BMC3- BMC2 and BMK4- BMK1 and BMK4-BMK2 were found to be closest with genetic distance 0.008 and 0.016 while populations BMC3-BMU2 BMC3-BMJ and BMH-BMC3 were observed to be the most divergent with genetic distance of 0.207, 0.22 and 0.29 by RAPD, ISSR and SRAP, respectively. BMK3 and BMK4 populations were closest by ISSR data.

The UPGMA dendrogram based on RAPD data denoted three principle clusters: I, II and III (Fig. 1a). Cluster I comprised of six populations, which could be divided into two sub-clusters IA (BMH, BMJ and BMD) and IB (BMU1, BMU2 and BMR). The cluster II comprised of five populations (BMK1, BMK2, BMK3, BMK4 and BMA). Third cluster included three populations (BMC1, BMC2 and BMC3). The cluster IA, IB and cluster I, II were supported with bootstrap value 34 and 82, respectively. On the other hand, the cluster III was supported with other two clusters with high bootstrap value of 100 (Fig. 1a).

Table 2 Levels of polymorphism and comparison of discriminating power of RAPD, ISSR and SRAP markers in *Butea monosperma* populations

S. No.	Indexes with their abbreviations	Marker		
		RAPD	ISSR	SRAP
1	Number of assay units (U)	17	15	11
2	Number of polymorphic bands (n_p)	96	71	57
3	Number of monomorphic bands (n_m)	18	21	5
4	Percentage polymorphism (%P)	84.2	77.2	91.9
5	Average number of polymorphic bands per assay unit (n_p/U)	5.6	4.7	5.2
6	Number of loci (L): The theoretical maximum number of loci is equal to total number of bands (n_p+n_m) obtained for each marker type.	114	92	62
7	Number of loci per assay unit: n_u=L/U	6.7	6.1	5.6
8	Average Nei's genetic distance	0.13	0.10	0.13

The UPGMA dendrogram based on ISSR data denoted three principle clusters: I, II and III (Fig. 1b). Cluster I comprised of six populations, which could be grouped in two subclusters IA (BMK1, BMK2, BMK3, and BMK4) and IB (BMC1, BMC2). The cluster II comprised of five populations (BMH, BMJ, BMA and BMU2). Third cluster comprised of three populations (BMR, BMU1 BMD) and BMC3 was as outlier. The cluster IA and IB, cluster I and II and cluster II and III were supported with low bootstrap values of 37, 12 and 19, respectively (Fig. 1b).

The UPGMA dendrogram based on SRAP data depicted three principle clusters: I, II and III (Fig. 1c). Cluster I comprised of seven populations, which could be grouped in two subclusters IA (BMK1, BMK2, BMK3, and BMK4) and IB (BMD, BMH and BMJ). The cluster II comprised of four populations, IIA (BMA and BMR) and IIB (BMU1 and BMU2). Third cluster comprised of three populations (BMC1, BMC2 and BMC3). The cluster IA and IB and cluster I and II were supported with low bootstrap values 24 and 38, respectively. On the other hand, the cluster III was supported with other two clusters with high bootstrap value of 100 (Fig. 1c).

All populations were clustered according to their geographical regions in dendrogram obtained by the combined data of three sets of molecular markers and showed similar topology with dendrograms obtained by RAPD and SRAP data with some differences only with Aurangabad population (BMA). In dendrogarms obtained by RAPD data BMA was grouped within the four BMK populations while in dendrogram obtained by combined data set, all BMK populations were grouped together (Fig. 1d) along with BMA population in the same cluster. On the other hand, BMA population was grouped with BMR population in dendrogram obtained by SRAP data.

The PCA result was comparable to the cluster analysis of RAPD, ISSR, SRAP (Figure not shown) and combined data of three sets of molecular markers (Fig. 2). The first few principal components (PCs) explained nearly half of the total variation observed and eight PCs were needed to retain more than 90 % of the original variance for RAPD while first two PCs retained 80 % of the original variance and four PCs were needed to retain more than 90 % of the original variance for ISSR and SRAP. On the other hand, 13 PCs were needed to retain the total variance.

Partitioning of genetic variability by analysis of molecular variance (AMOVA) revealed the pattern in which total diversity was distributed 12.84 %, 22.04 %, 32.21 % (among groups), 39.72 %, 24.34 %, 20.48 % (among populations within groups) and 47.44 %, 53.63 %, 47.30 % (within populations) for RAPD, ISSR and SRAP, respectively (Table 3). Accordingly, F-statistics values were as 0.45, 0.31and 0.30 (F_{IS}), 0.52, 0.46 and 0.52 (F_{ST}) and 0.12, 0.22 and 0.32 (F_{IT}) for RAPD, ISSR and SRAP,

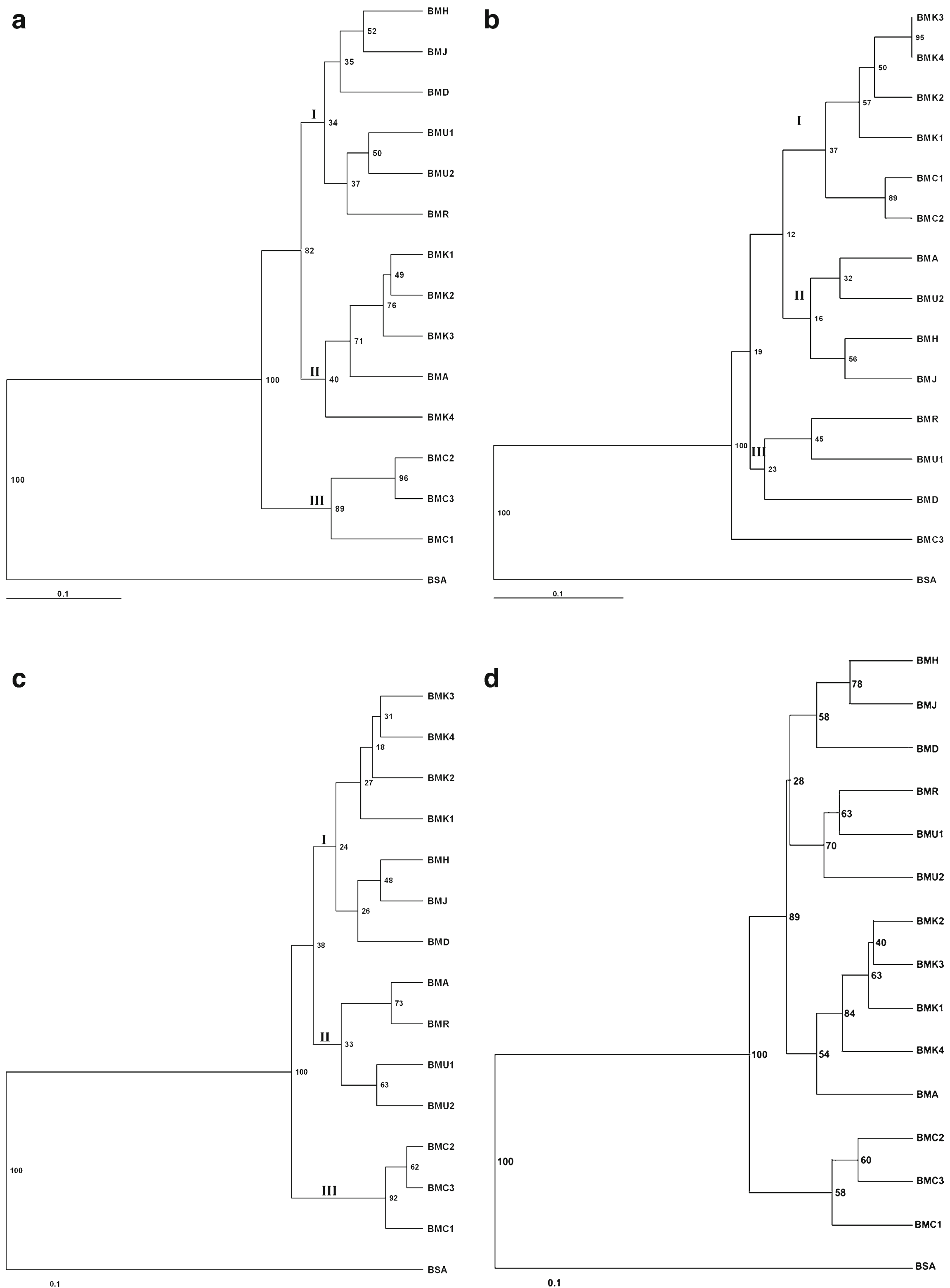


Fig. 1 UPGMA dendrogram based on Nei's genetic distance using (**a**) RAPD data (**b**) ISSR data (**c**) SRAP data and (**d**) Combined data of three markers analysis of 14 populations of *Butea monosperma* and one population of *Butea superba*. Bootstrap values are shown at nodes

respectively (Fig. 3). The overall gene flow among populations (*N*m) was found very low at 0.231, 0.293 and 0.231 by RAPD, ISSR and SRAP data, respectively (Fig. 3).

Mantel matrix correspondence test was used to test the correlation between the clustering pattern and similarity coefficient values obtained with each marker and combined

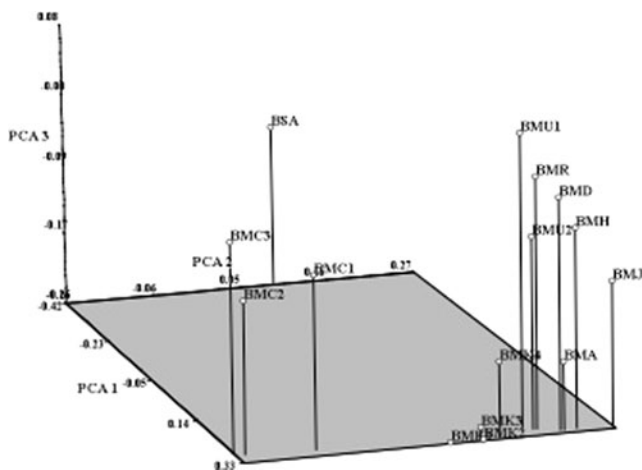


Fig. 2 PCA based on Nei's genetic distance matrix of combined data of three markers of 14 populations of *Butea monosperma*

data set and also to compare the patterns obtained with each marker (Table 4). Cophenetic correlation coefficient was statistically significant for RAPD, SRAP and combined data set (r=0.89, 0.81 and 0.88, respectively) except ISSR data (r=0.78, nearly good fit) (Table 4).

Discussion

In order to understand the extent of genetic diversity and differentiation among *B. monosperma* populations occurring in different geographical regions of India, three different PCR-based marker systems, namely, RAPD, ISSR and SRAP were used. Usefulness of these markers in detecting and characterizing genetic variations has already been demonstrated at inter- and intraspecific level in a large number of taxa (Li and Quiros 2001; Pither et al. 2003; Juchum et al. 2007; Xue et al. 2007).

The level of polymorphism by RAPD (84.2 %), ISSR (77.2 %) and SRAP (91.9 %) among the populations of *B. monosperma* studied in the present investigations corroborates with the results of Vaishali et al. (2008) where 86 % polymorphism was reported among *B. monosperma* populations collected from agro- ecological regions of India using RAPD markers. Likewise, Lacerda et al. (2001) detected 70.8 % polymorphism in *Plathymenia reticulate* using RAPD analysis and Aparajita et al. (2008) observed 95.05 % and 96.02 % polymorphism in *Albizzia* sp. by RAPD and ISSR markers, respectively; corroborating our results. On the other hand, it was considerably higher than the variation observed in previous RAPD and ISSR studies of other tropical legume trees and shrubs. Casiva et al. (2002) reported an average of 19.1 % polymorphism across populations of Argentinian *Acacia* species, Juarez-Munoz et al. (2002) found an average of 28.3 % polymorphism in

Table 3 Analysis of molecular variance (AMOVA) of RAPD, ISSR and SRAP amplicons for14 populations of *Butea monosperma*

Source of variation	RAPD				ISSR				SRAP			
	d.f	Sum of square	Variance components	Percentage of variation	d.f	Sum of square	Variance components	Percentage of variation	d.f	Sum of square	Variance components	Percentage of variation
Among groups	2	43.65	1.24 Va	12.84	2	28.75	1.43 Va	22.04	2	21.82	1.44 Va	32.21
Among populations within groups	2	25.55	3.84 Vb	39.72	2	13.75	1.58 Vb	24.34	2	8.15	0.91 Vb	20.48
Within populations	9	41.36	4.59 Vc	47.44	9	31.5	3.50 Vc	53.63	9	19.1	2.12 Vc	47.3
Total	13	110.57	9.68		13	74	6.52		13	49.07	4.48	

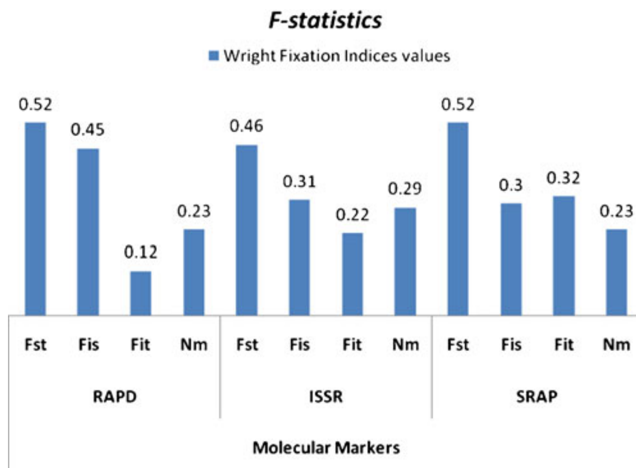


Fig. 3 Wright fixation indices or *F*-statistics subdivision: F_{IS}, F_{ST} (subpopulations), F_{IT} (total population) and gene flow (*N*m) among 14 *Butea monosperma* populations

Prosopis species, Juchum et al. (2007) reported 39 % polymorphism in *Dalbergia nigra* and Schierenbeck et al. (1997) quantified 43.3 % polymorphism in *Inga thibaudiana* employing RAPD assay. No such report was available in the literature for the evaluation of genetic diversity of legume trees using SRAP markers.

The average number of polymorphic fragments scored per primer was 5 and 5.1 in *B. monosperma* using RAPD and ISSR, respectively; which is comparable to other legume trees such as in *Flemingia macrophylla* (Heider et al. 2007), *Plathymenia reticulate* (Lacerda et al. 2001). However, a higher number of fragments was observed in *Albizzia* sp. using RAPD (10.9) and ISSR (11.9) marker (Aparajita et al. 2008). Similar comparison with other woody perennials could not be done due to lack of such reports in case of SRAP marker. Therefore, a comparison with studies employing SRAP on other plants was made. An average number of 8.4 bands per SRAP primer combination was low, compared to previous studies by Li and Quiros (2001) in *Brassica oleracea* L, Budak et al. (2004) in buffalo grass [*Buchloe dactyloides* (Nutt.) Englem.], Ferriol et al. (2004) in *Cucurbita moschata* [(Duchesne ex Lam.) Duchesne ex Poir] and Espósito et al. (2007) in pea where an average of 10–23 bands per primer combination was observed.

Table 4 Cophenetic correlation coefficient comparison of similarity matrices and cluster pattern from different dataset obtained using RAPD, ISSR, SRAP and combined data by Mantel test

Marker system	RAPD	ISSR	SRAP	Combined data set
RAPD	0.89	0.47	0.62	0.88
ISSR		0.78	0.52	0.8
SRAP			0.81	0.79
Combined data set				0.88

Moreover, high level of genetic variability represented by a large percentage of polymorphic loci was in agreement with the wide range of Nei's genetic distance irrespective of marker system used. Unfortunately, most studies on tropical legume tree and shrub genetic structure have used different diversity measures and thus it is very difficult to compare them directly with each other. Nevertheless, the average genetic similarity as well as the range for the Nei's genetic distance was comparable in case of the three markers used.

Cluster analyses of *B. monosperma* populations employing UPGMA led to grouping of all the populations into distinct clusters. The Mantel's test carried out with each of the markers used separately and also combined data showed a good fit suggesting that the clustering pattern has significant correlation with the similarity values obtained in case of RAPD, SRAP and the combined data. However, in case of ISSR, Mantel test showed marginally poor fit (*r*-value= 0.78), as this was also quite close to 0.8 taken as the cut off value for the fitness. Nevertheless, nearly all the populations could be genetically discriminated from each other in each of the three markers used except two populations from Kolhapur (BMK3 and BMK4), which were not resolved with ISSR markers. In general, populations collected from closer geographic distances showed higher similarity as they grouped together in the dendrograms. However, lack of close clustering of the populations collected from relatively closer areas was observed in case of ISSR markers (Fig. 1b) For example, three populations (BMC1, BMC2 and BMC3) collected from Coimbatore grouped together in RAPD and SRAP analysis (Fig. 1a and c) but BMC3 did not group closer to other two populations from Coimbatore in ISSR based analysis. Similarly, three northern populations BMJ, BMD and BMH were grouped together in RAPD and SRAP analysis but not so in case of ISSR analysis.

Analysis of Molecular Variance (AMOVA) was used to estimate the variance components along with the geographically nested structure of populations revealed that half of the total genetic variation was present within the individuals of populations, irrespective of the markers used. Interestingly, the other half of the total variation has shown variable patterns with three markers used. Andrianoelina et al. (2006) analyzed the genetic variation and related parameters for *D. monticola*, another woody legume and have found similar results as more than 80 % variation was present within the individuals of the populations. Likewise, the extent of genetic variation present among populations within the groups (16 %) was nearly one-third of that present among populations (36 %) (Pither et al. 2003; Awasthi et al. 2004).

F_{ST} values, a very useful indicator for the genetic differentiation, indicated a very high level of genetic differentiation in the populations of *B. monosperma*. Level of differentiation of populations obtained was comparable with three markers used, as its values ranged from 0.46 to 0.52.

Similarly, F_{IS} values (0.30–0.45) are indicating the extent of inbreeding and F_{IT} values (0.12–0.32) are indicating the deficiency of heterozygote in comparison to the total expected levels revealing comparable results.

In general, woody species maintain more variation within species and within populations than species with other life forms but have less variation among populations. Hamrick et al. (1991) identified a combination of species characteristics including breeding system, seed dispersal, life form and geographic distribution range as decisive factors for the level of genetic variation and its partitioning among and within populations. Of these factors, breeding biology had the most profound effect on the genetic structure of plant populations (Hamrick and Godt 1989). In general, species with large geographic range, out crossing breeding system, wind and animal- ingested seed dispersal have more genetic diversity within species and populations and less variation among populations. Therefore, specific evolutionary history of a species has been suggested to play an important role in determining the level and distribution of genetic diversity (Hamrick et al. 1991; Qiu and Parks 1994)

A substantial genetic differentiation observed among populations of *B. monosperma* may be partly due to a low level of gene flow. The migration estimate was low ($Nm<1$) irrespective of the marker used, indicating a very low level of gene exchange among populations. A low level or no gene flow among populations generally leads to genetic heterogeneity among populations (Wright 1943; Kimura and Weiss 1964) as observed here in *B. monosperma*. The geographic distance and different ecological environments in the habitats of eight collection sites for *B. monosperma* populations may be the possible explanation for low level of gene flow. Moreover, flowers of *B. monosperma* possess abundant nectar and are cross-pollinated by birds or squirrels. Squirrel-pollinated flowers show geitonogamy, as they have a narrow territorial range and visit mostly flowers on the same tree. Although geitnogamy is functionally cross-pollination involving a pollinating agent but genetically it is similar to autogamy since the pollen grains come from the same plant. Tandon et al. (2003) have reported weak self-incompatibility in *B. monosperma*. Lloyd (1992) also observed that a weak form of self-incompatibility provides flexibility under which cross-pollination cannot always be guaranteed. Therefore, the extent of genetic differentiation revealed in the present study is also indicative of a mixed mating system.

Further, different patterns of partitioning of genetic variation in the same set of individuals analyzed with different markers have been reported earlier (Aparajita et al. 2008; Wang et al. 2008). Adaptive and phenological features show higher variation among populations, than within the population (Medri et al. 2003). In other words, adaptive traits or the genomic regions controlling the adaptive traits, are likely to exhibit the results of selection pressures prevailing in that particular geographic region, having different ecological conditions thus, experiencing local selection pressures. Diversity in the neutral markers is more likely to reflect the history of the population such as migration, genetic shifts etc.

It is noteworthy that the vast array of marker systems available these days may or may not yield comparable results as the different marker systems target different genomic fractions undergoing entirely different processes in terms of their rate as well as kind. Use of more than one marker systems to ascertain the evolutionary processes acting differently on different loci is highlighted in the present study, particularly at the higher hierarchical levels. In other words, though the partitioning of variation in the individuals within the populations show similar pattern irrespective of the markers used, more than one marker systems targeting qualitatively different genomic regions are required to reveal a clearer understanding of the plant genomes and their evolutions.

Acknowledgements Authors are highly thankful and gratefully acknowledge Dr. KV Bhat, Principal Scientist, National Bureau of Plant Genetic Resources (NBPGR) New Delhi for helping in data analysis and to Department of Biotechnology, New Delhi (India) for financial support to carry out the present study.

References

Andrianoelina O, Rakotondraoelina H, Ramamonjisoa L, Maley J, Danthu P, Bouvet JM (2006) Genetic diversity of *Dalbergia monticola* (Fabaceae) an endangered tree species in the fragmented oriental forest of Madagascar. Biodivers Conserv 15:1109–1128

Aparajita S, Senapati SK, Rout GR (2008) Identification and genetic relationships among nine *Albizzia* species based on morphological and molecular markers. Plant Biosyst 142:30–39

Awasthi AK, Nagaraja GM, Naik GV, Kanginakudru S, Thangavelu K, Nagaraju J (2004) Genetic diversity and relationships in mulberry (genus *Morus*) as revealed by RAPD and ISSR marker assays. BMC Genet 5:1–9

Budak HRC, Shearman I, Parmaksiz RE, Gaussoin TP, Riosdan DI (2004) Molecular characterization of Buffalograss germplasm using sequence-related amplified polymorphism markers. Theor Appl Genet 108:328–334

Burli DA, Khade AB (2007) A comprehensive review on *Butea monosperma* (Lam.) Kuntze. Pharmacogn Rev 2:333–337

Casiva PV, Saidman BO, Vilardi JC, Cialdella AM (2002) First comparative phenetic studies of Argentinean species of *Acacia* (Fabaceae), using morphometric, isozymal, and RAPD approaches. Am J Bot 89:843–853

EspÓsito MA, Martin EA, Cravero VP, Cointry E (2007) Characterization of pea accessions by SRAP's markers. Sci Hortic-Amsterdam 113:329–335

Excoffier L, Smouse PE, Quattro JM (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes: Application to human mitochondrial DNA data. Genetics 131:479–491

Ferriol M, Picó B, Fernández de Córdova P, Nuez F (2004) Molecular diversity of a germplasm collection of squash (*cucurbita moschata*) determined by SRAP and AFLP markers. Crop Sci 44:653–664

Hamrick JL, Godt MJW (1989) Allozyme diversity in plant species. In: Brown AHD, Clegg MT, Kahler AL, Weir BS (eds) Plant population genetics, breeding, and genetic resources. Sinauer, Massachusetts, pp 43–63

Hamrick JL, Godt MJW, Murawski DA, Loveless MD (1991) Correlations between species traits and allozyme diversity: implications for conservation biology. In: Falk D, Holsinger K (eds) Genetics and conservation of rare plants. Oxford Press, London, pp 75–86

Heider B, Andersson MS, Schultze-Kraft R (2007) RAPD variation among North Vietnamese *Flemingia macrophylla* (Willd.) Kuntze ex Merr. Accessions. Biodivers Conserv 16:1617–1631

Juarez-Munoz J, Carrillo-Castañeda G, Arreguín R, Rubluo A (2002) Inter and intragenetic variation of four wild populations of *Prosopis* using RAPD-PCR fingerprints. Biodivers Conserv 11:921–930

Juchum FS, Leal JB, Santos LM, Almeida MP, Ahnert D, Corrêa RX (2007) Evaluation of genetic diversity in a natural rosewood population (*Dalbergia nigra* Vell. Allemão ex Benth.) using RAPD markers. Genet Mol Res 6:543–553

Kimura M, Weiss GH (1964) The stepping-stone model of population structure and the decrease of genetic correlation with distance. Genetics 49:561–576

Lacerda DR, Acedo MDP, Lemos Filho JP, Lovato MB (2001) Genetic diversity and structure of natural populations of *Plathymeni reticulate* (Mimosoideae), a tropical tree from the Brazilian Cerrado. Mol Ecol 10:1143–1152

Li G, Quiros CF (2001) Sequence-related amplified polymorphism (SRAP), a new marker system based on a simple PCR reaction: its application to mapping and gene tagging in *Brassica*. Theor Appl Genet 103:455–461

Lloyd DG (1992) Self- and cross-fertilization in plants. II. The selection of self-fertilization. Int J Plant Sci 153:370–380

Mantel N (1967) The detection of disease clustering and a generalized regression approach. Cancer Res 27:209–220

Mazumder PM, Das MK, Das S, Das S (2011) *Butea monosperma* (Lam) Kuntze-A comprehensive review. Int J Pha Sci Nanotech 4(2):1390–1393

Medri C, Ruas PM, Higa AR, Murakami M, De Fátima RC (2003) Effects of forest management on the genetic diversity in a population of *Araucaria angustifolia* (bert.) O. Kuntze. Silvae Genet 52:202–205

Mitra AP (1988) The wealth of India: a dictionary of Indian raw materials & industrial products: raw materials, vol. 2: B. Publications & Information Directorate (CSIR), New Delhi

Nei M, Li WH (1979) Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc Natl Acad Sci USA 76:5269–5273

Pal P, Bose S (2011) Phytopharmacological and Phytochemical Reviews of *Butea monosperma*. Int J Pha Biomed Sci 2(3):1374–1388

Pither R, Shore JS, Kellman M (2003) Genetic diversity of the tropical tree *Terminalia amazonia* (Combretaceae) in naturally fragmented populations. Heredity 91:307–313

Qiu YL, Parks CR (1994) Disparity of allozyme variation levels in three *Mangnolia* (Mangnoliaceae) species from the southeastern United States. Am J Bot 81:1300–1308

Rohlf FJ (1992) NTSYS-pc. Numerical taxonomy and multivariate analysis system, version 1.7. Exeter Software, New York

Saghai-Maroof MA, Soliman KM, Jorgensen RA, Allard RW (1984) Ribosomal DNA spacer-length polymorphisms in barley: Mendelian inheritance, chromosomal location and population dynamics. Proc Natl Acad Sci USA 81:8014–8018

Schierenbeck KA, Skupski M, Lieberman D, Lieberman M (1997) Population structure and genetic diversity in four tropical tree species in Costa Rica. Mol Ecol 6:137–144

Schnabel A, Hamrick JL (1990) Nonrandom associations between sex and 6-phosphogluconate dehydrogenase isozyme genotypes in *Gleditsia triacanthos* L. J Hered 81:230–233

Schneider S, Roessli D, Excoffier L (2000) ARLEQUIN, version 2.0: a software for population genetics data analysis. Genetic and Biometry Laboratory, Department of Anthropology, University of Geneva, Switzerland

Semagn S, Bjornstad A, Ndjiondjop MN (2006) An overview of molecular markers methods for plants. Afr J Biotechnol 5(25):2540–2568

Sharma KK, Ramani R (1999) An update on synoptic catalogue of lac insects (Homoptera: Tachardidae). J Bombay Nat Hist Soc 96:438–443

Sneath PHA, Sokal RR (1973) Numerical taxonomy. W.H. Freeman and Company, San Francisco

Song C, Bao M (2006) Genetic diversity of RAPD marker for natural *Davidia involucrata* populations. Front For China 1:95–99

Storfar A (1996) Quantitative genetics: a promising approach for the assessment genetic variation in endangered species. Trends Ecol Evol 11:343–348

Tandon R, Shivanna KR, Mohan Ram HY (2003) Reproductive biology of *Butea monosperma* (Fabaceae). Ann Bot 92:715–723

Vaishali, Khan S, Sharma V (2008) RAPD based assessment of genetic diversity of *Butea monosperma* from different agro-ecological regions of India. Indian J Biotechnol 7:320–327

Wang LL, Ping ZL, Qin GY, Xia WM, Ming CL, Lan YJ, Yan W, Min YF, Zhi WL (2008) DNA fingerprinting and genetic diversity analysis of late-bolting radish cultivars with RAPD, ISSR and SRAP markers. Sci Hortic-Amsterdam 116:240–247

Wright S (1943) Isolation by distance. Genetics 28:114–138

Wright S (1951) The genetical structure of populations. Ann Eug 15:323–354

Xue X, Wang Y, Korpelainen H, Li C (2007) Genetic diversity of *Picea asperata* populations based on RAPDs. Plant Biol 9:101–108