Intra-specific Genetic Diversity of Different Accessions of *Cassia occidentalis* by RAPD Markers

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Abstract

The RAPD technique has been successfully used in a variety of taxonomic and genetic diversity studies. The genetic diversity of ten accessions of *C. occidentalis* collected from different districts of Haryana has been reported in this paper using twelve random amplified polymorphic DNA primers. 111 bands were scored corresponding to an average of 9.25 bands per primer with 79 bands showing polymorphism (71.17%). Nine out of twelve primers gave more than 60% polymorphism. Jaccard similarity coefficient ranged from 0.54 to 0.73. A dendrogram constructed based on the UPGMA clustering method revealed two major clusters. Cluster-1 comprises of nine accessions which was further differentiated into two sub-clusters while Cluster–2 includes only accession C10. This study revealed rich genetic diversity among *C. occidentalis* accessions from Haryana region in India.

Keywords: RAPD; *C. occidentalis*; genetic diversity; medicinal plant.

1. Introduction

Cassia occidentalis L. (known as Kasondi) is an Ayurvedic medicinal plant with ethnobotanical importance. This plant belongs to family Caesalpiniaceae, found principally in tropical and warm temperate regions. *C. occidentalis* is a commonly found wild plant in India. This plant differs a lot in morphological features when collected from different regions of India according to climate, soil type and other factors. This plant is an annual shrub in Haryana (Northern India) but a perennial herb in Chennai (Southern India) [1]. Similarly, due to seasonal and geographical variations, a lot of phytochemical differences were also observed on this plant in different studies [2, 3]. Phytochemical differences are directly or indirectly linked to the genetic diversity. DNA markers based fingerprinting can distinguish species rapidly using small amounts of DNA and therefore can assist to deduce reliable information on their phylogenetic relationships. Various approaches are available for DNA fingerprinting such as AFLP (Amplified Fragment Length Polymorphism), SSR (Simple Sequence Repeats) and RAPD (Random Amplified Polymorphic DNA) [4]. RAPD is convenient to conduct with good polymorphism and can be used in analyzing genetic diversity and the relation between species. It has been used in analyzing the relationships between strains belonging to same genera and genetic diversity in many plants, especially medicinal plants [4]. Although RAPD is of dominant nature, several strategies have been put forward to minimize the dominance effects on genetic variation analysis [5, 6]. In occasional cases, RAPD is poor in reproducibility but this can usually be solved by optimization of reaction conditions [7, 8]. RAPD analysis requires only a small amount of genomic DNA and can produce high level of polymorphism and may facilitate more effective diversity analysis in plants. It provides information that can help to define the distinctiveness of species and phylogenetic relationships at molecular level. Use of such techniques for germplasm characterization may facilitate the conservation and utilization of plant genetic resources, permitting the identification of unique genotypes or sources of genetically diverse genotype.

In our previous studies, *C. occidentalis* plant was shown to exhibit important medicinal activities [2, 9]. These studies support the need for genetic diversity analysis of this plant in Haryana region to explore the hidden role of genes according to different factors. In the present study, genetic diversity of different accessions of *C. occidentalis* was studied by random RAPD markers and their genetic relatedness was also reported by constructing dendrogram based on UPGMA (Unweighted Paired Group Method using Arithmetic Averages) analysis.

2. Methods

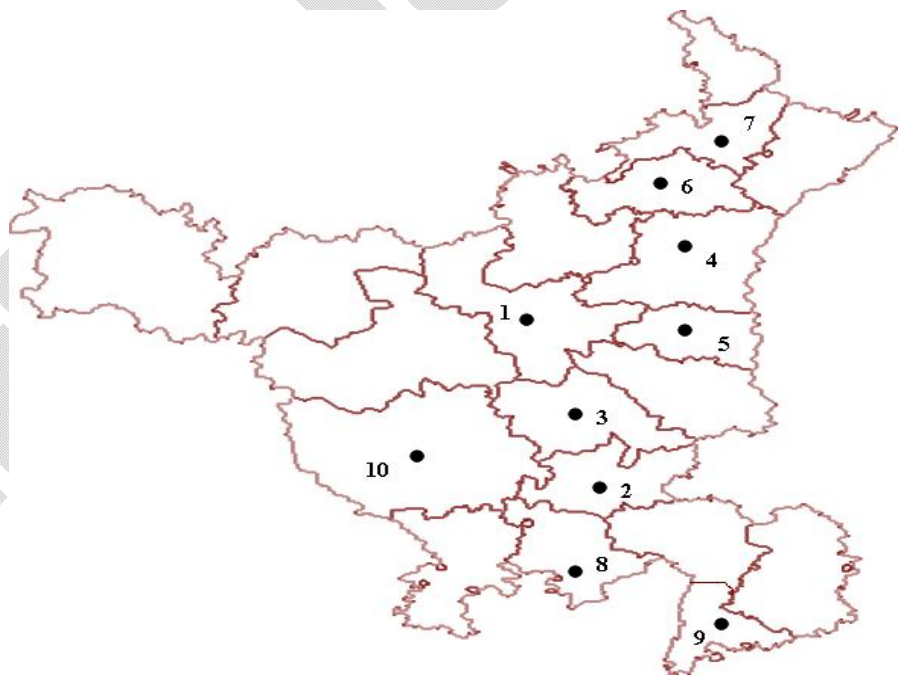
2.1 Plant material

A total of 10 plant samples (one from each location) were collected from ten different districts of Haryana as shown in Figure 1. Plant samples were collected from ten different districts of Haryana: Jind, Jhajjar, Rohtak, Karnal, Panipat, Kurukshetra, Ambala, Rewari, Gurgaon and Bhiwani. Different plant accessions were numbered as C1 to C10. The average rainfall, latitude and longitude of these districts are given in Table 1. These districts have different climatic and soil conditions. The nutrient composition, water holding capacity, nature and type of soil is variable in these districts.

Table 1: Latitude, longitude and average rainfall of different districts of Haryana.

S. No.	Name of district	Latitude	Longitude	Average rainfall (mm)
1	Jind	29° 19' N	76° 23' E	515
2	Jhajjar	28° 33' N	76° 28' E	580
3	Rohtak	28° 54' N	76 ° 38' E	592
4	Karnal	29° 42' N	77° 02' E	696
5	Panipat	29° 38' N	76° 58' E	680
6	Kurukshetra	29° 52' N	76° 26' E	582
7	Ambala	30° 21' N	76° 52' E	1076
8	Rewari	28° 12' N	76° 40' E	553
9	Gurgaon	28° 37' N	77° 04' E	596
10	Bhiwani	28° 46' N	76° 18' E	420

Figure 1: Map of Haryana showing sites of collection.



(1 = Jind; 2 = Jhajjar; 3 = Rohtak; 4 = Karnal; 5 = Panipat; 6 = Kurukshetra; 7 = Ambala; 8 = Rewari; 9 = Gurgaon; 10 = Bhiwani)

2.2 Genomic DNA isolation

Genomic DNA was isolated from fresh leaves of the plants using Promega Maxwell extractor, treated with RNase, assessed on 0.8% agarose gel, quantified using Nanodrop spectrophotometer (ND-1000, version 3.1.1, USA) and diluted to 25 ng/µl for PCR amplification.

2.3 PCR amplification using RAPD primers

Fifty random decamer RAPD primers were screened which were obtained from UBC (University of British Columbia). The PCR reactions were accomplished in a 25 µl reaction mixture containing 1 X assay buffer, one unit of Taq DNA polymerase (Bangalore Genei Pvt. Ltd., India), 200 µM of each dNTP (Bangalore Genei Pvt. Ltd., India), 0.2 µM primers and 50 ng of template DNA. The PCR reaction was carried out in DNA thermal cycler (Model-CGI-96, Corbett Research, Australia). The PCR amplification conditions for RAPD consisted of initial extended step of denaturation at 94^0C for 4 minutes followed by 43 cycles of denaturation at 94^0C for 1 min, primer annealing at 37^0C for 1 min and elongation at 72^0C for 4 min. The PCR products were visualized on 1.2% agarose gel.

2.4 Statistical analysis

The RAPD bands were scored for presence (1) or absence (0) and each band was regarded as locus. All calculations were done using computer program NTSys PC version 2.02 [10]. Pairwise similarity matrices were generated by Jaccard's coefficient of similarity by using SIMQUAL format of NTSys software. A dendrogram was constructed by using the UPGMA with SAHN module of NTSys software to show a phenetic representation of genetic relationship as revealed by the similarity coefficient. Polymorphism information content (PIC) was also calculated according to Anderson and his co-workers [11].

3. Results

Fifty random decamer primers purchased from UBC were screened taking DNA of two *C. occidentalis* samples before performing RAPD analysis in all the genotypes. Out of fifty primers used for screening, 38 did not amplify any fragment (Figure 3). While, other 12 primers generated amplicons ranging from 3 (UBC 177) to 13 (UBC 106 & UBC 112). The reproducibility of the bands generated by these 12 primers was confirmed by replicating the amplification twice and if needed thrice. Only the bands showing reproducible amplification were considered for scoring (Table 2) and for further analysis. The number of polymorphic bands ranged from 3 to 13 with range of polymorphisms 37.5% (UBC 61) to 100% (UBC 178, UBC 121, UBC 171) (Table 1). The total number of bands generated by twelve amplifying primers was 111 with an average amplification of 9.25 bands per primer. The average polymorphism generated by these bands was 71.17%. The size of the amplicons generated varied from 385 bp to 3400 bp. In the present study, the PIC ranges from 0.166 (UBC 177) to 0.394 (UBC 121) (Table 2) were also calculated.

Table 2: List of single arbitrary primers showing total and polymorphic amplicons generated along with PIC of each pattern for 10 genotypes of *C. occidentalis.*

Primers	Total no. of bands (a)	Total no. of polymorphic bands (b)	% Polymorphism (b/a x 100)	PIC
UBC 106	13	8	61.53	0.215
UBC 23	10	7	70	0.242
UBC 103	6	4	66.66	0.183
UBC 112	13	5	38.46	0.211
UBC 61	8	3	37.5	0.173
UBC 104	8	6	75	0.227
UBC 178	9	9	100	0.246
UBC 135	11	8	72.72	0.241
UBC 69	11	9	81.81	0.261
UBC 121	11	11	100	0.394
UBC 177	3	2	66.66	0.166
UBC 171	7	7	100	0.342
Total	**111**	**79**	**71.17 (Aver.)**	

The Jaccard's pairwise similarity coefficient values ranged from 0.54 (C 7 and C 10) to 0.73 (C 5 and C 3, C 5 and C 4, C 5 and C 2) (Table 3). The clusters constructed through NTSys (2.02 PC) presented in the form of dendrogram has been shown in Figure 2. The dendrogram has put all the genotypes in two major groups (group A and B). Cluster-1 comprises of nine accessions which was further differentiated into two sub-clusters while Cluster–2 includes only accession C10.

Table 3: Jaccard's similarity coefficient among different accessions of *C. occidentalis.*

	C 1	C 2	C 3	C 4	C 5	C 6	C 7	C 8	C 9	C 10
C 1	1.00									
C 2	0.68	1.00								
C 3	0.71	0.72	1.00							
C 4	0.66	0.70	0.70	1.00						
C 5	0.67	0.73	0.73	0.73	1.00					
C 6	0.60	0.62	0.63	0.60	0.63	1.00				
C 7	0.67	0.64	0.65	0.61	0.64	0.67	1.00			
C 8	0.60	0.63	0.68	0.61	0.62	0.68	0.66	1.00		
C 9	0.63	0.66	0.67	0.66	0.65	0.64	0.65	0.62	1.00	
C 10	0.57	0.58	0.66	0.58	0.61	0.58	0.56	0.54	0.56	1.00

Figure 2: UPGMA dendrogram of ten accessions of *C. occidentalis* based on 12 random RAPD primers.

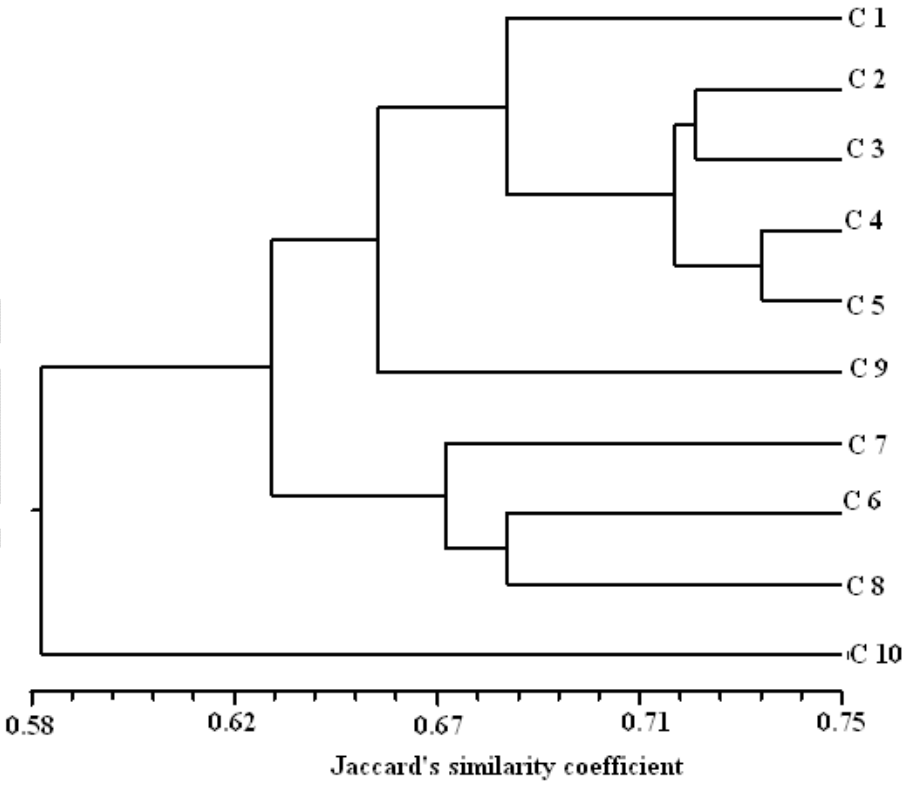
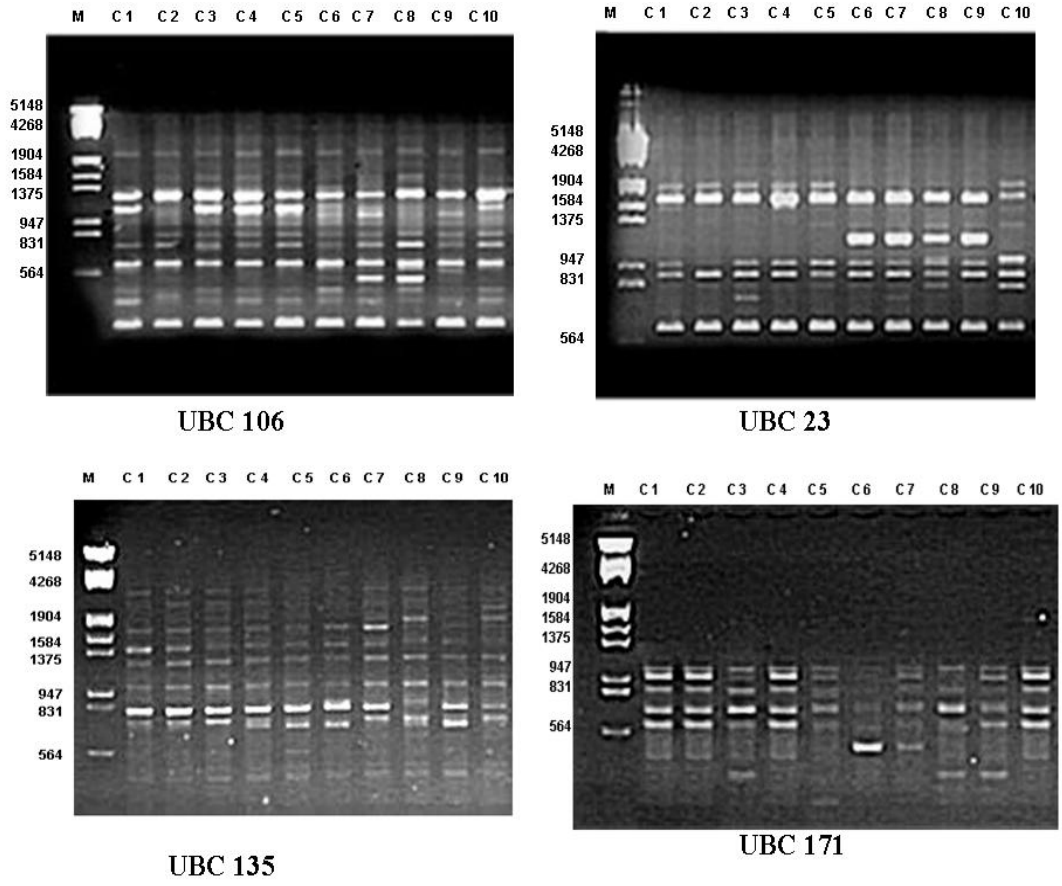


Figure 3: Gel photographs showing polymorphism obtained by different primers.



4. Discussion

RAPD technology is a common and well-proven tool in genetic studies and a convenient procedure for detecting total genetic variation and its partitioning within and among populations. The RAPD technique had been successfully used in a variety of taxonomic and genetic diversity studies [12, 13]. The technical simplicity of RAPD technique facilitated its use in the analysis of genetic relationship in several genera [14-16]. The major concerns regarding RAPD generated phylogeny include homology of bands showing the same rate of migration, causes of variation in fragment mobility and origin of sequence in the genome. In spite of this limitation, RAPD marker has the greatest advantage in its capability to scan across all regions of the genome, hence highly suited for phylogeny studies at species level [14, 15]. In the present study, the marker technology was used to detect genetic variation within *C. occidentalis* accessions.

In the present study, the medicinal plant *C. occidentalis* showed a high percentage of genetic polymorphism of 71.17% respectively, which was near to the percentage for *Changium smyrnidoies* (69%) [17], but higher than that of *Dacydium pierrei* (33.3%) [18] and *Cathaya argrophylla* (32%) [19]. Similarly, the genetic diversity index was also highly variable from 0.54 to 0.73 in case of *C. occidentalis* accessions. These studies indicate that RAPD is sufficiently informative and powerful to access genetic variability of natural populations of *C. occidentalis*. Thus, RAPD markers will provide a useful tool in the future design of collection strategies for germplasm conservation.

The genetic diversity of the plants is closely related to their geographic distribution. Species with a wide geographic area generally have more genetic diversity [14]. This RAPD analysis showed high genetic diversity in *C. occidentalis* accessions growing in different

environments and low diversity in *C. occidentalis* accessions in the same or adjacent regions, with a few exceptions. For example, *C. occidentalis* accession C 4 and C 6 are from Karnal and Kurukshetra respectively, which are not geographically much far apart, but their genetic distance was great in the present RAPD analysis. Similarly, in case of *C. tora*, C 15 and C 14 samples of Panipat and Karnal showed significant genetic divergence.

During intraspecific genetic variability study, the divisions of 10 samples of *C. occidentalis* into two subclusters as shown in Figure 2 allowed very little chances for gene flow among accessions that were geographically distant, but the probability of naturally occurring genetic cross and gene flow should be high among accessions growing near each other. So, this study concluded that the high genetic diversity among accessions in adjacent regions was mostly attributable to artificial introduction, not natural genetic differentiation.

The level and distribution of genetic diversity detected by RAPD are in overall agreement with recent studies in India [20, 21, 22, 23, 24]. RAPD, being a multi-locus marker with the simplest and fastest technology, have been successfully employed for determination of intra-species genetic diversity in several plant species [25, 26]. In case of *C. occidentalis*, C 10 sample from Bhiwani did not group with any other accession in dendrogram confirming its genetic distinctness from all other accessions included in this investigation.

The calculated PIC based on the probability that two unrelated genotypes amplified from the test population will be placed into different typing groups. PIC estimates the degree of polymorphism of marker, which essentially is the proportion of individuals that are heterozygous for a marker. PIC is a good measure of the heterozygosity. It is an index of how many alleles a certain marker has and how those alleles divide. High PIC value indicates rich heterozygosity which in turn is associated with a high degree of polymorphism [27]. In case of *C. occidentalis*, good range of PIC value was observed, which showed significant genetic diversity among *C. occidentalis* accessions.

5. Conclusion

Identification of intra-population diversity also forms a very essential pre-requisite for the promising genetic diversity analysis. The observations and interpretations of this investigation are interesting as a preliminary exploration analysis. The present findings make a strong point to enlarge the scope and size of collection throughout the distribution area of *C. occidentalis* in order to detect and quantify the prevalent genetic diversity existing within this species at molecular level. To the best of our knowledge, this is the first report on the characterization of *C. occidentalis* genotypes based on commercially available primers from Haryana region. The powerful capability of molecular technique to distinguish closely related genotypes based on their RAPD patterns has been brought out by this study.

Competing Interests

The authors declare that they have no competing interests.

Authors' Contributions

All authors contributed equally in the experimentation and preparation of the manuscript.

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References

1. Rani MS, Emmanuel S, Sreekanth MR, Ignacimuthu S, 2010. Evaluation of in vitro antioxidant and hepatoprotective activity of *Cassia occidentalis* Linn. against paracetamol induced liver toxicity in rats. International Journal of Pharmacy & Pharmaceutical Science, 2(3): 67-70.

2. Yadav JP, Arya V, Yadav S, Panghal M, Kumar S, Dhankhar S, 2010. *Cassia occidentalis*: a review on its ethnobotany, phytochemical and pharmacological process Fitoterapia, 81: 223-230.

3. Arya V, Yadav S, Kumar S, Yadav JP, 2010. Antimicrobial activity of *Cassia occidentalis* L. (leaf) against various human pathogenic microbes. Life Sciences and Medicine Research, 2010: LSMR-9.

4. Lanying Z, Yongqing W, Zhang L, 2009. Genetic diversity and relationship of 43 *Rhododendron* sp. based on RAPD analysis. Botany Research International, 2(1): 1-6.

5. Lynch M, Milligan BG, 1994. Analysis of population genetic structure with RAPD markers. Molecular Ecology, 3: 91-99.

6. Stewart CN, Excoffier L, 1996. Assessing population genetic structure and variability with RAPD data: application to *Vaccinium macrocarpon*. Journal of Evolutionary Biology, 9: 153-171.

7. Szmidt AE, Wang X, Liu M, 1996. Empirical assessment of allozyme and RAPD variation in *Pinus sylvestris* L. using haploid tissue analysis. Heredity, 76: 412-1120.

8. Weising K, Nybonn H, Wolff K, Meyer W, 1995. DNA fingerprinting in plants and fungi. SCR Press, Boca Raton, USA.

9. Arya V, Yadav JP, 2010. Antioxidant and total phenolics in leaves extracts of *Cassia tora* L. Pharmacologyonline, 2: 1030-1036.

10. Rohlf FJ, 1998. NTSYS-PC Numerical taxonomy and multivariate analysis system. Version 2.02e. EXETER Software, Setauket.

11. Anderson JA, Churchill GA, Autrique JE, Tanksley SD, Sorrells ME, 1993. Optimizing parental selection for genetic linkage maps. Genome, 36: 181–186.

12. Vyas GK, Sharma R, Kumar V, Sharma TB, Khandelwal V, 2009. Diversity analysis of *Capparis decidua* (Forssk.) Edgew. using biochemical and molecular parameters. Genetic Resources and Crop Evolution, 56: 905-911.

13. Ahmad G, Mudasir, Kudesia R, Shikha, Srivastava MK, 2010. Evaluation of genetic diversity in pea (*Pisum sativum*) using RAPD analysis. Genetic Engineering and Biotechnology Journal, 2010: GEBJ-16.

14. Wilikie SE, Issac PG, Slater RJ, 1993. Random amplified polymorphic DNA (RAPD) markers for genetic analysis in *Allium*. Theoretical and Applied Genetics, 87: 668-672.

15. Nair NV, Nair S, Sreenivasan TV, Mohan M, 1999. Analysis of genetic diversity and phylogeny in *Saccharum* and related genera using RAPD markers. Genetic Resources and Crop Evolution, 46: 73-79.

16. Demeke T, Adams RP, Chibber R, 1992. Potential taxonomic use of random amplified polymorphic DNA – a case study in *Brassica*. Theoretical and Applied Genetics, 84: 990-994.

17. Fu CX, Qiu YX, Kong HH, 2003. RAPD analysis for genetic diversity in *Changium smyrnioides* (Apiaceae), an endangered plant. Botanical Bulletin of Academia Sinica, 44: 13-18.

18. Su YJ, Wnag T, Huang C, Zhu JM, Zhou Q, 1999. RAPD analysis of different population of *Dacydium pierrei*. Acta Sciencia National University of Sunyatseni, 38: 99-101.

19. Wang XQ, Zou YP, Zhang DM, Hong DY, 1996. RAPD analysis of genetic diversity of *Cathaya argyrophylla*. Science in China, 26: 436-441.

20. Vaishali, Khan S, Sharma V, 2008. RAPD based assessment of genetic diversity of *Butea monosperma* from different agro-ecological regions of India. Indian Journal of Biotechnology, 7: 320-327.

21. Harisaranraj R, Prasitha R, Babu SS, Suresh K, 2008. Analysis of inter-species relationships of *Ocimum* sp. using RAPD markers. Ethnobotanical Leaflets, 12: 609-13.

22. Ranade SA, Srivastava AP, Rana TS, Srivastava J, Tuli R, 2008. Easy assessment of diversity in *Jatropha curcas* L. plant using two single-primer amplification reaction (SPAR) methods. Biomass and Bioenergy, 32(6): 533-540.

23. Chaurasia AK, Subramaniam VR, Krishna B, Sane PV, 2009. RAPD based genetic variability among cultivated varieties of Aonla (Indian Gooseberry, *Phyllanthus emblica*). Physiology and Molecular Biology of Plants, 15(2): 169-173.

24. Sahasrabudhe A, Deodhar M, 2010. Standardization of DNA extraction and optimization of RAPD-PCR conditions in *Cinia indica*. International Journal of Botany, 6: 293-298.

25. Malviya N, Yadav D, 2010. RAPD analysis among pigeon pea [*Cajanus cajan* (L.) Mill sp.] cultivars for their genetic diversity. Genetic Engineering and Biotechnology Journal, 2010: GEBJ-1.

26. Gupta R, Verma K, Joshi DC, Yadav D, Singh M, 2010. Assessment of genetic relatedness among three varieties of finger millet with variable seed coat color using RAPD and ISSR markers. Genetic Engineering and Biotechnology Journal, 2010: GEBJ-2.

27. Zimmer EA, Roalson EH, 2005. Molecular Evolution Producing the Biochemical Data. Part B, Volume 395, Academic Press, Elsevier, USA, 1-896.