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Genetic characterization of *Abrus precatorius* L. varieties using SDS-PAGE

A. John De Britto*, P. Benjamin Jeya Rathna Kumar* and *D. Herin Sheeba Gracelin

Plant Molecular Biology Research Unit,
Post Graduate and Research Department of Plant Biology and Biotechnology,
St. Xavier's College (Autonomous), Palayamkottai - 627 002, Tamil Nadu, India
*E-mail: bjohnde@yahoo.co.in

ABSTRACT

Present study was undertaken to estimate genetic characterization of seven *Abrus precatorius* L. varieties using SDS-PAGE analysis. Total seed proteins were extracted and separated on 13% Polyacrylamide gels using standard protocols. Protein fragments of various molecular weights were separated in *Abrus* varieties. Individual protein fragments were considered as allele/loci. Variations as well as similarities were observed in protein profile. Overall 56% of similarity between varieties was observed. Dendrogram showed two major clusters. Varieties white and red were grouped in one major cluster and the remaining varieties were grouped with another major cluster.

KEY WORDS: *Abrus precatorius*, Genetic diversity, SDS-PAGE

INTRODUCTION

Abrus precatorius L. is commonly known as Rosary pea that belongs to the family Fabaceae (Leguminosae). It is a beautiful, much-branched, slender, perennial, deciduous, woody, prickly twining or climbing herb, commonly found as twining herb in mixed deciduous forests, in moist shady localities and grows best in fairly dry regions at low elevations. Leaves, roots and seeds are used for medicinal purposes. Seeds have the potential of good insecticide (Khanna and Kaushik, 1989) and possess antimicrobial activity (Saxena and Vyas, 1986). *Abrus* seeds have different colors. The red variety with black eye is the most common, but there are black, white and green varieties as well. Each variety of seeds has unique medicinal properties. In Siddha medicine the white variety is used to prepare oil that is claimed to be an aphrodisiac (Raamachandran, 2008).

Characterization is the description of a character or quality of an individual (Merriam-Webster, 1991). In genetic terms, characterization refers to the detection of variation as a result of differences in either DNA sequences or specific genes or modifying factors.

Genetic characterization refers to the description of attributes that follow a Mendelian inheritance or that involve specific DNA sequences. The application of biochemical assays such as those that detect differences between isozymes or protein profiles, the application of molecular markers and the identification of particular sequences through diverse genomic approaches all are considered as genetic characterization methods. Because of its nature, genetic characterization clearly offers an enhanced power for detecting diversity (including genotypes and genes). The genetic characterization of individuals and the interrelationship between them has

for many years been determined by presence of morphological characteristics or by use of biochemical tests. Developments and refinements have led to the present day situation where whole arrays of molecular technique are available to characterize the individual on genetic basis.

Among biochemical techniques SDS-PAGE is widely used due to its simplicity and effectiveness for describing the genetic structure of crop germplasm (Murphy *et al*., 1990; Javaid *et al*., 2004; Anwar *et al.,* 2003.). The analysis of storage protein variation in wheat has proved to be a useful tool not only for diversity studies but also to optimize variation in germplasm collections (Masood *et al.,* 2000) The total cell proteins are extracted from the plant parts leaves, shoot or seeds and then subjected to electrophoresis. SDS-PAGE has made more sophisticated studies possible. It enables us to identify variation in the physical and chemical properties of proteins (Gardner *et al*., 1991). Hence the present research was carried out to evaluate the genetic diversity and identify the quality genotype among seven *Abrus* varieties based on protein profiles using SDS-PAGE.

MATERIALS AND METHODS

The experimental material comprised of seven colored *Abrus* varieties. The seeds of all varieties are collected from the Sathurakiri hills, Virudhunagar District, Tamilnadu during September 2011. The hills are located on the eastern side of Western Ghats between 9.42'- 9.44'latitude and 77. 375'– 77. 415' longitude (1:50,000). The selected seed varieties of our studies are White color (W), Red color (R), Sandal color (S), Violet color (V), Red with Black color (RB), Green color (G) and Black color (B) (Figure 1). The experiments were carried out in Plant Molecular Biology Research Unit at St. Xavier's College, Palayamkottai, Tamilnadu.

Protein isolation

For protein isolation, at least 10 seeds from each variety were grounded to a fine powder with mortar and pestle. 250 mg of powder was homogenized with 500µl of protein extraction buffer (0.0625M Tris-HCl,pH 6.8, 2%(w/v) SDS, 5% (v/v) 2-mercaptoethanol, 10% (w/v) glycerol, 0.002% (w/v) bromophenol blue). The extracts were transferred to an eppendorf tube, and incubated for 2h. After incubation the extracts was suspended in a boiling water bath for 2 min, allow to cool and centrifuged at 10000 rpm for 10 min. 10-20µl of supernatant was taken and separated by SDS-PAGE.

SDS - PAGE analysis

SDS - PAGE of seed protein was carried out in vertical slab gel discontinuous buffer system following the method of Laemmli (1970) using 13% acrylamide gel concentration. A total volume of 10µl protein extract solution was loaded into each well and electrophoresis was carried out at 60V until the bromophenol blue dye reaches the bottom of the gel.

After electrophoresis the gels were stained with staining solution comprising 0.2% (W/V) Comassie Brilliant Blue (CBB) R 250 dissolved in 7% (V/V) acetic acid, 50% (V/V) methanol and 43% (V/V) Distilled water over night at room temperature. Gels were destained in a solution containing 7 % (V/V) acetic acid and 30% (V/V) ethanol and 63 % (V/V) Distilled water. Gels were shacked gently until the background of the gel became clear and protein bands were clearly visible. After

destaining the gels were photographed using gel documentation systems.

Data analysis

For genetic diversity analysis, every scorable band was considered as single allele/locus and was scored 1 for presence or 0 for absence. Based on electrophoretic band spectra, Jaccard's similarity index (JSI) was calculated by the formula, JSI = (2× No. of common bands)/Total no. of bands. The dendrogram was constructed based on this similarity index table using statistical software NTSYS- PC, version 2.01 (Rohlf, 1999).

RESULTS AND DISCUSSION

Genetic diversity evaluation

In this study SDS-PAGE of seed proteins was performed in order to investigate genetic diversity among *Abrus* varieties. The seven *Abrus* varieties used in the present study showed various banding pattern using SDS-PAGE technique. Figure (1) showed the electrophorogram of the protein banding pattern of different *Abrus* varieties. A total of 18 bands were obtained among which band number 3, 6, 10, 13 and 17 were common in all varieties but other bands show variation. Molecular weight of bands ranged from 205kDa -3.5kDa (Figure 2).

Cluster analysis on the basis of SDS-PAGE

The genetic similarity coefficient matrix of seven varieties based on SDS-PAGE using UPGMA method was used to construct a dendrogram using a computer program NTSYS-PC, version 2.0, to find the diversity among given varieties. The results of the cluster analysis are given in a dendrogram (Figure 3) on the basis of similarity coefficient (Table l). The dendrogram revealed that the two main groups UC and LC; the group LC is further divided into two sub clusters LC1 and LC2, sub cluster LC1 contains Sandal (S) and Violet (V) varieties. It showed 90% similarity. Another sub cluster LC2 divided into LC2A and LC2B. LC2A and LC2B showed 84% similarity. LC2A contains only one variety i.e., Black with Red (RB). LC2B showed two varieties, Green (G) and Black (B) and it showed maximum 95% similarity. Regarding to the Upper Cluster (UC) it contains White (W) and Red (R) varieties. Totally 56% similarity was observed.

The variation in storage protein banding pattern was revealed by SDS-PAGE. 13 bands were used for analysis and genetic diversity was estimated based on the number of different protein peptides between the two compared. From the results of the SDS-PAGE, the overall blueprint of seed storage-proteins showed the low degree of heterogeneity. A low level of genetic diversity may be attributed to narrow genetic base. Gene silencing may be the reason of variations in high molecular weight protein subunits (Lawrence and Shephred, 1980).

Similarly Siddiqui and Naz, (2009) evaluated the genetic diversity of 10 Pakistani wheat genotypes. They concluded that the greatest similarity (95%) was observed between Shahkar-95 and Mehran-89, while the lowest similarity (16%) was observed between Parwaz-94 and Abadgar-93. Nazia Akbar *et al*. (2010) estimated genetic diversity in local and exotic genotypes of *Capsicu* and recommended Fehsil Bibber 1 and Ajay Bibber 1 genotypes for improvement of chili crop. Asad *et al*. (2003) genetically characterized some maize varieties using SDS-PAGE and

concluded that Kisan-90 variety is different from the other varieties.

In the present study some *Abrus* varieties are genetically characterized using SDS-PAGE. Fufa *et al*., (2005) also do this kind of work. The variety Green (G) and Black (B) showed 95% similarity with one another. But in the present investigation the White and Red varieties showed more dissimilarity with other six varieties considered as quality genotypes.

In the present study the protein patterns was used as a tool for intra specific variation studies. This study was understood to identify the degree of genetic diversity based on protein profile. Adoption of this technology would be useful to plant protection regulatory systems, especially for plant variety and registration of new plant varieties, breeding programs and protection purposes, although a wide array of DNA-based molecular procedures introduced in the last two decades allow genetic diversity to be estimated with greater precision. SDS-PAGE is a simple technique to assess the genetic diversity.

CONCLUSION

The seed storage protein profiles could be used as a valuable marker to evaluate the genetic diversity and used to identify the quality genotypes, improve and conserve the efficiency of those genotypes. In the present investigation, the white and red varieties are identified as quality genotypes for improvement and conservation based on the seed storage protein profiles in SDS-PAGE.

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Table 1: Similarity coefficients among seven *Abrus* varieties

	White	Red	Sandal	Red with Black	Violet	Green	Black
White	1.0						
Red	0.92	1.0					
Sandal	0.47	0.54	1.0				
Red with Black	0.45	0.52	0.90	1.0			
Violet	0.55	0.63	0.74	0.85	1.0		
Green	0.58	0.66	0.76	0.81	0.86	1.0	
Black	0.55	0.63	0.81	0.71	0.83	0.95	1.0

Figure 1: ***Abrus precatorius*** **– selected seed varieties**

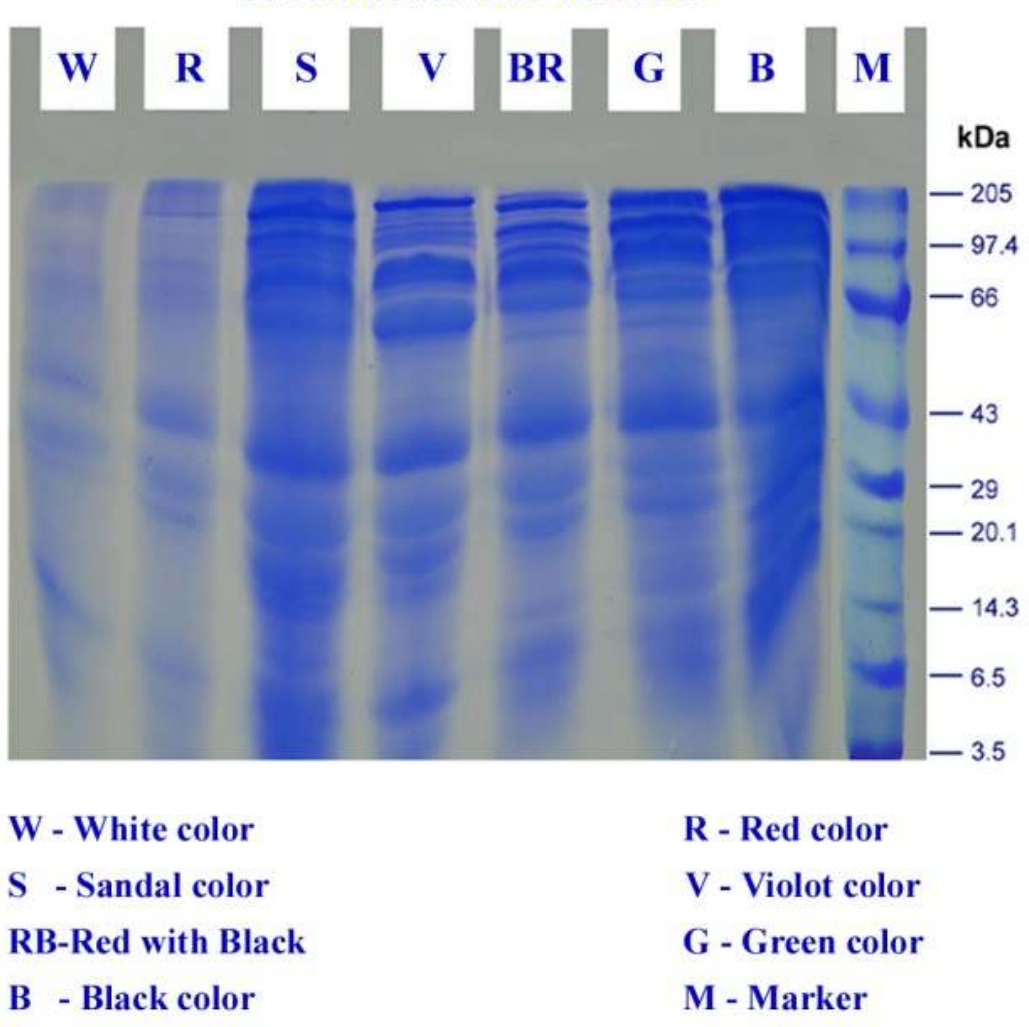

Figure 2: Electrophorogram showing protein banding pattern of selected ***Abrus*** **varieties**

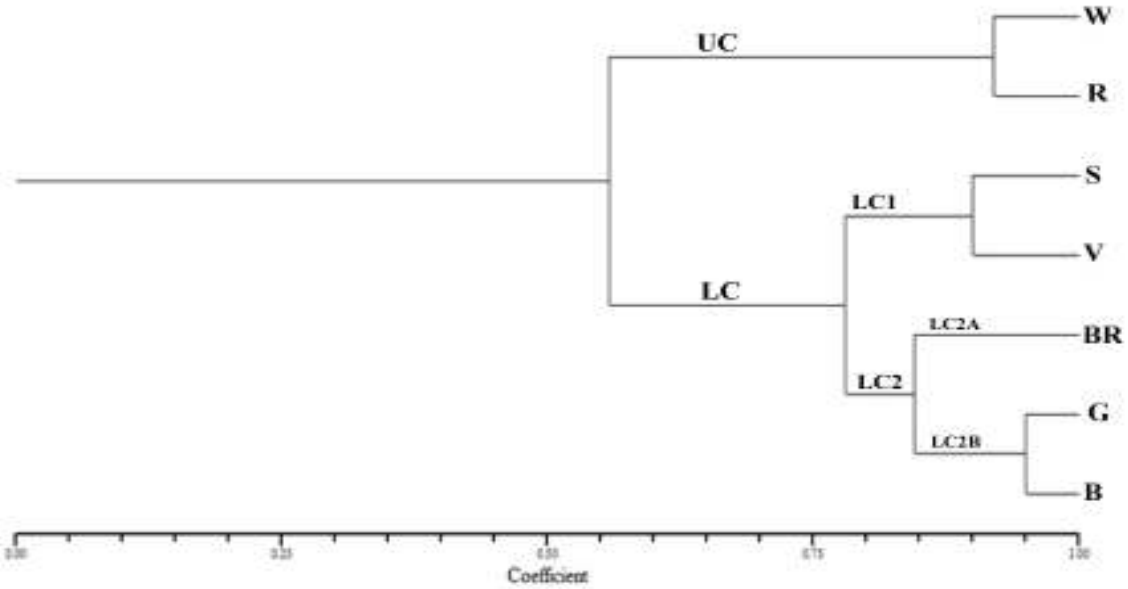

Figure 3: UPGMA cluster analysis showing the diversity and relationship among *Abrus* varieties based on SDS-PAGE

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