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Genetic diversity analysis of *Butea monosperma* from different agroecological regions of India using ISSR marker system and its comparison with rapd

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Abstract
In the present paper, we report the use of ISSR markers and their comparison with RAPD to analyze the genetic diversity of *Butea monosperma* from different agroecological regions viz. arid, semi-arid, dry sub-humid and moist sub-humid. For this purpose, 26 ISSR primers were tested. Of them 7 reproducible primers yield 58 (83%) polymorphic bands. The average value of Nei's gene diversity and resolving power were 0.71 and 7.47 respectively. The UPGMA based dendrogram clustered all individuals into three distinct clusters with one sample from Bangalore placed outside the major cluster and at one end of the dendrogram. The coefficient of genetic differentiation (G_{ST}) shows the lesser genetic variation among population and within population then from RAPD. The estimated value of correlation coefficient ie 0.84 indicated a good correlation and shows that these two methods are informative and could accurately detect the structure of genetic diversity and its partition within and between groups.

Keywords: *ISSR, RAPD, Butea monosperma, genetic diversity, agroecological regions*

1. Introduction
Butea monosperma (commonly called Flame of forest, Dhak or Palash), native to Indo-Malayan region is an economically important species with great medicinal value. The tree is of great medicinal value e.g. the leaves have astringent and aphrodisiac properties (Cherdshewasart W and Nimsakul N., 2003), flowers possess antistress properties (Bhatwadekar *et al*., 1999), seeds are anthelmintic (Prashant *et al* 2001) and bark have antifungal properties (Bhandara *et al* 1989). Despite its great medicinal uses, little is known about the genetic diversity of *B. monosperma.*
DNA based markers provide useful information regarding genetic diversity and relationship between accessions. ISSRs, like RAPDs are quick and easy to handle but they seem to have the reproducibility because of the longer length of their primers (Bornet B and Branchard M.., 2001). The combined use of RAPD and ISSR marker allows higher genome coverage since RAPD markers have been described as associated with functionally important loci and ISSR markers amplify hyper variable non-coding regions. RAPD and ISSR marker technique together have been successfully applied for the study of genetic diversity in wild tree species (Gemas *et al*., 2004). Studies describing polymorphism in *B. monosperma* have been described using RAPD marker (Vaishali *et al* 2008). In the present paper, we report the genetic diversity of *B. monosperma* using ISSR markers and to compare these data with the prior RAPD data.

2. Materials and Methods
2.1. Plant material from different agroecological regions of India:
To study the genetic diversity, leaf samples of *B. monosperma* were collected from four major agroecological zones viz. arid, semi-arid, dry sub-humid and moist sub-humid as classified by Sehgal (Sehgal *et al* 1992). For this purpose a total of 16 sites were selected which belonged to 9 agro-climatic sub zones distributed in five states viz. Uttarakhand, Uttar Pradesh, Madhya Pradesh, Rajasthan and Karnataka (Table 1). Fully mature leaf samples of approximately same size were collected and the samples were dried and stored at room temperature.

2.2. Genomic DNA isolation from plant material:
The dried leaf samples were subjected for the DNA isolation using CTAB as a major cationic agent following the standard protocol of Doyle and Doyle (Doyle JJ and Doyle JL., 1990) with some modification as described in earlier study (Vaishali *et al.*2008).

Table 1: Distribution of samples of *B. monosperma* in different agro-ecological zones of India

S. no.	Major zones	Sub zones	Place	State	Latitude	Longitude
1	Arid	E-1.2	Jodhpur	Rajasthan	N 26º23'	E 73º 80'
2	Semi -arid	D-4.2	Chittorgarh	Rajasthan	N 24º52'	E 74º 38'
3		D-4.2	Bhilwara	Rajasthan	N 22º25'	E 74º 38'
4		D-4.2	Kota	Rajasthan	N 24º14'	E 75º 49'
5		D-4.2	Jhalawarh	Rajasthan	N 24º40'	E 76º 10'
6		D-4.2	Pratapgarh	Rajasthan	N 24º20'	E 74º 40'
7		D-4.2	Singoli	M.P	N 25º00'	E 75º 22'
8		D-4.3	Meerut	U.P	N 29º10'	E 77º 42'
9		D-3.3	Banasthali	Rajasthan	N 26º60'	E 75º 54'
10		D-4.4	Bangalore	Karnataka	N 12º59'	E 77º 40'
11	Dry sub-humid	CD-5.4	Saharanpur	U.P	N 29º58'	E 77º 33'
12		CD-5.4	Kanpur	U.P	N 26º28'	E 80º 20'
13		CD-5.4	Lucknow	U.P	N 26º50'	E 81º 00'
14		CD-4.1	Banaras	U.P	N 25º22'	E 83º 00'
15	Moist sub- humid	CM-6.2	Pantnagar	Uttarakhand	N 29º31'	E 79º 30'
16		CD-6.1	Roorkee	Uttarakhand	N 29º52'	E 77º 59'

2.3. ISSR-PCR amplification:

A total of 26 random decamer primers (custom synthesized by Bangalore Genei Pvt. Ltd., GC content >50%) were used for ISSR analysis. DNA amplification reaction was performed in 25 µl reaction volume which contained an end concentration 2.5 mM each of the dNTPs, 1 U/µl Taq polymerase enzyme, 25 ng DNA template and 10 ng primer in Taq polymerase assay buffer (1 X) (10 X buffer contains 100 mM Tris-Cl, 500 mM KCl, 15 mM $MgCl_2$ and 0.1 % gelatin). Amplification reaction was carried out in a Bio-Rad Thermal cycler with the following thermal profile: one cycle of 4 min at 94 ºC (initial denaturation) followed by 45 cycles of 15 sec at 94 ºC (denaturation), 45 sec at 40 ºC (primer annealing) and 90 sec at 72 ºC (primer elongation), and finally one cycle of 4 min at 72 ºC (final extension).

The amplification products were size-separated by standard horizontal electrophoresis in 1.5% agarose gels and stained with ethidium bromide. The reproducibility of the DNA profiles was tested by repeating the PCR amplifications twice with each of the primers analysed. The robust bands were found to be repeatable, and were the products considered in this study.

Table 2: Polymorphic ISSR primers with their corresponding gene diversity and resolving power

	Primer code	Sequence	G+C %	Total no. of bands	No. of poly. bands	% poly.	Expected gene diversity	Resolving power
1	15BV17C1	$(AG)_8C$	52	4	3	75	0.62	3.49
2	16BV17A2	$(GA)_8A$	47	9	5	55	0.59	5.85
3	18BV18C4	$(GA)_8YC$	50	10	9	90	0.55	11.22
4	19BV19C5	$(AC)_8YAC$	47	6	4	66	0.98	0.62
5	20BV17A6	$(AGC)_5CA$	64	16	15	93	0.80	13.22
6	26BV16T12	$(CA)_7GT$	50	11	10	90	0.72	7.72
7	40BV17T26	$(GA)_8CTT$	47	14	12	85	0.71	10.22
	Average			10	8.2	79.1	0.71	7.47

2.4. Data analysis:

Polymorphic ISSR bands were scored as present (1) or absent (0) in each accession. Similarity matrix using the similarity coefficient of Jaccard (Jaccard P., 1908) was constructed from the whole ISSR data. Grouping of accessions was done using NTSYS-PC version 2.11s (Rohlf FJ., 2000) software based on UPGMA algorithm (Sneath PHA and Sokal RR., 1973). In addition, Shannon's diversity index was also employed to characterize the gene diversity and distribution of the variation. Shannon's index of gene diversity was calculated as $H_O = -\Sigma\, p_i \log_2 p_i$, in which pi is the frequency of a given RAPD fragment. H_O was calculated at two levels: the average diversity within population (H_{POP}) and the total diversity (H_{SP}). The proportion of the diversity among populations was estimated as (H_{SP}-H_{POP})/H_{SP}. The gene flow was inferred indirectly using Wright's (1931) formula $N_M = 0.25(1-G_{ST})/G_{ST}$.

3. Results and Discussion

3.1. Genetic diversity analysis of B. monosperma from different geographical regions using ISSR markers:

To investigate the applicability of ISSR-PCR in the genetic analysis of *B. monosperma*, 26 ISSR primers were tested of which 12 primers gave amplification of DNA. Other primers generally resulted in smear formation or no amplification. Of the 12 primers amplified, only 7 primers gave reproducible and scorable results, hence considered for further analysis (Figure 1). The seven primers yield a total of 70 bands. Of these, 58 (83%) were polymorphic (Table 2). The estimated Nei's gene diversity ranged from the lower value of 0.55 with primer-3 to a maximum value of 0.98 with primer-4 with a mean value of 0.71. Resolving power which indicates the ability of primers to distinguish different accessions, varied from 0.62 to 13.22 with primer-4 and primer-5 respectively with a mean value of 7.47.

Table 3: Similarity coefficient of different accessions of *B. monosperma* using ISSR primers

	Jodhpur	Bhilwara	Kota	Jhalawar	Pratapgarh	Banasthali	Meerut	Chittorgarh	Pantnagar	Roorkee	Saharanpur	Lucknow	Banaras	Indore	Kanpur	Bangalore
Jodhpur	1															
Bhilwara	0.57	1														
Kota	0.62	0.8	1													
Jhalawar	0.62	0.66	0.64	1												
Pratapgarh	0.62	0.55	0.64	0.71	1											
Meerut	0.62	0.58	0.67	0.57	0.75	1										
Banasthali	0.55	0.51	0.67	0.64	0.64	0.71	1									
Chittorgarh	0.53	0.58	0.6	0.53	0.6	0.67	0.64	1								
Pantnagar	0.51	0.55	0.5	0.57	0.5	0.57	0.57	0.64	1							
Roorkee	0.62	0.66	0.67	0.57	0.6	0.71	0.67	0.67	0.64	1						
Saharanpur	0.44	0.62	0.53	0.53	0.57	0.57	0.53	0.6	0.75	0.67	1					
Lucknow	0.53	0.46	0.55	0.62	0.66	0.55	0.55	0.55	0.55	0.55	0.58	1				
Banaras	0.6	0.6	0.62	0.58	0.73	0.55	0.55	0.44	0.37	0.55	0.51	0.67	1			
Indore	0.73	0.55	0.6	0.5	0.6	0.67	0.57	0.64	0.57	0.6	0.57	0.58	0.66	1		
Kanpur	0.6	0.67	0.76	0.58	0.55	0.66	0.58	0.66	0.58	0.69	0.58	0.53	0.53	0.58	1	
Bangalore	0.57	0.6	0.55	0.55	0.44	0.51	0.58	0.62	0.55	0.58	0.51	0.46	0.5	0.55	0.64	1

3.2. Cluster analysis using ISSR primers:

ISSR data were used to make pair wise comparison of the accessions based on shared and unique amplification products to generate a similarity matrix with NTSYS-PC (version 2.11s). Of the 16 samples analyzed, two accessions collected from Kota and Bhilwara displayed the greatest genetic similarity, with a similarity coefficient value of 0.80 (Table 3). The UPGMA based dendrogram clustered all individuals into three distinct clusters with one sample from Bangalore placed outside the major cluster and at one end of the dendrogram (Figure 2). The major clusters were sub-divided into 2-3 different sub-clusters within clusters. The sample from Bangalore was not grouped along with other members of semi-arid major agroecological zone and exists as separate branch at one end of cluster.

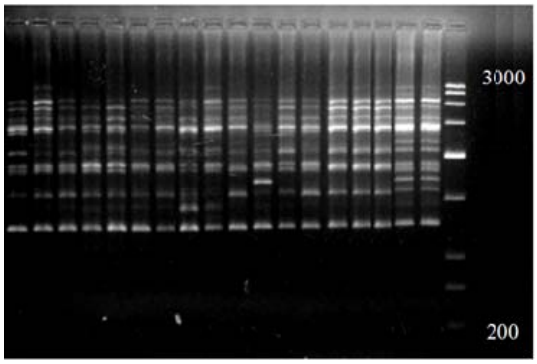
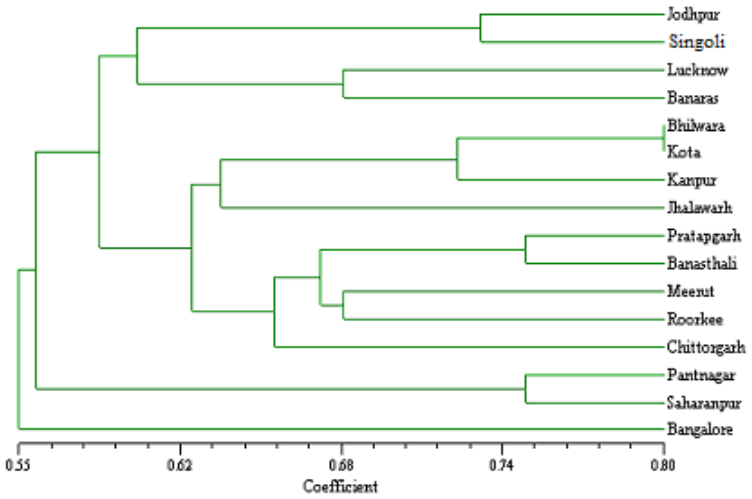


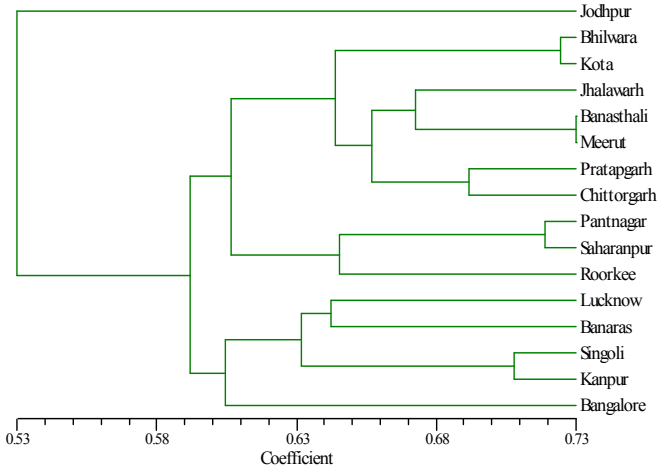
Fig 1: ISSR-PCR profile of different accessions of *B. monosperma* with primer

3.3. Shannon's diversity indices for B. monosperma using ISSR primers:

Shannon index of genotypic diversity for ISSR data was estimated between group and within groups based on their Geographical regions. The four groups viz. arid, semi-arid, dry sub-humid and moist sub-humid differed in the amount of observed heterozygosity (H_O) (Table 4). The mean value of observed heterozygosity (H_O) within semi-arid region was 1.76. Similarly, the mean value of H_O for dry sub-humid and moist sub-humid region was 1.87 and 0.98 respectively. The H_O could not be calculated for arid zone, as there is only one sample from this region. Based on the Shannon's index of genetic diversity, the Nei's coefficient of genetic differentiation is used to estimate the proportion of total genetic diversity residing among population (Nei, 1973). The investigated population of *B. monosperma* showed 49% genetic variation among individuals population and 51% within population with a gene flow of 0.26. In population genetics, a value of gene flow (N_M) < 1.0 (fewer than one migrant per generation into a population) or equivalently, a value of gene differentiation (G_{ST}) >0.25 is generally regarded as the threshold quantities beyond which significant population differentiation occurs .



A



B

Fig 2: UPGMA cluster analysis of B. monosperma collected from the different agroecological zones of India using ISSR primers (A), using RAPD and ISSR primers combined data set (B).

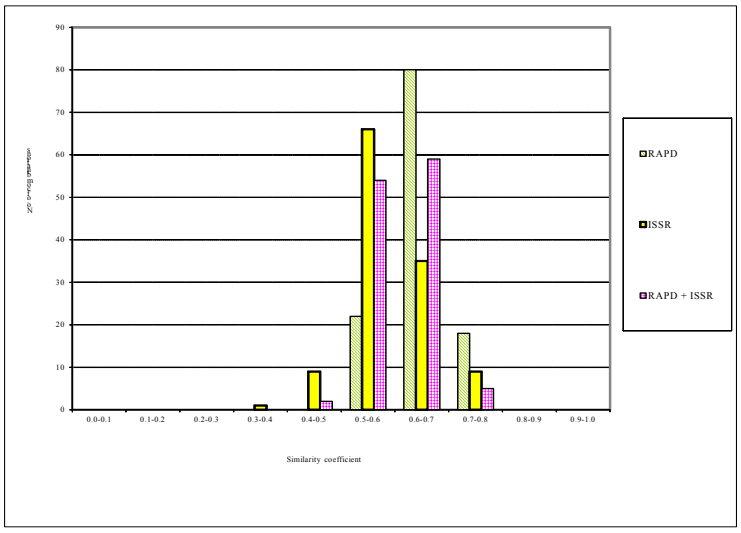


Fig 3: Histogram of genetic similarity values calculated for all pair wise combinations among 16 isolates of *B. monosperma* based on RAPD, ISSR and combined primer data

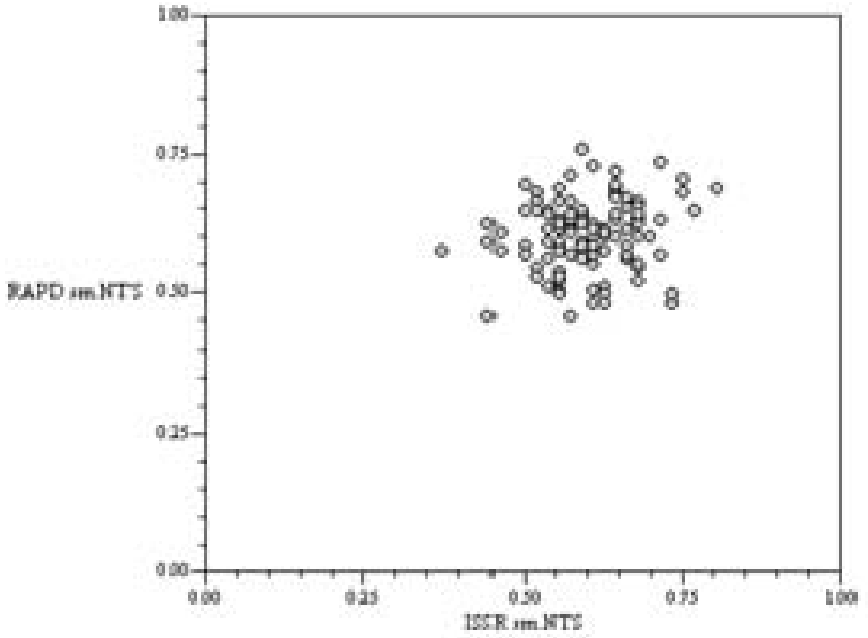


Fig 4: Comparative matrix plot based on similarity coefficient value generated by RAPD and ISSR primers in *B. monosperma*

S. no.	Primer code	Sequence	Arid	Semi-arid	Dry sub-humid	Moist sub humid	H POP	HSP	HPOP/ HSP	GST
RAPD										
1	73SP10G4	AATCGGGCTG	-	1.47	2.16	1.03	1.16	2.36	0.49	0.50
2	74SP10G5	AGGGGTCTTG	-	3.32	3.02	2.76	2.27	4.56	0.49	0.50
3	76SP10G7	GAAACGGGTG	-	3.02	2.59	2.07	1.92	3.25	0.58	0.41
4	77SP10G8	GTGACGTAGG	-	1.29	1.59	0.69	0.89	1.81	0.49	0.50
5	79SP10G10	GTGATCGCAG	-	1.61	1.73	1.03	1.09	2.71	0.40	0.59
6	80SP10T11	CAATCGCCGT	-	1.25	1.73	0.34	0.83	1.68	0.49	0.50
7	81SP10G12	TCGGCGATAG	-	2.52	2.80	2.07	1.85	3.83	0.48	0.51
8	83SP10G14	TCTGTGCTGG	-	1.48	0.77	0.34	0.65	1.89	0.34	0.65
9	84SP10C15	TTCCGAACCC	-	2.75	2.01	2.07	1.74	3.05	0.57	0.42
10	85SP10A16	AGCCAGCGAA	-	4.20	2.85	2.07	2.28	4.92	0.46	0.53
11	87SP10T18	AGGTGACCGT	-	1.90	1.59	1.03	1.13	2.44	0.46	0.53
12	89SP10C20	GTTGCGATCC	-	2.07	1.94	0.69	1.17	2.56	0.58	0.54
	Average			2.24	2.06	1.34	1.41	2.92	0.48	0.51
	Nm									**0.24**
ISSR										
1	15BV17C1	$(AG)_8C$	-	0.58	0.34	-	0.23	0.69	0.33	0.66
2	16BV17A2	$(GA)_8A$	-	1.18	1.03	0.69	0.72	1.38	0.52	0.47
3	18BV18C4	$(GA)_8YC$	-	1.87	1.59	1.38	1.21	2.22	0.54	0.45
4	19BV19C5	$(AC)_8YAC$	-	0.43	1.03	-	0.36	0.69	0.53	0.46
5	20BV17A6	$(AGC)_5CA$	-	4.31	4.10	1.38	2.45	4.83	0.50	0.49
6	26BV16T12	$(CA)_7GT$	-	1.79	2.63	1.38	1.45	2.85	0.50	0.49
7	40BV17T26	$(GA)_8CTT$	-	2.17	2.37	2.07	1.65	2.84	0.57	0.42
	Average		-	1.76	1.87	0.98	1.15	2.21	0.50	0.49
	Nm									**0.26**

3.4. Comparison of genetic diversity based on RAPD and ISSR marker system:

The ISSR data were processed with RAPD data from (Vaishali *et al*. 2008) and a collective dendrogram was constructed (Figure 2). In the combined dendrogram, the 16 samples of *B. monosperma* were grouped into three main clusters with sub-clusters. The cluster one represented a single sample of Jodhpur (arid) situated as a branch at one end of the dendrogram. It is understandable as this is the only sample that belonged to arid zone. Overall there was no clear clustering pattern of geographically closer accessions indicating that the association between genetic similarity and geographical distance was less significant. Moreover, the results also showed that the groupings of accession are not well defined according to their geographical origin. For examples, the sample from Bangalore belonged to semi-arid agroecological region, but it did not cluster along with the other samples of this region but instead took independent position in dendrogram. This showed that climatic conditions and physical parameters may affect the plant genome as the plant is adapted and these changes are inherited through genome to the next generation.

The polymorphism obtained with RAPD and ISSR markers have different underlying sources at the molecular level and may differ in their informativeness for the exploration of genetic diversity and the establishment of relationships among ecotypes like barley, allium, brassica etc. (Ferdaous *et al* 2011, Mukherjee *et al* 2013, Sara *et al* 2013). *B. monosperma* showed relatively higher mean gene diversity as compared with other

tree species (Zhao *et al* 2006, Li *et al* 2007) with reduced level of gene flow. This may be due to the specific topology of the region and geographical constraints (Ge *et al* 2005).

The results obtained on the basis of RAPD and ISSR showed similar tendencies. However, despite being of nearly identical tendency, similarity coefficient values based on ISSR data were higher than those of RAPD, which was supported by earlier studies on strawberry, tellitia indica, ocimum (Kuras *et al* 2004, Shabana *et al* 2013, Shu-Yun *et al* 2013). Using both types of marker system, the genetic similarity values were distributed within limited range among 16 accessions of *B. monosperma* (Figure 3). The similarity values based on RAPD were skewed towards higher similarity, whereas those based on ISSR were skewed towards lower similarity. The results are in good agreement with earlier studies where ISSR showed relatively lower value of genetic similarity coefficient value than those of RAPD (Archak *et al* 2003). This confirmed the better discrimination power of ISSR.

The genetic diversity within and among group using both types of marker system revealed higher genetic diversity with almost same pattern of portioning of genetic diversity. The Shannon's diversity indices within group are in accordance with RAPD primers. However, the ISSR analysis showed relatively lower value of Shannon's indices at different levels than those with RAPD analysis. However, in both the cases higher values of G_{ST} were observed which indicated that the genetic diversity was higher within population rather than among population. The higher genetic diversity within groups is probably structured by geographical conditions, ecological factors and mating system of the plant.

The compatibility to these two techniques in differentiating the accessions of *B. monosperma* was confirmed by estimating the Pearson correlation coefficient. For this purpose the similarity matrices for both marker systems were taken into consideration. The estimated value of correlation coefficient was found to be 0.84. The coefficient value of this range indicated a good correlation between data generated by both the systems. This was also reflected in similarity matrix comparison plot (Figure 4). Hence, RAPD and ISSR are effective, promising and informative for estimating the extent of genetic diversity as well as to determine the pattern of genetic relationships between different accessions of *B. monosperma*, with polymorphism levels sufficient to establish informative fingerprints with relatively fewer primer sets.

5. References

1. Archak S, Gaikwad AB, Gautam D, Rao EVVB, Swamy KRM, Karihaloo JL *et al.* Comparative assessment of DNA fingerprinting techniques (RAPD, ISSR, and AFLP) for genetic analysis of cashew (*Anacardium occidentale* L.) accessions of India. Genome, 2003, 46: 362-369.
2. Bhandara BM, Kumar NS, Samaranayak KM. An antifungal constituent from the stem bark of *Butea monosperma*. Ethnopharmacology 1989; 25:73-75
3. Bhatwadekar AD, Chintawar SD, Logede NA, Simani RS, Kasture VS, Kasture SB *et al*. Antistress activity of *Butea monosperma* flowers. Indian Journal of Pharmacology 1999; 31:153-155.
4. Bornet B, Branchard M. Nona chord inter simple sequence repeat (ISSR) markers: Reproducible and specific tools for genome fingerprinting. Plant Molecular Biology Reporter 2001; 19:209-215.
5. Cherdshewasart W, Nimsakul N. Clinical trial of *Butea superba* an alternative herbal treatment for eractile dysfunction. Asian Journal of Andrology 2003; 5:245-253.
6. Doyle JJ, Doyle JL. Isolation of plant DNA from fresh tissue. Focus1990; 12:13-15.
7. Guasmi F, Elfalleh W, Hannachi H, Fères K, Touil L, Marzougui N *et al*. The Use of ISSR and RAPD Markers for Genetic Diversity among South Tunisian Barley. ISRN Agronomy 2011; 2012: 10.
8. Ge XJ, Zhang LB, Yuan YM, Hao G and Chiang TY. Strong genetic differentiation of the East Himalayan *Megacodon stylophours* (Gentianaceae) detected by inter simple sequence repeats (ISSR). Biodiversity and Conservation 2005; 14:849-861.
9. Gemas VJV, Almadanim MC, Tenreiro R, Martins A, Fevereiro P. Genetic diversity in the olive tree cultivated in Portugal revealed by RAPD and ISSR markers. Genetic Resources and Crop Evolution 2004; 51:501-511.
10. Jaccard P. Nouvelles recherches sur la distribution florale. Bulletin Society Vaudoise Sciences Naturelles 1908; 44:223-270.
11. Kuras A, Korbin M, Zurawicz E. Comparison of suitability of RAPDs and ISSR techniques for determination of strawberry relationship. Plant Cell Tissue and Organ Culture 2004; 79:189-193.
12. Li JM, Dong M Zhong ZC. Population genetic differentiation in the invasive plant *Mikaania micrantha* in china. Journal of Plant Ecology 2007; 31(4):680-688.
13. Mukherjee A, Sikdar B, Ghosh B, Banerjee A, Ghosh E, Bhattacharya M *et al*. RAPD and ISSR analysis of some economically important species, varieties, and cultivars of the genus Allium (Alliaceae). Turkish Journal of Botany 2013; 37:605-618.
14. Prashant D, Asha MK, Amit A, Padmaya R. Anthelmintic activity of *Butea monosperma*. Fitoterapia, 2001; 72:421-422.
15. Rohlf FJ. Numerical Taxonomy and Multivariate Analysis System, version 2.11 manual. New York, Applied Biostatistics, Inc, 2000.
16. Safari S, Mehrabi AA, Safari Z. Efficiency of RAPD and ISSR markers in assessment of genetic diversity in Brassica napus genotypes. International Journal of Agriculture and Crop Sciences. IJACS 2013; 5(3):273-279.
17. Sehgal JL, Mandal DK, Mandal C, Vadivelu S. Agro Ecological Regions of India. National Bureau of Soil Survey & Land Use Planning (NBSS &LUP) & Indian Council of Agricultural Research (ICAR), Nagpur, 1992.
18. Parveen S, Saharan MS, Verma A, Sharma I. Comparative analysis of RAPD and ISSR marker assays for detecting genetic polymorphism in Tilletia indica. European Journal of Experimental Biology 2013; 3(1):380-387.
19. Shu-Yun C, Ting-Xuan D, Yuan-Tsung C, Shyh-Shyan W, Shang-Ling O, Wen-Ling C *et al.* Genetic diversity among Ocimum species based on ISSR, RAPD and SRAP markers., AJCS 2013, 7(10):1463-1471.
20. Sneath PHA, Sokal RR. Numerical Taxonomy. WH Freeman, San Francisco, CA, 1973.
21. Khan VS and Sharma V. RAPD Based Assessment of Genetic Diversity of *Butea monosperma* from Different Agro-Ecological Regions of India. Indian Journal of Biotechnology 2008; 7:320-327.
22. Zhao WG, Zhang JQ, Wang YH, Chen TT, Yin YL, Huang YP *et al.* Analysis of genetic diversity in wild population of mulberry from western part of Northeast China determined by ISSR markers. Journal of Genetics and Molecular Biology 2006; 17(4):196-203.