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Biochemical and molecular characterization of different *Abrus precatorius* L. genotypes varying in seed coat colour

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Abstract
The aim of the present study intended mainly to investigate an interrelationship among three varieties of *Abrus precatorius* L. Varying in seed coat colour i.e. red, white and black. Biochemical study includes seed protein profiling by SDS PAGE and three isoenzymes (Catalase, Peroxidase and Superoxide dismutase) pattern by native PAGE. Molecular study carried out by RAPD. There were ten primers used, of which six were reproducible and significant. In protein profiling study total of 38 polypeptide bands were recorded ranging in molecular weight from 15.00 kDa to 95.00 kDa. Out of these 38 polypeptide bands, 23 were common among all three genotypes and 5 bands were polymorphic. Dendrogram of RAPD shows that red and white varieties have more similarities and grouped into the same cluster and the black variety was somewhat differ and grouped into the second cluster.

Keywords: *Abrus precatorius,* dendrogram, molecular characterization, page, isozymes

1. Introduction
Molecular characterization can be effectively used to study genetic diversity. Genetic diversity is useful for several reasons, e.g., identifying cultivars, analyzing the pattern of gene flow, detecting phylogenetic relationships among closely related species, identifying duplication of accessions, selecting areas for additional plant exploration[1] and selecting diverse parents for crossing in a breeding program [2].
Electrophoretic analysis of proteins and isoenzyme offers an efficient and cost effective method towards cultivar identification and varietal purity tests in seeds [3-6]. Polyacrylamide gel electrophoresis (PAGE) is generally favored technique for rapid of the seed proteins and isozymes [7, 8] due to its validity and simplicity for describing genetic variations [9]. SDS-PAGE is most economical simple, rapid, accurate and extensively used biochemical techniques for analysis of cultivar identification this technique has been used effectively to decipher genetic diversity among/ between genotypes in different plant species [10, 11]. The phylogenetic relationships in many genera have been studied by isozymes electrophoresis [12, 13].
Seed protein electrophoresis has been successfully used in define species relationships in various groups of plants [14]. The technology of the molecular biology has been developed over the 20 years and provided new methods for observing the genetic differences among species. These techniques offer and give many advantages over the conventional methods [15].
Therefore, the present study was designed to clarify the genetic relationships among three varieties of *Abrus precatorius* varying in their seed coat colour (red, black and white).

1.1 Plant description

Table 1: *Abrus precatorius* plant Taxonomic Hierarchy: (NCBI Taxonomy browser, Taxon ID: 3816)

Plant Taxonomic Hierarchy			
	Kingdom	:	Plantae – plantes, Planta, Vegetal, plants
	Subkingdom	:	Viridaeplantae – green plants
	Infrakingdom	:	Streptophyta – land plants
	Division	:	Tracheophyta – vascular plants, tracheophytes
	Subdivision	:	Spermatophytina – spermatophytes, seed plants, phanérogames
	Infradivision	:	Angiospermae – flowering plants, angiosperms, plantas com flor, angiosperma, plantes à fleurs,
	Class	:	Magnoliopsida
	Superorder	:	Rosanae
	Order	:	Fabales
	Family	:	Fabaceae – peas, legumes
	Genus	:	*Abrus precatorius* L. – rosarypea, crab's eye, jequerity, pois rouge, precatory bean, rosary pea, tento muido

2. Materials and Method

Three varieties of *Abrus precatorius* vary from the seed coat colour subjected to analysis using available characterization methods. Viable seeds of the studied varieties were planted at Department of Biotechnology, Junagadh Agricultural University, Junagadh and different parts were used for the study.

2.1 Protein analysis

Seed proteins pattern of three varieties were studied by electrophoresis analysis SDS-PAGE technique [16].

2.2 Isozymes analysis

Isozymes variations identified using native polyacrylamide gel electrophoresis. Three isozymes (Catalase, Peroxidase and Superoxide dismutase) were studied. These isozymes were separated on polyacrylamide gel according to method given by Stegmann with some modification [17].

2.4 DNA extraction

Genomic DNA of three varieties of *Abrus precatorius* were extracted from fresh young leaves according to method given by Dellaporta with some modification [18].

2.5 Random amplified polymorphic DNA (RAPD-DNA)

Ten primers were used to generate RAPD markers according to method given by William with some modifications[19]. Of these ten primes, six primers have given significant and reproducible results are given in Table 2. The percentage of polymorphism can be calculated according to this equation.

$$\% \text{ of polymorphism} = \text{Polymorphic bands} / \text{Total bands} \times 100$$

2.6 Molecular characterization

The presence or absence of each band was treated as a binary character in a data matrix (coded 1 and 0 respectively) [20]. The bands with same mobility were treated as identical bands. The RAPDPCR data were entered into MS-Excel sheet and subsequently analysed using NTSY Spc version 2.02i. The data matrix was read by NTSY Spc version 2.02i and analysed by the SIMQUAL (similarity for qualitative data) program with Jaccard's similarity coefficient. The similarity matrix values were converted into Dendrogram using UPGMA (unweight pair group method with the arithmetic average) clustering method.

3. Results

3.1 Protein analysis

The electrophoretic banding patterns of extracted proteins have been studied in the three varieties of *Abrus precatorius*. These patterns were Plate.1. The following is a brief description of the banding profiles for all varieties. A total of 38 polypeptide bands were recorded. The size of these polypeptide bands ranged from 15.00 kDa to 95.00 kDa and Rm values were ranged from 0.229 to 0.600. Out of these 38 polypeptide bands, 23 were common among all three genotypes and 5 bands were polymorphic. There were some specific bands, which were peculiar to each *Abrus precatorius* genotypes. Black genotype had 3 specific polypeptide bands, which were absent in other two (Red & White) genotypes. Out of these three. One band with approximate molecular weight of 28 kDa was having high intensity and remarkably absent in other two verities.

Jaccard's similarity coefficient value ranged from 0.70 to 1.00. The most closely related genotypes were red and white varieties with the highest similarity index (1.00) whereas, black genotype showed same similarity index with red and white genotype (0.833) (Table.1). The similarity matrix was subjected to UPGMA clustering to generate dendrogram (fig.1). Lowest Jaccard's similarity value represents maximum diversity. Genotype with black seed coat colour was more diverse as compared to genotypes with red and white seed coat colour.

3.2 Isoenzymes

3.2.1 Isozymes Catalase

The electrophoretic analysis of catalase, peroxidise and SOD isozymes using native PAGE gel for the three varieties of *Abrus precatorius* recorded in Plate.2. Dendrogram for all the isozymes are given in figure.2.

Total 40 bands were observed in the catalase isozyme profiling (Rm value; 0.068, 0.110, 0.157, 0.217 & 0.263). Maximum number of bands was observed in white variety (17) and minimum bands were in black variety (09). The Jaccard's similarity value represents that the most closely related genotypes were *Abrus* white and red with the highest similarity index (0.476) whereas, black genotype showed minimum similarity index with white genotype (0.368).

3.2.2 Isozymes Peroxidase

During this study, total 56 isoenzyme markers with Rm values 0.278, 0.378, 0.456, 0.552 & 0.736 were obtained. Maximum numbers of bands were observed in white variety (20) and minimum bands were in black variety (17). Jaccard's similarity value revealed that highest similarity index was found among red and black varieties (0.947).

3.2.3 Isozymes Superoxide dismutase

Total 58 bands of Rm values 0.100, 0.238, 0.284, 0.432, 0.497, 0.584, 0.662 and 0.860 were observed during this study. Maximum numbers of bands were found in white *Abrus precatorius* (20). Jaccard's similarity value represents that maximum similarity index was found among red and black variety (0.727).

3.3 DNA finger5print

In present investigation, three *Abrus precatorius* varieties were subjected to RAPD analysis using 10 arbitrary decamer primers was used for screening, and only those primers were selected for the present study which provides significant and reproducible amplification products under similar condition and its detail is given in table.2. Jaccard's similarity coefficient was calculated to obtain genetic variation among three genotypes (table.4). A dendrogram was constructed based on UPGMA cluster analysis (figure.3).

A total of 6 primers produced clear and reproducible amplified products. These primers produced a total of 119 amplified fragments, of which, 11 bands were polymorphic. The highest numbers of 26 bands were produced by OPC-05 primer followed lowest 13 bands by OPC-09. The polymorphic information content (PIC) was calculated for each primer (Table.3) and it was varied between 0.793 (OPC-09) and 0.890 (OPC-05) with an average of 0.855 per primer.

The RAPD data were subjected to statistical analysis for the calculation of Jaccard's similarity coefficient and cluster analysis by UPGMA (Unweighted pair-group method with arithmetic averages) using NTSYSpc-2.02i software. The dendrogram revealed that three varieties of Abrus were grouped into two main clusters viz. cluster-I and cluster-II. The first cluster-I consist of red and white and the black

variety was grouped into cluster-II. The highest similarity index (0.968) was observed between red and white genotypes and the lowest (0.903) similarity index was recorded between black and red.

4. Discussion

Biochemical and molecular techniques are provided approaches for evaluating genetic diversity in plants. These methods are favoured because they are independent of the developmental stage of the plant [21]. Biochemical evidences such as seed storage protein electrophoresis and isozyme polymorphisms are convenient evidences for assessing genetic relationships [22, 23].

The variation in SDS-PAGE of seed protein profiles have successfully been used to differentiate between species [24] and provide a valid source of taxonomic evidence for addressing the relationships at the different taxonomic levels [25].

SDS-PAGE analysis study revealed significant variation in polypeptide banding pattern. Bands with same mobility were considered as identical fragments, regardless of their staining intensity. A total of 38 polypeptide bands were recorded. The size of these polypeptide bands ranged from 15.00 kDa to 95.00 kDa and Rm values were ranged from 0.229 to 0.600. Jaccard's similarity coefficient value ranged from 0.70 to 1.00. The most closely related genotypes were red and white varieties with the highest similarity index (1.00) whereas, black genotype showed same similarity index with red and white genotype (0.833). Genotype with black seed coat colour was more diverse as compared to genotypes with red and white seed coat colour.

Previous reports of Chittora, 2012 [26] support these results as the found nearly similar banding patterns in red, black and white varieties of *Abrus precatorius.* Kumar *et al* 2008 [27], has done this type of study in white variety for abrin protein characterization.

Isozymes polymorphisms are used effectively to assess genetic relationships among individuals, populations and closely related species [28, 29]. The applications of isozymes polymorphism are still important for population genetic studies and in addressing infra-specific relationships [30].

There are three isozymes were used to differentiate among the three varieties of *Abrus precatorius*. Isozyme study by native PAGE reveals the differentiation among the three varieties. The results concluded that the peroxidase and superoxide dismutase shows similar dendrogram pattern where red and white varieties came under same cluster and overall the black variety represents more differentiation among all three varieties.

As proteins and isozymes electrophoretic markers have major limitation of lack of enough polymorphism among closely related cultivars [31]. For this reason, DNA based genetic markers have been integrated into several plant systems and are playing a very important role in molecular genetics and plant breeding [31, 32]. Randomly amplified polymorphic DNA (RAPD) technique has been used in many different applications involving the detection of DNA sequence Polymorphisms to identify varieties and to assess the genetic diversity [33, 34, 35].

In this study ten primers used to differentiate among the three varieties of *Abrus precatorius*, of which six primes were reproducible and significant. These primers produced a total of 119 amplified fragments, of which, 11 bands were polymorphic. The highest numbers of bands were produced by OPC-05 (26) primer followed lowest bands by OPC-09(13). The polymorphic information content (PIC) was varied between 0.793 (OPC-09) and 0.890 (OPC-05) with an average of 0.855 per primer.

Cluster analysis was conducted to generate a dendrogram Fig. 2. All three varieties were divided into two groups. The first group includes red and white varieties and the second group includes black variety.

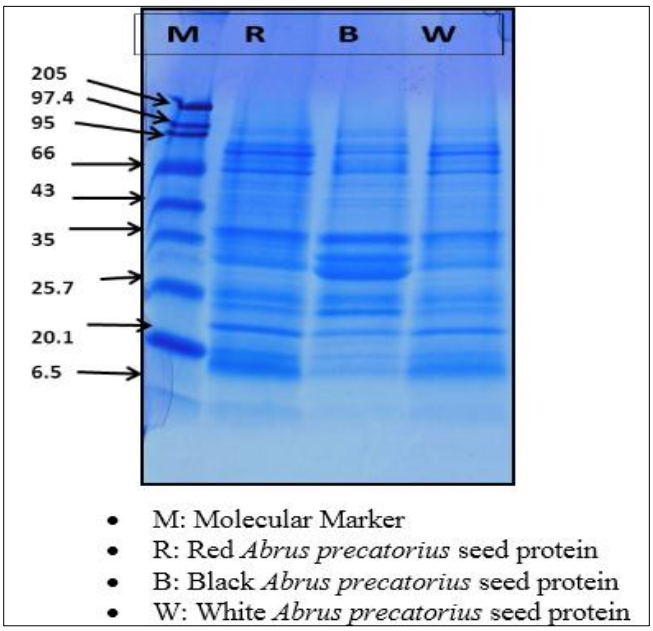

Plate 1: SDS PAGE of seed protein from red, black and white *Abrus precatorius* L.

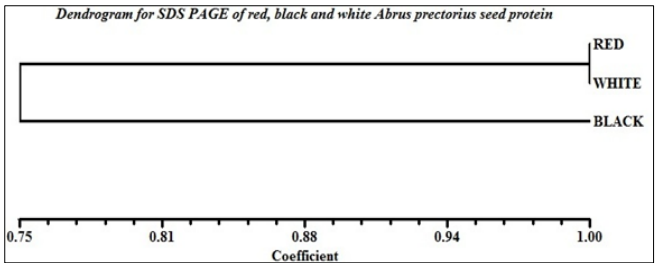

Fig 1: Dendrogram for SDS PAGE from red, black and white *Abrus precatorius* seed protein.

Table 1: Jaccard's similarities from SDS analysis

	Red	Black	White
Red	1.000		
Black	0.833	1.000	
White	1.000	0.833	1.000

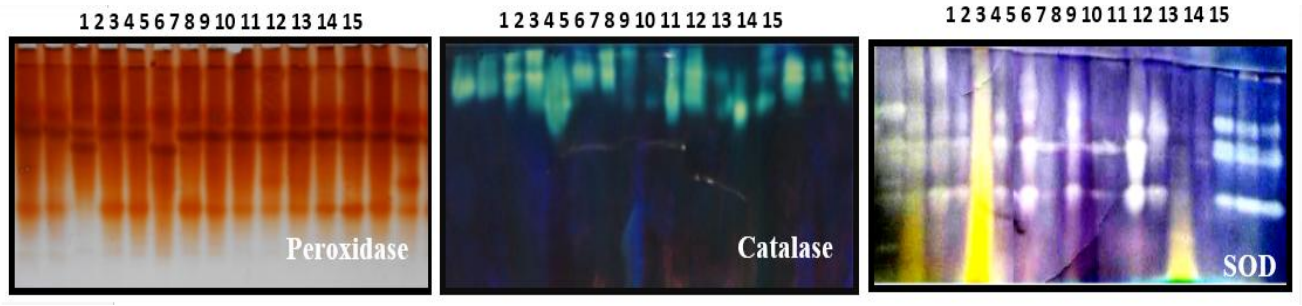

Plate 2: The electrophoresis pattern of three isoenzymes from different plant parts of *Abrus precatorius* (Red, White & Black).

1	Red Root	4	Red stem	7	Red leaf	10	Red pod	13	Red seed
2	Black root	5	Black stem	8	Black leaf	11	Black pod	14	Black seed
3	White root	6	White stem	9	White leaf	12	White pod	15	White seed

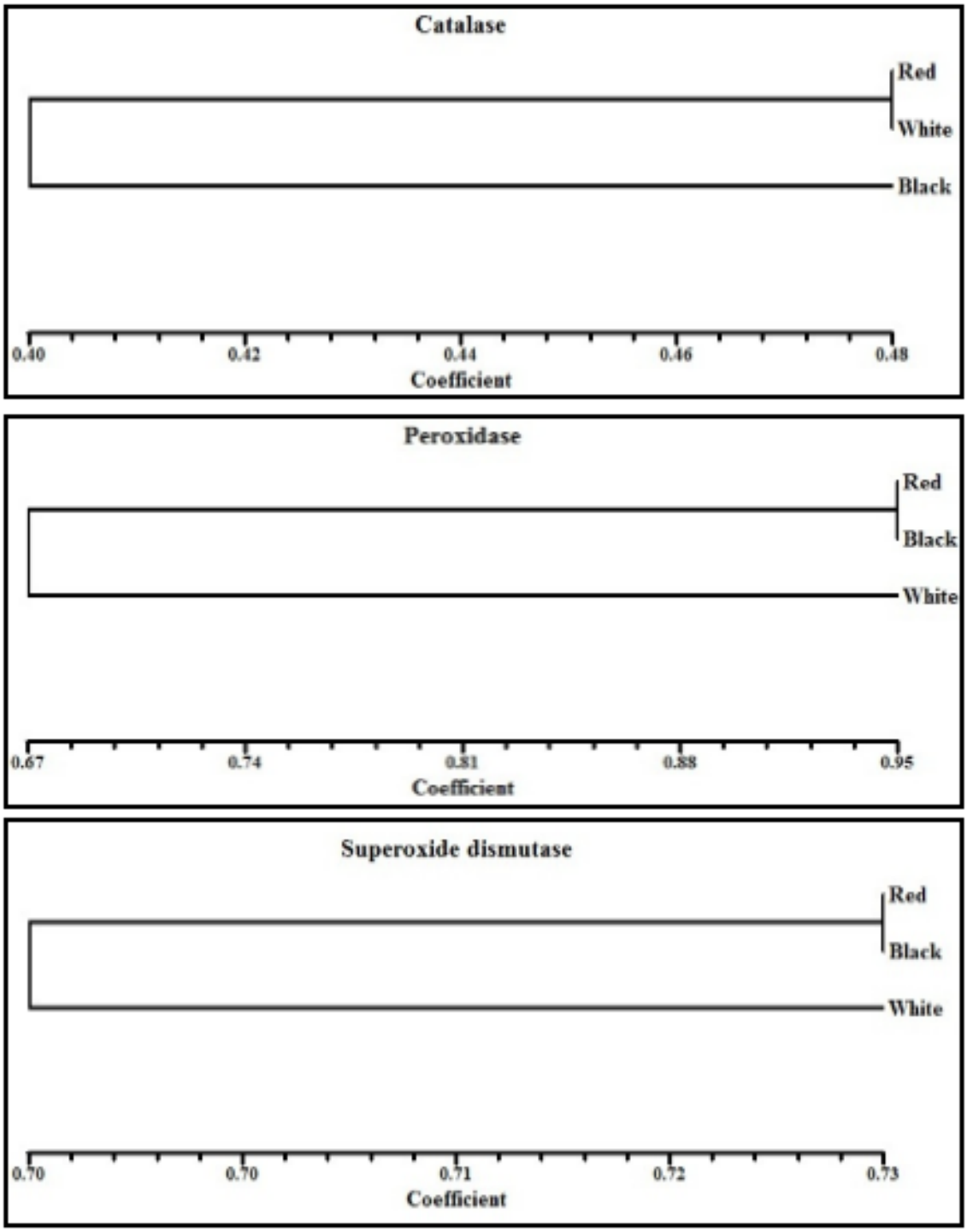

Fig 2: Dendrogram for various isozymes

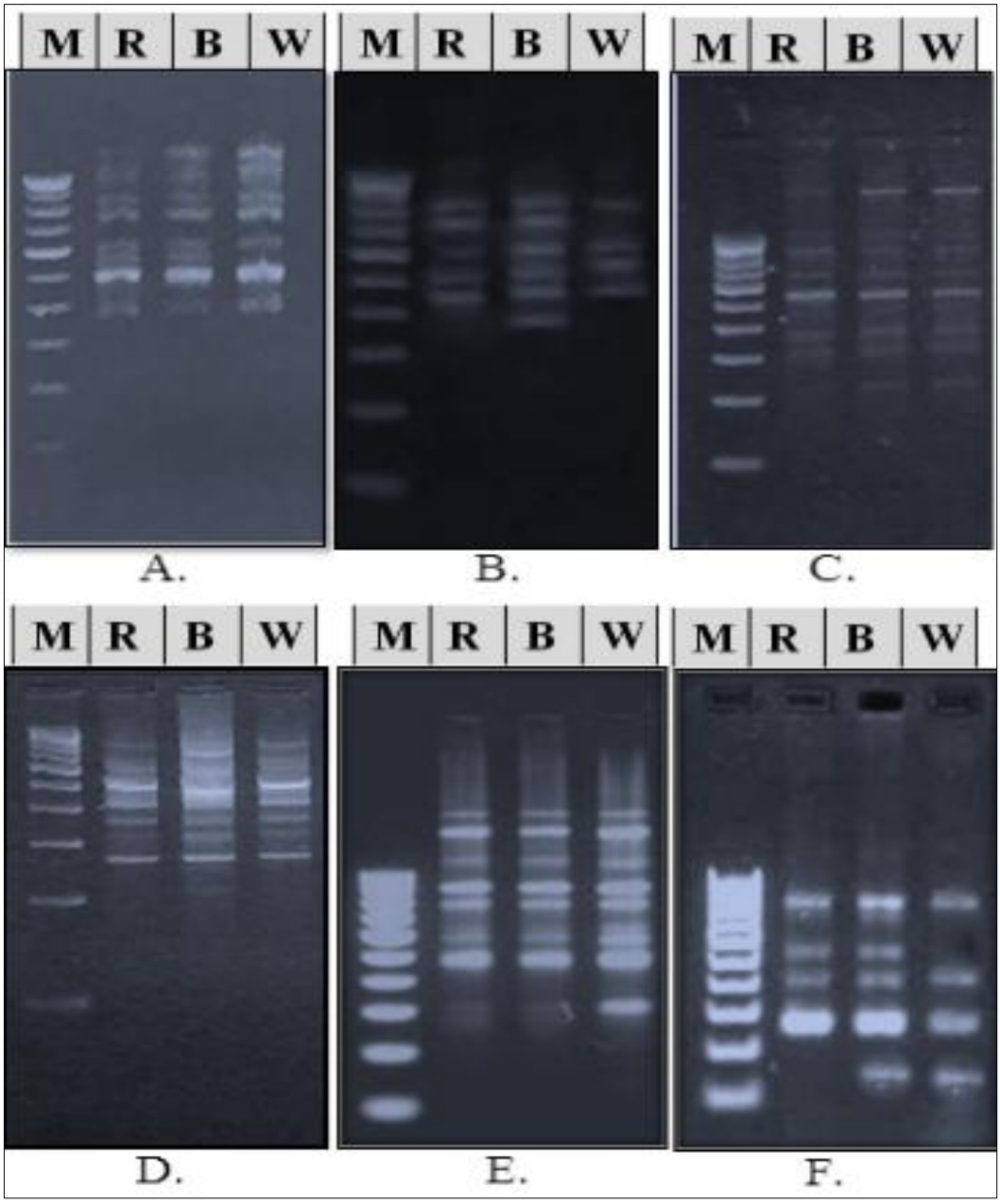

Plate 3: RAPD profiling of *Abrus precatorius* L. Genotypes showing banding patterns using different primers

A	OPA 02	B	OPA 07	C	OPA 16
D	OPA 18	E	OPA 05	F	OPA 09

Table 2: List of RAPD primers used in the present study

S. No.	RAPD Primer	Sequence (5`→3`)	Tm	GC (%)
1	OPA-02	5'-ACAACTGGGG-3'	25.0	60%
2	OPA-07	5'- GAAACGGGTG -3'	25.0	60%
3	OPA-16	5'- AGCCAGGAA -3'	25.0	60%
4	OPA-18	5'- AGGTGACCGT -3'	25.0	60%
5	OPC-05	5'-GATGACCGCC -3'	27.0	70%
6	OPC-09	5'- CTCACCGTCC -3'	27.0	70%

Table 3: Size, number of amplified bands, per cent polymorphism and PIC obtained by RAPD primers.

No.	RAPD primers	Total No. of Allele/bands (A)	No. of Polymorphic bands (B)	% polymorphism (B/A)	No of Unique band	PIC value
1	OPA-02	23	2	8.69	0	0.868
2	OPA-07	15	2	13.33	1	0.835
3	OPA-16	21	1	4.76	0	0.871
4	OPA-18	21	3	14.30	1	0.871
5	OPC-05	26	2	7.69	0	0.890
6	OPC-09	13	1	7.69	0	0.793
	Total	119	11	9.24		

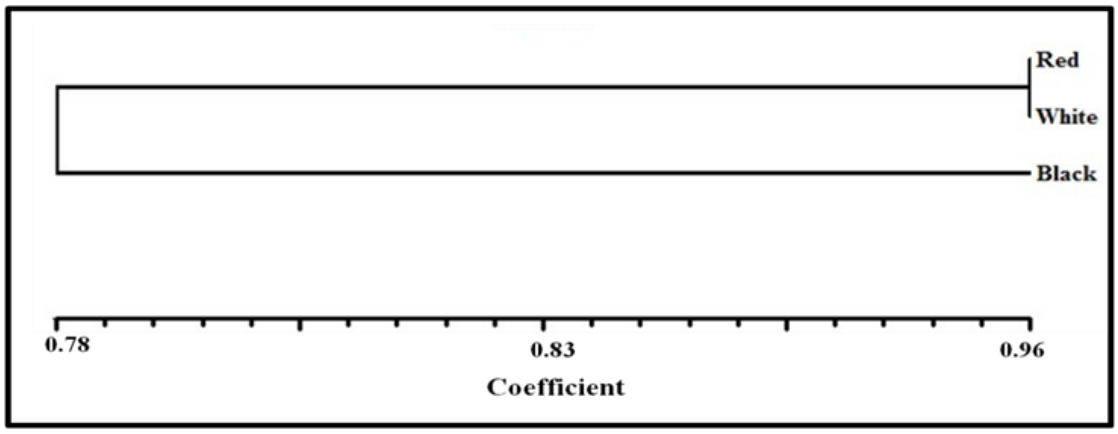

Fig 3: Dendrogram for RAPD profile of *Abrus precatorius* genotypes

Table 4: Jaccard's similarity coefficient of three genotypes of *Abrus precatorius* based on RAPD data.

	Red	Black	White
Red	1.000		
Black	0.903	1.000	
White	0.968	0.917	1.000

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Conflicts of interest: None

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