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Research Paper

OPTIMIZATION OF SDS-PAGE CONDITIONS AND ANALYSIS OF SEED PROTEIN DIVERSITY IN *ABRUS PRECATORIUS* GENOTYPES WITH DIFFERENT SEED COAT COLOUR

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SDS-PAGE technique was used for the study of seed protein polymorphism among three genotypes of Abrus precatorius with different seed coat colour. A total of 44 polypeptide bands were recorded. Out of these 26 were common among all three genotypes and 18 (40.90%) were polymorphic. The data analysis using UPGMA clustering revealed that genotypes with black and white seed coat colour were closer as compared to genotype with red seed coat colour. Each of three genotypes of A. precatorius had some polypeptide bands which were peculiar to them only. This enabled distinguishing all three genotypes on the basis of specific polypeptide fragments using SDS-PAGE analysis.

Keywords: *Abrus precatorius*, Genotype, Genetic markers, SDS-PAGE

INTRODUCTION

Abrus precatorius L. commonly known as Coral pea or Rati. is a medicinal plant belonging to family Fabaceae. It is a climbing perennial herb which grows wild in the dry regions at low elevations. This plant is mainly valued traditionally and scientifically for its anti-tumor potential (Sudaroli and Chatterjee, 2007). Three common genotypes based on seed coat colour (Red, Black and White) have been reported in *A. precatorius* (Karnick, 1978). Colour and pattern of seed coat are basic phenotypic features often used to distinguish genotypes of higher plant species (McClean *et al*., 2002). Red seeded genotype is reputed for its anti-tumor (Ramnath *et al*., 2002) and anti-inflammatory properties (Rosin *et al*., 1994), whereas, white seeded genotype is used against leucoderma, leprosy, ulcer and tumor (Khare, 2004). Control of phenotypic characteristics such as seed coat pattern and colour by specific gene have been identified in a number of plant species (Acampora *et al*., 2007). These genes might exhibit epistatic interaction with other genes which may define many seed coat colours and patterns within the species.

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Genetic markers such as morphological traits, biochemical characteristics (isozyme, protein profiles) and DNA based molecular markers are powerful tools for the analysis of genetic diversity and relatedness among genotypes, species and large populations of plants. Although, morphological trait can be used for assessing genetic diversity but it is often influenced by the environmental factors (Siddiqui and Naz, 2009). Biochemical markers such as proteins and isozymes have served as an important tools to detect genetic relationships in plants Mukhlesur *et al*., 2004). Protein polymorphism serves as genetic markers as they are direct products of active genes and are quite polymorphic and generally heritable (gepts, 1990). The polymorphism observed in the protein profiles reflects the changes in the active part of the genome. Although protein polymorphism can be analysed through a variety of techniques, polyacrylamide gel electrophoresis (PAGE) is generally favored technique for rapid analysis (Fergusan and Grabe, 1986; Smith and Smith, 1986; Ratmond *et al*., 1991) due to its validity and simplicity for describing genetic variations (Ahmed and slinkard, 1992). This technique has been used effectively to decipher genetic diversity among/ between genotypes in different plant species (Cook, 1984; Mukherjee and Datta, 2008).

In case of *A. precatorius* isozyme polymorphism among its natural populations (with Red, Black and Pink seed coat colour) has been reported. Studies on biochemical characteristics of red and white coated sedds have been carried out while SDS-PAGE based protein profiles and isozyme pattern in three accessions of *A. precatorius* have been studied. The present investigation was undertaken to study the seed protein polymorphisms among three genotypes of *A. precatorius* with different seed coat colour using SDS-PAGE.

MATERIALS AND METHODS

Plant Materials

Three genotypes of *A. precatorius* L. consisting of different seed coat colour (Black, White and Red) were chosen for the present investigation. For Protein extraction seeds were collected from the field grown plants, maintained in the Field Gene Bank in the nursery of University College of Science, Mohanlal Sukhadia University, Udaipur, India.

Extraction of Seed Proteins

In this investigation, five different protein extraction methods were used to extract protein from seeds as follows.

(a) Phosphate buffer (pH=7.0)

Seeds (1.0 g) were ground to fine powder using 10.0 ml 0.1M phosphate buffer (pH=7.0) containing - mercaptoethanol (10mM) and Phenylmethane Sulphonylfluoride (2.8mM). The extract was centrifuged at 10,000 rpm for 15 min at 4°C. The supernatant was again centrifuged at 10,000 rpm for 10 min at 4°C. The resulting supernatant was used as protein sample.

(b) Tris-HCl extraction buffer (pH=8.0)

1.0 g seeds were ground to fine powder using 2.8 ml buffer containing 200 mM Tris-Cl (pH 8.0), 100 mM NaCl, 10 mM EDTA, 0.1% MgCl2, 15% glycerol, 2.8 mM PMSF and 10 mM - mercaptoethanol (added freshly). It was centrifuged at 10,000 rpm for 15 min at 4°C. The supernatant was again centrifuged at 10,000 rpm for 10 min at 4°C. The resulting supernatant was used as protein sample.

(c) Sucrose-SDS buffer

In this method, 1.0 g seeds were ground to fine powder using 2.8 ml buffer containing 5% Sucrose, 4% SDS, 2.8 mM PMSF and 10 mM -mercaptoethanol (added freshly). It was centrifuged at 10,000 rpm for 15 min at 4°C. The supernatant was again centrifuged at 10,000 rpm for 10 min at 4°C. The resulting supernatant was used as protein sample.

(d) Tris-HCl extraction buffer / Acetone precipitation

1.0 g seeds were ground to fine powder using 2.8 ml buffer containing 200 mM Tris-Cl (pH 8.0), 100 mM NaCl, 10 mM EDTA, 0.1% MgCl2, 15% glycerol, 2.8 mM PMSF and 10 mM -mercaptoethanol (added freshly). It was centrifuged at 10,000 rpm for 15 min at 4°C. The supernatant was again centrifuged at 10,000 rpm for 10 min at 4°C. After centrifugation supernatant was precipitated with 5 volume chilled acetone. Mixed well and kept the tubes at -20°C for overnight. After the complete precipitation the protein was recovered at 7,000 rpm for 10 min at 4°C. The resultant pellet was dried and dissolved in 1.0 ml buffer.

(e) Sucrose-SDS buffer/Acetone precipitation

1.0 g seeds were ground to fine powder using 2.8 ml buffer containing 5% Sucrose, 4% SDS, 2.8 mM PMSF and 10 mM -mercaptoethanol (added freshly). It was centrifuged at 10,000 rpm for 15 min at 4°C. The supernatant was again centrifuged at 10,000 rpm for 10 min at 4°C. After centrifugation supernatant was precipitated with 5 volume chilled acetone. Mixed well and kept the tubes at -20°C for overnight. After the complete precipitation the protein was recovered at 7,000 rpm for 10 min at 4°C. The resultant pellet was dried and dissolved in 1.0 ml buffer.

Determination of protein concentration

The concentrations of proteins extracted by the five different methods were determined spectrophotometricaly using the Bradford method (Bradford, 1976).

Sample Application and Gel Electrophoresis

Protein sample was mixed with 4X gel loading dye to make its final concentration of 1X in mixture and was heated at 95°C in water bath for 10 min prior to loading. Protein sample (100 µg) was loaded in each lane. Protein molecular weight marker (Bangalore Genei, India) was used as reference. Protein samples were electrophoresed at 8 V/cm for about 4 h at constant current.

Staining of Gel

Preparative gels were visualized by staining with Coomassie Brilliant Blue R-250.

Data analyis

Gels were placed on a white light transilluminator and photographed. The polypeptide bands were analyzed using UVIBandMap software.

RESULTS

In the above investigation, five different methods of protein extraction were applied. Results have shown that the protein extracted with Tris-HCl extraction buffer (pH=8.0) consistently resulted in higher protein yields (32 mg/g of seeds) as compared to other methods (Table 1). When protein samples extracted from all the methods were subjected to electrophoresis, it was found that protein samples precipitated with acetone (method 4 and 5) could not provide discrete banding pattern and showed high degree of background noise while the other three protein samples (method 1, 2 and 3) showed more significant, scorable and discrete banding pattern. On comparing the number of resolved

polypeptide fragments, we found that protein extracted using Tris-HCl extraction buffer (pH=8.0) yielded maximum number of polypeptide fragments as compared to all other methods (Table 1). Therefore, Tris-HCl extraction buffer (pH=8.0) was finally selected for all further extractions.

The total seed protein extracts of all three genotypes (Black, Red and White) when subjected to SDS-PAGE analysis revealed significant variation in polypeptide banding pattern. Bands with same mobility were considered as identical fragments, regardless of their staining intensity. A total of 44 polypeptide bands were recorded. The size of these polypeptide bands ranged from 20.283 kDa to 143.715 kDa. Out of these polypeptide bands 26 were common among all three genotypes and 18 bands were polymorphic.

Each of three genotypes of *Abrus precatorius* had some polypeptide bands which were peculiar to them only. Red genotype had 5 specific polypeptide bands (126.445 kDa, 119.38 kDa, 88.819 kDa, 39.815 kDa and 38.164 kDa), which were absent in other two (Black and White) genotypes. Polypeptide bands of the molecular weight 127.23 kDa and 43.550 kDa were present only in white genotype, whereas two polypeptide bands (128.015 kDa and 41.949 kDa) were present only in black genotype. The phylogenetic analysis based on protein pattern placed white and black seeded genotypes very close to each other on the basis of 7 non-unique shared bands (12.950 kDa, 113.885 kDa, 91.376 kDa, 84.619 kDa, 40.037 kDa, 37.973 kDa and 35.328 kDa) which were absent in red genotype.

Jaccard.s similarity coefficient value ranged from 0.89 to 0.95. The similarity matrix was subjected to UPGMA clustering to generate dendrogram. Lowest Jaccard.s similarity value represents maximum diversity. Genotypes with black and red seed coat colour were more diverse as compared to genotype with white seed coat colour. The most closely related genotypes were *Abrus* white and black with the highest similarity index (0.95) whereas, black genotype showed minimum similarity index with red genotype (0.89).

DISCUSSION

Seed protein analysis using SDS-PAGE is particularly considered as a reliable technique because seed storage proteins are highly independent of environmental fluctuations. The high stability of seed protein profile and its additive nature makes it a promising tool for distinguishing genotypes of particular plant species. Therefore, in the present studies SDS-PAGE technique was employed for analysis of seed protein diversity in *A. precatorius* genotypes with different seed coat colour (Black, Red and White). SDS-PAGE technique has been successfully applied in many different plant species to estimate genetic diversity and phylogenetic relationship among genotypes.

In wheat, Siddique and Naz (2009) showed 5-84.0% polymorphism among 10 genotypes. Similarly, Khan *et al*. (2010) observed 0-60.0% genetic polymorphism with in twenty genotypes of walnut. Using SDS-PAGE, Inamullah *et al*. (2010) have showed 0-80% polymorphism among eleven genotypes of *Oryza sativa*. In *Capsicum*, 0-100% polymorphism was observed in nineteen genotypes (Akbar *et al*., 2010) while in Kabuli Chickpea genotypes significant polymorphism was reported (Hameed *et al*., 2009). Present studies carried out with three genotypes of *A. precatorius* revealed 5-11% polymorphism. SDS-PAGE analysis provided strong basis for the discrimination of genotypes on the basis of specific polypeptide fragments.

Table 1: Influence of Protein Extraction Buffer upon the amount of Total soluble Protein Content

S. No.	Buffer	Additive	Total soluble protein content in mg/g of seeds	Total number of resolved polypeptide bands
1	0.1 M Phosphate Buffer (pH=7.0)	2.8 mM PMSF 10 mM B-mercaptoethanol	30	26
2	0.2 M Tris-Cl Buffer (pH=8.0)	EDTA 0.1% MgCl2 100 mM NaCl 10 mM 15% glycerol 2.8 mM PMSF 10 mM β-mercaptoethanol	32	44
3	0.2 M Tris-Cl Buffer (pH=8.0)	5 % Sucrose 4 % SDS 2.8 mM PMSF 10 mM β-mercaptoethanol	13.3	22
4	0.2 M Tris-Cl Buffer (pH=8.0)	100 mM NaCl 10 mM EDTA 0.1% MgCl2 15% glycerol 2.8 mM PMSF 10 mM β-mercaptoethanol + Resulting Supernatant precipitated with Acetone	26.5	16
5	0.2 M Tris-Cl Buffer (pH=8.0)	5 % Sucrose 4 % SDS 2.8 mM PMSF 10 mM β-mercaptoethanol + Resulting Supernatant precipitated with Acetone	11.2	16

Table 2: Comparative Evaluation of Protein profiles among three genotypes of *A. precatorius*

S. No.	Molecular Weight	Red	White	Blac?
1	143.715	+	+	+
2	136.650	+	+	+
3	128.015			+
4	127.230		+	
5	126.445	+		
6	120.950		+	+
7	119.380	+		
8	113.885		+	+
9	104.465	+	+	+
10	91.376		+	+

Table 2 (Cont.)

S. No.	Molecular Weight	Red	White	Blac?
11	88.819	+		
12	84.619		+	+
13	77.362	+	+	+
14	72.826	+	+	+
15	67.281	+	+	+
16	63.580	+	+	+
17	58.405	+	+	+
18	49.160	+	+	+
19	44.405	+		+
20	43.550		+	
21	42.467	+	+	+
22	41.949			+
23	40.037		+	+
24	39.815	+		
25	38.164	+		
26	37.973		+	+
27	36.394	+	+	+
28	35.328		+	+
29	34.390	+		+
30	33.034	+	+	+
31	32.280	+	+	+
32	31.246	+	+	+
33	30.564	+	+	+
34	29.934	+	+	+
35	29.379	+	+	+
36	28.400	+	+	+
37	27.469	+	+	+
38	26.629	+	+	+
39	25.664	+	+	+
40	24.315	+	+	+
41	23.442	+	+	+
42	22.731	+	+	+
43	21.649	+	+	+
44	20.283	+	+	+

Table 3: Jaccard. s Similarity Coefficient Values of Three Genotypes of *A. precatorius*

Red	White	Black	
Red	1.0000		
White	0.9400	1.0000	
Black	0.8900	0.9500	1.0000

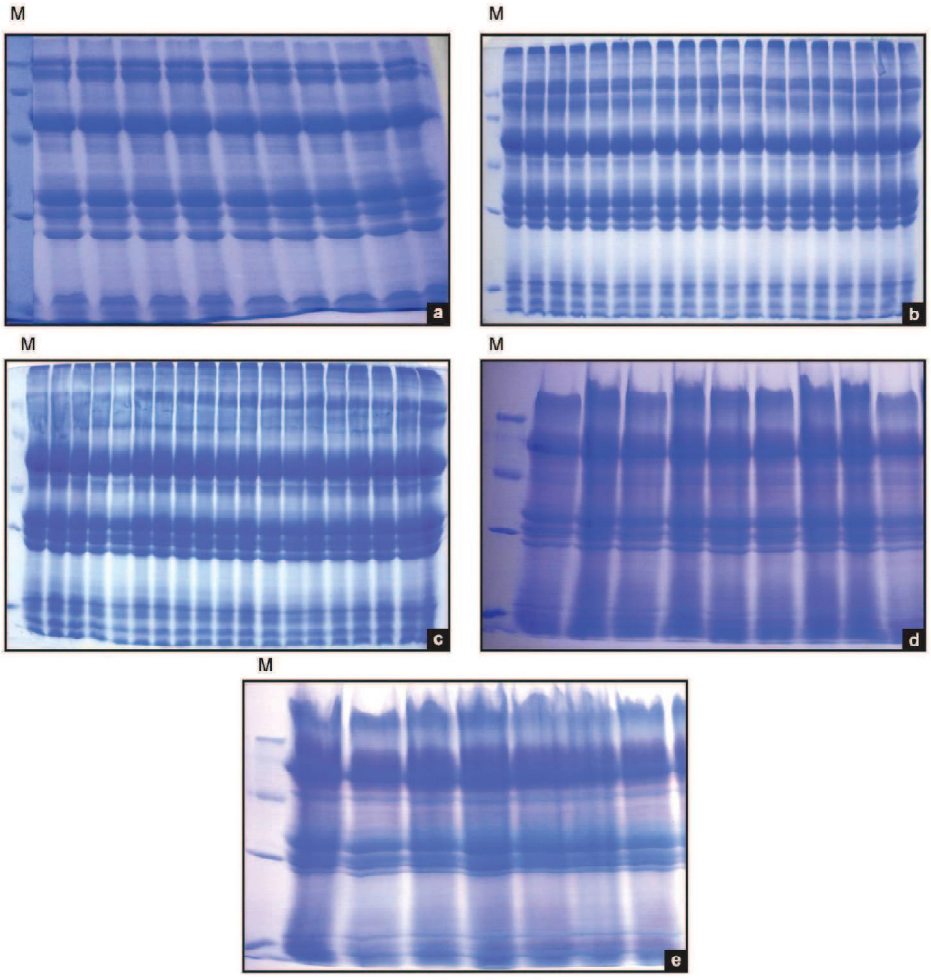

Figure 1:
(a) Protein profile of Abrus precatorius obtained using phosphate buffer (pH = 7.0)
(b) Protein profile of A. precatorius obtained using Tris-HCl extraction buffer (pH=8.0)
(c) Protein profile of A. precatorius obtained using Sucrose-SDS extraction buffer
(d) Protein profile of A. precatorius obtained using Tris-HCl extraction buffer / Acetone precipitation.
(e) Protein profile of A. precatorius obtained using Sucrose-SDS extraction buffer / Acetone precipitation.

M = Molecular weight marker.

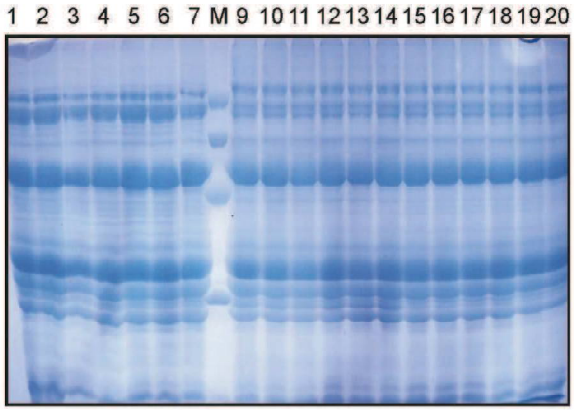

Figure 2:
Comparative evaluation of protein profiles of three genotypes (Black, Red and White) of A. precatorius.
M = Molecular weight marker; Lane 1-7 = Red genotype;
Lane 9-14 = White genotype; Lane 15-20 = Black genotype

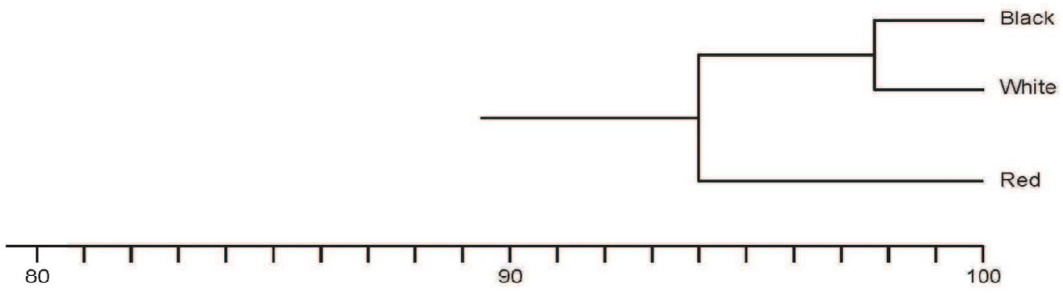

Figure 3:
Dendrogram obtained from polypeptide bands analysis using UPGMA demonstrating relationship among three genotypes of A. precatorius.

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