Assessment of Genetic Diversity among Wild Accessions of *Abrus precatorius* L. using RAPD and ISSR Markers

Gurinder Jit Randhawa[1*], Monika Singh[1] and Veena Gupta[2]

[1]*National Research Centre on DNA Fingerprinting, National Bureau of Plant Genetic Resources, Pusa Campus, New Delhi-110012, India*

[2]*Germplasm Conservation Division, National Bureau of Plant Genetic Resources, Pusa Campus, New Delhi-110012, India*

Genetic diversity was estimated among eight wild accessions of *Abrus precatorious* L. collected from different areas of Maharashtra, Orissa and West Bengal using Random Amplified Polymorphic DNA (RAPD) and Inter Simple Sequence Repeat (ISSR) markers. Out of 40 random primers used, 28 were polymorphic, generating a total of 175 amplification products with an average of 6.25 products per polymorphic primer. Out of 35 ISSR primers, 20 were found polymorphic, generating a total of 81 amplification products with an average of 4.01 bands per polymorphic primer. Mean polymorphic information content values were 0.77 and 0.67 for RAPD and ISSR, respectively. Pair wise Jaccard's similarity coefficient values ranged from 0.357 to 0.740 for RAPD markers and 0.245 to 0.829 for ISSR markers, indicating high genetic variability among these genotypes. The accessions IC418115 from Thane and IC392840 from Mayurbhanj displayed the maximum genetic similarity. The cluster analysis discriminated all the accessions from Maharashtra in one group. The degree of genetic variation detected using RAPD and ISSR analysis among eight accessions suggests that these markers can be employed for genetic diversity studies in *Abrus precatorius*.

Key Words: *Abrus precatorius* L., Genetic diversity, ISSR, Medicinal plant, RAPD

Introduction

The genus *Abrus* is a small pantropic genus (Isely, 1990) of the tribe Viceae of Papilionoidae-Leguminosae (Hutchinson and Dalziel, 1958). It grows in tropical climates such as India, Sri Lanka, Thailand, the Philippine Islands, South China, tropical Africa and the West Indies. *Abrus precatorius* L. is a wild plant that grows best in fairly dry regions at low elevations. *Abrus precatorius* is an important medicinal plant of India that holds a reputed position in Ayurvedic System of Medicine. The leaves of *Abrus* contain glycyrrhizin, triterpenol saponins, flavonol glycosides, isoflavoquinones, abrine etc; (Windholz, 1983). The seeds of *Abrus* have high protein content, *i.e.*, 17-20 mg/ml (Gupta *et al.*, 2006a), carbohydrates, lipids, saponins, and sterols; seeds are also abortifacient, anodyne, aphrodisiac, antimicrobial, diuretic, emetic, expectorant, febrifuge, hemostat, laxative, purgative, refrigerant and sedative. Vermifuge paste of seeds is applied locally in sciatica, stiffness of shoulder joints and paralysis. Roots are used for gonorrhoea, jaundice and haemoglobinuric bile (http://www.motherherbs.com/abrus-precatorius.html). The oil extracted from seeds promotes the growth of human hair. The seeds of *Abrus* under the name of *Rati*, weighing about 1 carat each, have been used in India since ancient times for the purpose of weighing gold (http://www.botanical.com).

There is an urgent need to look for new sources of varieties to widen the genetic base of the high yielding varieties to make them more adaptable to the local environment. Wild populations found within the same agro climatic conditions of the cultivated varieties offer the advantage of being utilized more easily for the breeding purpose than those found in far away places. So to conserve the precious genetic resources of wild populations for their proper utilization, it is imperative to assess the genetic variability present among wild populations along with their genetic inter-relationships with the cultivated relatives (De Bustos *et al.*, 1998). Both natural and man-made factors have been responsible for limiting the distribution and genetic diversity of medicinal plant species and are causing them to become rare or even extinct. Prior to breeding programmes for increasing the pharmacological alkaloid content, it is imperative to assess the range of chemical and genetic variability in natural populations of *Abrus precatorius* and to establish the possible concordance/ discordance relationship between the genetic and chemical profiles. Random Amplified Polymorphic DNA (RAPD) and Amplified Fragment Length Polymorphism (AFLP), the Polymerase Chain Reaction (PCR)-based markers are most widely used genetic tools for assessment of the genetic variation in the medicinal plants (Singh *et al.*, 1999; Bejaj *et al.*, 2002;

*Corresponding author: E-mail: gurinder.randhawa@rediffmail.com, gjr@nbpgr.ernet.in

Deshwal *et al.*, 2005; Padmesh *et al.*, 2006). RAPD markers are generated by using short, decamer oligonucleotides of random sequence (Williams *et al.*, 1990). These markers are generally dominant and detect variation in both coding as well as non-coding regions of the genome. RAPD analysis is technically simple and suitable for large-scale germplasm characterization and can be performed even in a moderately equipped laboratory (Rafalski and Tingey, 1993).

The ISSRs are semiarbitrary markers amplified by PCR in the presence of one primer complementary to a target microsatellite (Zietkiewicz *et al.*, 1994). ISSRs have been proven to be a rapid, simple, and inexpensive way to assess genetic diversity in plants (Kantety *et al.*, 1995; Tsumura *et al.*, 1996; Tanyo-lac, 2003), to identify closely related cultivars (Fang *et al.*, 1997, 1998; Eiadthong *et al.*, 1999; Martins *et al.*, 2003), and to study evolutionary processes, such as reproductive systems (Liston *et al.*, 2003) and gene flow (Wolfe *et al.*, 1998). ISSRs have also been recently utilized as markers to assess genetic diversity in medicinal plants (Fracaro and Echeverrigaray, 2006; Apte *et al.*, 2006).

So far, no report is available on the molecular characterization and determination of genetic relationship among the accessions except that of Gupta *et al.* (2006b), in which genotypic variations were analyzed in three *Abrus precatorius* accessions using three isozymes, *viz.*, esterase, acid phosphatases and peroxidase.

In the present study, DNA profiling of eight accessions of *Abrus precatorius* L. has been carried out using RAPD and ISSR markers with an objective of evaluating genetic relationships among the accessions collected from different areas of Maharashtra, Orissa and West Bengal.

Materials and Methods

Plant Materials

The experimental material comprised the seed samples of eight wild accessions of *Abrus precatorius* L. that were collected from three states of India, *viz.*, Maharashtra, Orissa and West Bengal under National Agricultural Technology Programme (NATP) and conserved in the National Gene Bank, NBPGR, New Delhi. Seed characteristics such as seed colour, seed weight, seed size and shape were analysed. The detailed description of the *Abrus* accessions along with their seed characteristics used in the present study is given in Table 1.

Genomic DNA Extraction

The collected seeds of *Abrus* were germinated as per ISTA rules (2003) after scarification using BP between towel papers method at 25°C. Total genomic DNA was isolated from young leaves from the 2-3 weeks old seedlings of

Table 1. Details of *Abrus precatorius* L. accessions used

Accession no.	Collection no.	Seed weight (g)	Seed size (mm)	Seed shape	Seed colour	Source (village/state)
IC418096	BBJ-606	2.56	0.8	Ovoid	Red with black eye	Ramwadi, Thane (Maharashtra)
IC418097	BBJ-607	2.11	0.7	Ovoid-subglobose	Dull white with brown eye	Jambhulpada, Thane (Maharashtra)
IC418103	BBJ-613	2.64	0.8	Subglobose	White	Bebgi, Thane (Maharashtra)
IC418115	BBJ-625	2.57	0.8	Ovoid	White	Dabosa, Thane (Maharashtra)
IC418120	BBJ-630	2.07	0.7	Ovoid-subglobose	Red with black eye	Vaki, Thane (Maharashtra)
IC392840	SARC/DP-10	2.55	0.9	Ovoid	Red with black eye	Aonlabadi, Mayurbhanj (Orissa)
IC391930	SARC/AS-48	2.61	0.9	Ovoid	Red with black eye	Jaladia, Keonjhar (Orissa)
IC427646	SBC-2/50	2.52	0.5	Subglobose	Dull white with brown eye	Satjele Emblibadi, 24 Paragana (West Bengal)

Abrus accessions using modified SDS (sodium dodecyl sulphate) protocol described by Dellaporta *et al.* (1983) along with the modifications. Different percentages of Poly Vinyl Pyrrolidone (MW 40,000) were used in the extraction buffer and 0.2% was found satisfactory to tackle the problem of phenolics. The isolated DNA was quantified by VersaFluor™ Fluorometer and its quality was checked on 0.8% agarose gels by horizontal electrophoresis.

RAPD and ISSR Analyses

RAPD analysis was performed using 40 random sequence decamer primers (Operon Technologies Inc., Alameda, USA) to screen polymorphism among *Abrus* accessions. Amplification reactions were carried out in 25μl reaction mixture containing 25 ng of genomic DNA, 1x*Taq* DNA polymerase buffer (50 mM KCl, 10 mM Tris-HCl pH 8.3), 1.5 mM $MgCl_2$, 200 μM dNTPs (Promega, USA), 10 pmoles of random primer and 1 unit of *Taq* DNA polymerase (Bangalore Genei, India). PCR amplification of the isolated DNA was done in a PTC-200 Thermal Cycler (MJ Research Inc, USA). The sequential amplification steps involved: 1 cycle at 94°C for 5 min (initial denaturation); followed by 40 cycles of 1 min at 94°C (denaturation), 1 min at 37°C (primer annealing) and 2 min at 72°C (primer extension). The last cycle was followed by 7 min final extension at 72°C. Thirty five ISSR primers (The University of British Columbia, Canada) were used for ISSR analysis. ISSR amplifications were performed in a total volume of 25 μl with 25 ng of genomic DNA, 1x *Taq* DNA polymerase buffer (50 mM KCl, 10 mM Tris-HCl pH 8.3), 1.0 mM $MgCl_2$, 200 μM dNTPs (Promega, USA), 10 pmoles of primer and 1 unit of *Taq* DNA polymerase (Invitrogen, USA). The amplification reactions were carried out on PTC-200 Thermal Cycler (MJ Research Inc, USA) with the following thermal profile: one cycle of 94°C for 4 min (initial denaturation) followed by 35 cycles of 45 sec at 94°C (denaturation), 45 sec at 39°C (primer annealing), and 2 min at 72°C (primer extension), and finally, one cycle of 7 min at 72°C.

The amplified PCR products were size fractionated on 1.6% agarose gel in 1x Tris-acetate EDTA (TAE) buffer stained with ethidium bromide. The electrophoretic patterns were visualized under UV light and photographed using Gel Documentation System (Alpha Innotech Corporation, USA). The reproducibility of the amplification was confirmed by repeating each experiment twice.

Data Analysis

Allele sizes (in nucleotide base pair) were determined on the basis of its migration relative to standard size marker, *i.e.*, 1Kb DNA ladder (MBI Fermentas). RAPD and ISSR bands were scored for a binary data matrix, coded by 1 and 0, respectively for their presence and absence across all the *Abrus* accessions, for each primer. The pair wise genetic similarities among all the pairs of samples were estimated with Jaccard's similarity coefficient (Jaccard, 1908). The statistical analysis was performed using NTSYS-pc software (version 2.11S). UPGMA (Unweighted Pair Group Method of Arithmetic Means) was employed to construct the dendrogram using SAHN program to group accessions into discrete clusters based on RAPD and ISSR data.

Polymorphism information content (PIC) was also determined for each SSR primer set as described by Smith *et al.* (1997).

$$PIC = 1-\Sigma f_i^2$$

where f_i is the frequency of ith allele.

Results and Discussion

Genetic Diversity among Abrus accessions using RAPD and ISSR Markers

Eight accessions of *Abrus precatorius* L. used in the present study were collected from different areas of Maharashtra, Orissa and West Bengal and were characterized morphologically with respect to seed weight, seed size, seed shape and seed colour to find the relationship with molecular markers. Forty RAPD primers were screened for polymorphism in *Abrus precatorius* L. accessions, of which 28 primers (70.0%) generated polymorphic patterns (Table 2). A total of 175 bands were produced with an average of 6.25 bands per polymorphic primer. Among these, 152 (86.85%) were polymorphic ranging between 2 to 12; the maximum number of bands was detected with OPC 18 (Fig. 1). The average PIC was 0.77, ranging from 0.44 to 0.89. The lowest and highest PIC values were recorded for primer OPX 04 and OPC 19, respectively. In ISSR analysis, 35 ISSR primers were used to study polymorphism and 20 of them (57.15%) showed polymorphism (Table 3). Polymorphic primers generated a total of 81 fragments with an average of 4.05 products per polymorphic primer. Out of these, 73 (90.12%) were polymorphic. The number of products amplified by the polymorphic primers varied from 1 to 8 (Fig. 2), the maximum number of products were detected with the primers UBC 811 and UBC 834. The average

Table 2. List of polymorphic primers and their sequence used for RAPD analysis

Primer Code	Sequence (5′-3′)	Bands scored		Amplicon size range (in bp)	PIC
		Total	Polymorphic		
OPC 02	GTGAGGCGTC	6	1	550-2200	0.83
OPC 03	GGGGGTCTTT	5	5	400-1300	0.76
OPC 04	CCGCATCTAC	8	7	500-1900	0.84
OPC 05	GATGACCGCC	7	2	280-2000	0.84
OPC 06	GAACGGACTC	6	2	450-2000	0.80
OPC 07	GTCCCGACGA	4	4	500-1300	0.77
OPC 09	CTCACCGTCC	3	3	200-850	0.62
OPC 10	TGTCTGGGTG	5	3	350-1200	0.74
OPC 11	AAAGCTGCGG	9	9	400-1500	0.86
OPC 15	GACGGATCAG	4	3	700-1550	0.74
OPC 16	CACACTCCAG	8	8	400-1800	0.85
OPC 17	TTCCCCCCAG	3	3	900-1500	0.62
OPC 18	TGAGTGGGTG	12	12	200-1900	0.88
OPC 19	GTTGCCAGCC	10	10	200-1750	0.89
OPF 17	AACCCGGGAA	4	4	500-1200	0.74
OPG 08	TCACGTCCAC	7	7	450-1500	0.84
OPG 20	TCTCCCTCAG	9	9	250-2000	0.85
OPK 20	GTGTCGCGAG	6	6	500-1800	0.79
OPM 14	AGGGTCGTTC	5	5	800-1500	0.76
OPP 19	GGGAAGGACA	10	10	600-2000	0.88
OPS 07	TCCGATGCTG	6	6	400-1800	0.82
OPX 04	CCGCTACCGA	2	2	1200-1500	0.44
OPX 05	CCTTTCCCTC	7	7	750-2000	0.83
OPX 07	GAGCGAGGCT	2	2	1500-1900	0.50
OPY 17	GACGTGGTGA	10	6	250-1400	0.88
OPY 20	AGCCGTGGAA	6	6	600-1500	0.80
OPZ 19	GTGCGAGCAA	2	2	750-1000	0.50
OPZ 20	ACTTTGGCGG	9	8	500-2000	0.87

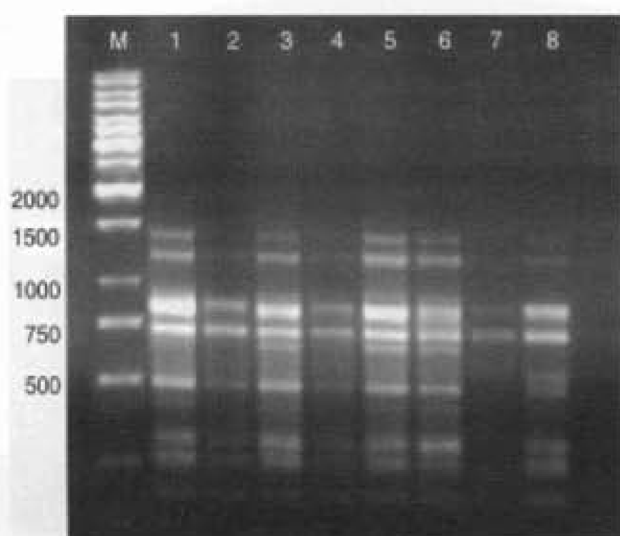

Fig. 1a

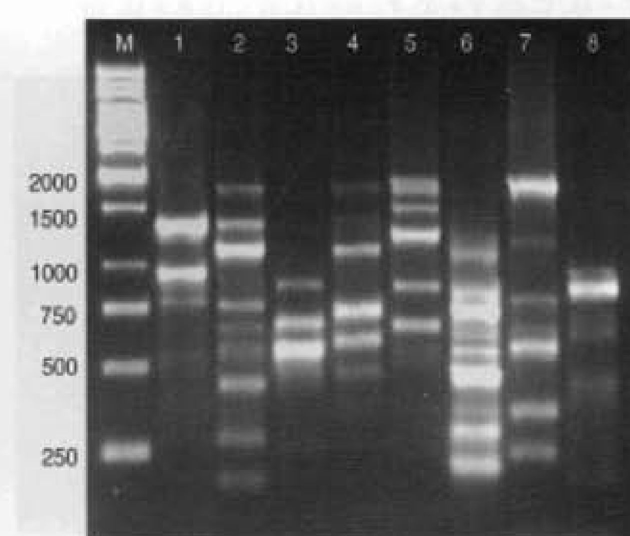

Fig. 1b

Fig. 1: RAPD profile of 8 *Abrus precatorius* L. accessions amplified by primers OPC 04 (Ia) and OPC 18 (1b) Lane-1:IC418096, Lane-2:IC418097, Lane-3:IC418103, Lane-4:IC418115, Lane-5:IC418120, Lane 6:1C392840, Lane-7:IC427646 and Lane-8:IC391930, Lane M-1kb DNA ladder

PIC was 0.67, and the lowest and highest PIC values were 0.32 (UBC 815) and 0.85 (UBC 811), respectively.

Genetic Similarity Matrix and Cluster Analysis

RAPD and ISSR data were used to make pair wise comparisons of the accessions based on shared and unique amplification products to generate a similarity matrix. Similarity coefficient values based on Jaccard's coefficient ranged from 0.357 to 0.740 with RAPD and from 0.245 to 0.829 with ISSR markers, reflecting broad range of genetic similarity (Tables 4, 5). The accessions IC418115 from Thane (Maharashtra) and IC392840 from

Table 3. List of polymorphic primers and their sequence used for ISSR analysis

Primer Code	Sequence (5'-3')	Bands scored		Amplicon size range (in bp)	PIC
		Total	Polymorphic		
UBC 801	ATA TAT ATA TAT ATA TT	4	2	800-2000	0.75
UBC 805	TAT ATA TAT ATA TAT AC	3	3	1200-2500	0.60
UBC 809	AGA GAG AGA GAG AGA GG	6	4	900-1400	0.80
UBC 811	GAG AGA GAG AGA GAG AC	8	8	450-2000	0.85
UBC 812	GAG AGA GAG AGA GAG AA	7	7	600-1800	0.84
UBC 813	CTC TCT CTC TCT CTC TT	5	5	500-1200	0.75
UBC 814	CTC TCT CTC TCT CTC TA	2	2	1000-1500	0.41
UBC 815	CTC TCT CTC TCT CTC TG	2	1	800-1500	0.32
UBC 817	CAC ACA CAC ACA CAC AA	3	2	550-1000	0.54
UBC 820	GTG TGT GTG TGT GTG TC	2	1	1500-1800	0.50
UBC 827	ACA CAC ACA CAC ACA CG	4	4	1000-1500	0.74
UBC 828	TGT GTG TGT GTG TGT GA	4	4	850-1200	0.72
UBC 829	TGT GTG TGT GTG TGT GC	2	2	450-1000	0.50
UBC 833	ATA TAT ATA TAT ATA TYG	2	1	1250-1500	0.84
UBC 834	AGA GAG AGA GAG AGA GYT	8	7	500-2200	0.84
UBC 836	AGA GAG AGA GAG AGA GYA	7	6	800-2000	0.84
UBC 840	GAG AGA GAG AGA GAG AYT	6	6	400-1600	0.83
UBC 843	CTC TCT CTC TCT CTC TRA	2	1	900-1500	0.50
UBC 845	CTC TCT CTC TCT CTC TRG	2	2	1000-1400	0.50
UBC 886	VDV CTC TCT CTC TCT CT	5	5	750-2000	0.78

V = (A, C, G); Y = (C, T); N = (A, G, C,T); R = (A, G)

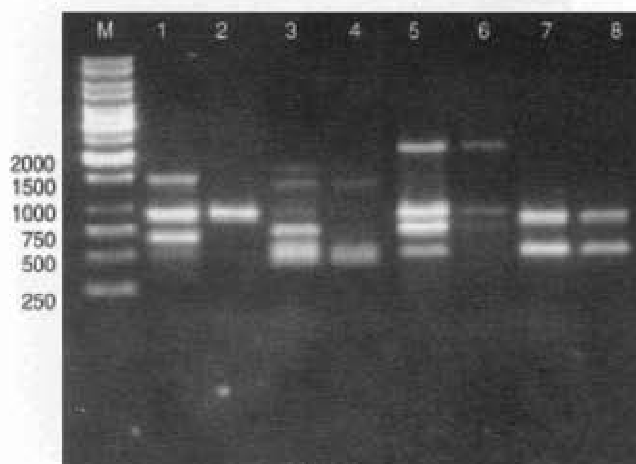

Fig. 2a

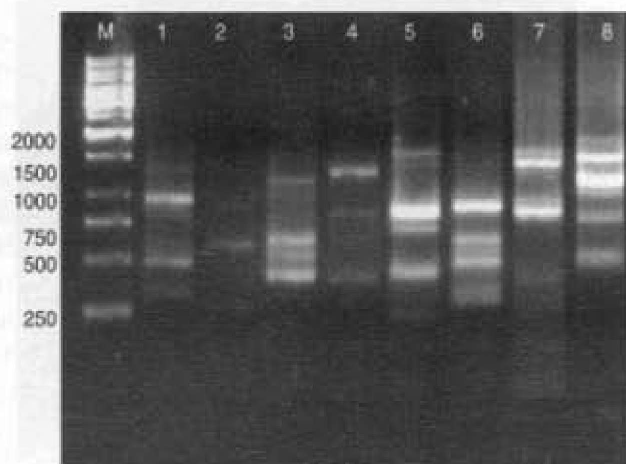

Fig. 2b

Fig. 2: ISSR profile of 8 *Abrus precatorius* L. accessions amplified using ISSR primers UBC 813 (2a) and UBC 840 (2b) Lane-1: IC418096, Lane-2: IC418097, Lane-3: IC418103, Lane-4: -IC418115, Lane-5: IC418120, Lane-6: IC392840, Lane-7: IC427646 and Lane-8: IC391930, Lane M: 1Kb DNA ladder

Mayurbhanj (Orissa) displayed the maximum genetic similarity where as IC418096 from Thane (Maharashtra) and IC391930 from Keonjhar (Orissa) showed the minimum genetic similarity as revealed by RAPD and ISSR.

In RAPD dendrogram, the accessions of *Abrus* clustered in two major groups (I and II) with subgrouping in cluster I (Fig. 3). The first cluster was divided into two sub groups a and b, one comprising of IC418096, IC418097 and IC427646 and other having IC418103, IC418120, IC418115 and IC392840. All the five accessions from Maharashtra were grouped in to two sub groups of the same cluster. The second cluster comprised of single accession belonging to Orissa, *viz.*, IC391930. In ISSR dendrogram, the two major clusters I and II were formed (Fig. 4), one consisting of six accessions IC418096, IC418103, IC418115, IC392840, IC418120 and IC418097 and other comprising IC427646 and IC391930. All the five accessions from Maharashtra were grouped in the same cluster. The cluster analysis based on RAPD and ISSR data revealed that the accessions IC418115 collected from Thane and IC392840 from Mayurbhanj shared the maximum similarity indicating that they are closely related where as IC418096 from Thane and IC391930 from Keonjhar were found far apart among the eight accessions. The cluster analysis discriminated all the accessions collected from Maharashtra in two sub-groups of single cluster although they showed morphological variations with respect to the seed characters. The accessions IC418115 collected from Thane (Maharashtra) and IC392840 from Mayurbhanj (Orissa) displayed the maximum genetic similarity where as IC418096 from Thane (Maharashtra) and IC391930 from Keonjhar (Orissa) showed the minimum genetic similarity as revealed by RAPD and ISSR data. The accessions IC392840 and IC391930 from Orissa having the similar seed characteristics were grouped into different clusters based on RAPD and ISSR data.

So far, there is no report available for molecular characterization and determination of genetic diversity in *Abrus precatorius* except that of Gupta *et al.* (2006b) study where the genotypic variation was studied in three *Abrus precatorius* accessions, *i.e.*, pink, red and white morphotypes using three isozyme systems, *viz.*, esterase, acid phosphatases and peroxidase. Thirty-two polypeptide bands ranging from Rm value 0.06 to 0.71 were detected. The similarity index analysis indicated that the pink genotype as possibly the natural hybrid of the white and red genotypes.

In the present study, eight accessions of *Abrus precatorius* L. collected from different areas of Maharashtra, Orissa and West Bengal were characterized morphologically with respect to seed weight, seed size, seed shape and seed colour and were used to find the

Table 4. Similarity matrix of *Abrus precatorius* L. accessions analyzed using RAPD data based on Jaccard's coefficient

	IC418096	IC418097	IC418103	IC418120	IC418115	IC392840	IC427646	IC391930
IC418096	1.000							
IC418097	0.655	1.000						
IC418103	0.583	0.452	1.000					
IC418120	0.594	0.472	0.722	1.000				
IC418115	0.516	0.420	0.665	0.686	1.000			
IC392840	0.506	0.390	0.644	0.719	**0.740**	1.000		
IC427646	0.561	0.586	0.409	0.466	0.415	0.396	1.000	
IC391930	**0.357**	0.476	0.248	0.302	0.265	0.313	0.416	1.000

Table 5. Similarity matrix of *Abrus precatorius* L. accessions analyzed using ISSR data based on Jaccard's coefficient

	IC418096	IC418097	IC418103	IC418120	IC418115	IC392840	IC427646	IC391930
IC418096	1.000							
IC418097	0.544	1.000						
IC418103	0.653	0.423	1.000					
IC418120	0.589	0.441	0.792	1.000				
IC418115	0.608	0.443	0.808	0.767	1.000			
IC392840	0.533	0.426	0.803	0.761	**0.829**	1.000		
IC427646	0.357	0.415	0.290	0.265	0.290	0.288	1.000	
IC391930	**0.245**	0.371	0.197	0.169	0.197	0.210	0.440	1.000

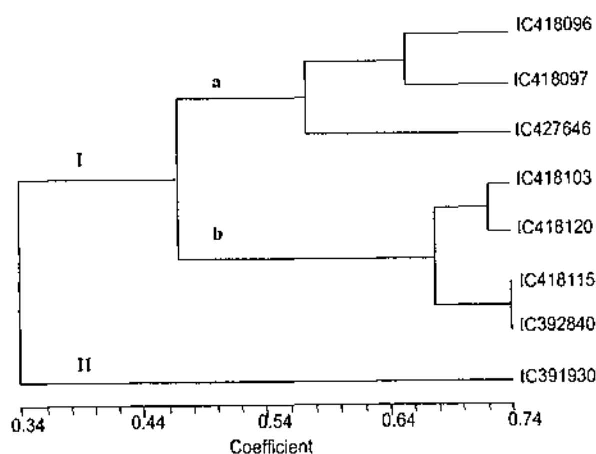

Fig. 3: Dendrogram generated by unweighted pair group method with arithmetic means based on RAPD data

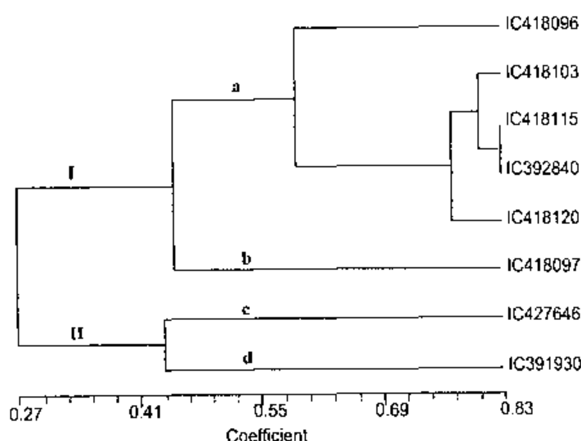

Fig. 4: Dendrogram generated by unweighted pair group method with arithmetic means based on ISSR data

relationship with molecular markers using RAPD and ISSR analyses. RAPD and ISSR analyses revealed an average of 86.85% and 90.12% polymorphism, respectively across all the eight *Abrus* accessions. In some of the recent reports, such high percentage polymorphisms using RAPD and ISSR markers have also been reported for other medicinal plants such as *Trigonella* (Dangi *et al.*, 2004) and *Mucuna pruriens* (Padmesh *et al.*, 2006).

The high levels of polymorphism detected in the accessions of *Abrus precatorius* may be attributed to the broad genetic base of the species that in the process of speciation might have acquired novel gene combinations for better adaptability in the changing environmental conditions. The high number of polymorphic products generated by certain RAPD primers may be due to the fact that in RAPD even small divergence between two accessions can result in distinct patterns as polymorphism may be due to single nucleotide change within the primer binding site, insertion or deletion with the amplified region so that part of the primer binding site in one of the strand is missing, complete absence of complementary sites, and the region between the binding sites on opposite strands is beyond the normal amplifiable length (Padmesh *et al.*, 2006).

Polymorphic information content (PIC) provides an estimate of the discriminatory power of a locus by taking into account, not only the number of alleles that are expressed, but also the relative frequencies of those alleles (Smith *et al.*, 1997). In the present study, a high level of polymorphism was observed among the eight *Abrus* accessions with both the marker systems, and average PIC values were 0.77 and 0.67 for RAPD and ISSR, respectively. The values of genetic similarity based on Jaccard's coefficient for all the eight accessions, ranged from 0.357 to 0.740 for RAPD markers and from 0.245 to 0.829 for ISSR markers, respectively, reflecting broad range of genetic dissimilarity (Tables 4, 5).

The cluster analysis discriminated all the accessions collected from Maharashtra in two sub-groups of single cluster although they showed morphological variations with respect to the seed characters. The accessions IC418115 collected from Thane (Maharashtra) and IC392840 from Mayurbhanj (Orissa) displayed the maximum genetic similarity where as IC418096 from Thane (Maharashtra) and IC391930 from Keonjhar (Orissa) showed the minimum genetic similarity as revealed by RAPD and ISSR data. Based on RAPD and ISSR data, the accessions IC392840 and IC391930 from Orissa exhibiting the similar seed characteristics were grouped into different clusters.

The present study indicates the utility of RAPD and ISSR markers for measuring genetic diversity and for DNA fingerprinting that would prove valuable for the breeding programme of *Abrus precatorius* L. These results are important for future genetic improvement, identification, and conservation of *Abrus precatorius* germplasm.

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