

Electrophoretic Study of Seed Storage Protein in Five Species of *Bauhinia*

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Abstract: Seed storage Protein profiles of five *Bauhinia* species were analysed by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). Total seed storage protein of *Bauhinia* species resolved on 10% SDS Polyarylamide gels showed variations in their banding pattern. Results of SDS-PAGE pattern of a few protein bands were up regulated whereas some other bands showed down regulation. Beside the common bands among the studied taxa, 7.37 kDa, 31.85 kDa and 261.143 kDa protein were found common in all the species. The maximum genetic affinities has been observed between *B. acuminata* x *B. purpurea*, while minimum variations in their band numbers. Maximum number of protein bands (12 bands) were observed in *B. tomentosa*, while minimum (7 bands) in *B. purpurea* and *B. variegata*. From present results, protein can differentiate between the studied *Bauhinia* species by producing some specific bands that could be used to distinguish any species from each other. It could be concluded that SDS-Protein is important for genetic analysis and indicate a considerable amount of genetic diversity between the different studied *Bauhinia* species.

Keywords: *Bauhinia* species, SDS-PAGE, Storage protein, Genetic affinity, Electrophoresis

I. Introduction:

The seed storage protein analysis helps in identification and characterization of diversity in crop varieties, cultivars and their wild varieties and also provides information on phylogenetic relationship of the accession [1, 2, 3]. Since many species are genetically closely related, it is often difficult to morphologically discriminate differences among species. Currently, biochemical methods especially SDS-PAGE is widely used in studies on phenotypically close taxa because SDS-PAGE of seed protein is a simple and effective method which is used as genetic markers in the study of genetic variation.

Electrophoresis of protein is a powerful tool for identification of genetic diversity and the SDS-PAGE is particularly considered as a reliable technology because seed storage proteins are highly independent of environmental fluctuations [4,5]. Seed protein patterns can also be used as a promising tool for distinguishing cultivars of particular crop species [6,7]. The SDS-PAGE is considered to be a practical and reliable method for species identification [8]

Since in mature seeds, type and amount of proteins are more constant than other plant tissues [9] therefore, the SDS-PAGE pattern of seed storage proteins of selected species showed polymorphism on the basis of difference in protein intensity among genotypes. The present investigation revealed variation in selected species with regard to their total seed storage protein profiles.

During the present investigation, five taxa viz. *Bauhinia acuminata* L., *Bauhinia purpurea* L., *Bauhinia racemosa* Lam., *Bauhinia tomentosa* L., *Bauhinia variegata* L., of the genus *Bauhinia* have been selected. *Bauhinia* Linn. is a genus with more than 300 species, of family Caesalpiniaceae. It is the largest genus in the legume tribe Cercideae, sub tribe Bauhiniinae [10,11]. *Bauhinia* genus consisting of trees, climbers and shrubs is distributed in India [12]. It has great medicinal value.

II. Materials and Methods

1.1 Plant Material: Fresh mature seeds of selected species of *Bauhinia* were collected from different localities of Patna.

1.2 Protein Extraction: Protein was extracted by method given by Jensen and Lixue [13]. Protein was extracted from overnight presoaked seeds in protein solubilization solution (62 mM Tris-HCl, pH 6.8, 10% glycerol, 2% SDS, p-mercaptoethanol and traces of bromophenol blue) then transferred to Eppendorf tube and centrifuged at 14000 rpm for 30 seconds. The supernatant was transferred to a fresh tube and placed into a boiling water bath for 4 minutes.

1.3 SDS-PAGE: SDS-PAGE was done by method suggested by Laemmli [14]. It was performed on a vertical slab gel. Bromophenol blue was added to the supernatant as tracking dye to watch the movement of protein in

the gel. Seed protein was analyzed through slab type SDS-PAGE using 10% Separating gel and 4% Stacking gel.

Molecular weight of different bands were calibrated with a mixture of standard protein markers include Myosin (261.143 kDa), Phosphorylase B (137.190kDa), BSA (102.564 kDa), Ovalbumin (54.854 kDa), Carbonic Anhydrase (37.670 kDa), Lysozyme (orange) (31.854 kDa), Lysozyme (21.769 kDa) and Aprotinin (7.337 kDa).

Protein Electrode buffer solution was poured into the bottom pool of the apparatus. Gel plates were placed in the apparatus carefully so as to prevent bubbles formation at the bottom of gel plated. Equal quantity of extracted protein from each sample along with Protein molecular weight marker (PAGE mark) were loaded with the micropipette into each wells of the gel. The apparatus was connected with constant electric supply. Electrophoresis was carried out at 20 mA current for 3-4 hours till the tracking dye reaches the bottom of the gel. After electrophoresis, the protein bands were visualized by staining with coomassie brilliant blue G-250 and destained with methanol, acetic acid and water (4:1:5).

1.3.1 Gel Documentation and Analysis: Finally gel was photographed. Molecular weight of protein bands were estimated by their relative mobility.

Pairing affinity or Similarity index was calculated by the method described by the formula.

$$PA = \frac{\text{Bands common to species I and species II}}{\text{Total bands of the species I and Species II}} \times 100$$

II. Result and Discussion

Seed storage protein was analyzed through SDS-PAGE using 10% Polyacrylamide gel. The pattern of the total protein content in five species of *Bauhinia* showed some variation among them. The Rf value between different species ranged from 0.08 to 0.91 (Table-1). The value depict the mobility of the protein on gel surface. Polymorphism was observed in three variable regions i.e. high, medium and low molecular weight. Molecular weight of proteins ranged from 7.37 kDa to 261.143 kDa). Band 1 (Rf=0.08, mol. wt. 261.143 kDa) and band 22 (Rf=0.90, mol.wt. 7.37 kDa) was exactly alike in all the species.

The SDS banding pattern of protein produced 22 bands distributed in all the species including marker with mol. wt. 7.337 kDa to 261.143 kDa (Fig.1-2). Maximum number of protein bands (12 bands) were observed in *B. tomentosa*, while minimum (7 bands) in *B. purpurea* and *B. variegata*. Beside the common bands among the studied taxa, 7.37 kDa, 31.85 kDa and 261.143 kDa protein were found common in all the species. 7.337kDa, 31.85 kDa, 49.95 kDa, 89.74 kDa 148.622 kDa, 254.77 kDa and 261.143 kDa, proteins were found in *B. racemosa* and *B. tomentosa* while 7.337 kDa, 31.85 kDa, 41.56 kDa, 54.854 kDa and 261.143 kDa proteins were found to be common in *B. acuminata* and *B. purpurea*. The pairing affinity index calculated on the basis of electrophoric patterns of seed protein. The percentage similarities for five species belonging to genus *Bauhinia* ranged from 20% to 45.45% (Table-2). Maximum amount of pairing affinity was observed between the two species viz. *B. acuminata* and *B. purpurea* (45.45%) while minimum affinity was observed between *B. racemosa* and *B. variegata* (20%).

According to the result of SDS-PAGE, the overall pattern of seed – storage protein showed the diversity of *Bauhinia* species. The diversity in seed storage protein has also been reported by Khan et al.,[15] for wheat varieties.

Collectively, seed storage protein profiling using SDS-PAGE has the potential to make a distribution between species. Clustering based on seed storage protein profile provides information about the phylogenetic relationship.

III. Conclusion

Five *Bauhinia* species were used in order to elucidate their genetic diversity by using SDS-proteins. It could be concluded that the present results, protein can differentiate between the studied *Bauhinia* species by producing some specific bands that could be used to distinguish any species from each others. These species variation were analysed to assess the protein polymorphism between different species of *Bauhinia*.

On the molecular level, present study gave the results with wide variations in their band numbers. It could be concluded that SDS-Protein is important for genetic analysis and indicate a considerable amount of genetic diversity between the different studied species of *Bauhinia*.

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TABLE - 1
The Rf value of the various bands that appeared on gel of *Bauhinia* species

Band No.	Rf value	Mol. Wt. In KDa	Marker	<i>B. acuminata</i>	<i>B. purpurea</i>	<i>B. racemosa</i>	<i>B. tomentosa</i>	<i>B. variegata</i>
1	0.08	261.143	+	+	+	+	+	+
2	0.14	254.77		-	-	+	+	-
3	0.20	178.34		-	-	+	-	-
4	0.24	148.622		-	-	+	+	-
5	0.26	147.740	+	+	-	-	-	-
6	0.28	137.190		-	-	-	+	-
7	0.30	128.04		-	-	+	-	-
8	0.34	126.60		-	+	+	-	-
9	0.38	113.36		-	-	-	+	-
10	0.42	102.564		+	-	-	+	+
11	0.44	97.90	+	-	-	-	-	-
12	0.47	91.65		-	-	-	-	+
13	0.48	89.74		+	-	+	+	-
14	0.51	54.854	+	+	+	-	-	-
15	0.54	51.80		+	-	-	-	-
16	0.56	49.95		-	-	+	+	-
17	0.58	41.56		+	+	+	-	-
18	0.64	37.67	+	-	+	-	+	+
19	0.75	31.85	+	+	+	+	+	+
20	0.81	21.767	+	-	-	-	-	+
21	0.82	21.50		-	-	-	+	-
22	0.90	7.337	+	+	+	+	+	+

TABLE- 2
Percentage Similarity Index between *Bauhinia* Species

S. No.	Species x Species	Percentage Similarity
1	<i>B. acuminata</i> x <i>B. purpurea</i>	45.45 %
2	<i>B. acuminata</i> x <i>B. racemosa</i>	33.33 %
3	<i>B. acuminata</i> x <i>B. tomentosa</i>	31.25 %
4	<i>B. acuminata</i> x <i>B. variegata</i>	33.33 %
5	<i>B. purpurea</i> x <i>B. racemosa</i>	38.46 %
6	<i>B. purpurea</i> x <i>B. tomentosa</i>	26.66 %
7	<i>B. purpurea</i> x <i>B. variegata</i>	40.00 %
8	<i>B. racemosa</i> x <i>B. tomentosa</i>	43.75 %
9	<i>B. racemosa</i> x <i>B. variegata</i>	20.00 %
10	<i>B. tomentosa</i> x <i>B. variegata</i>	35.71 %

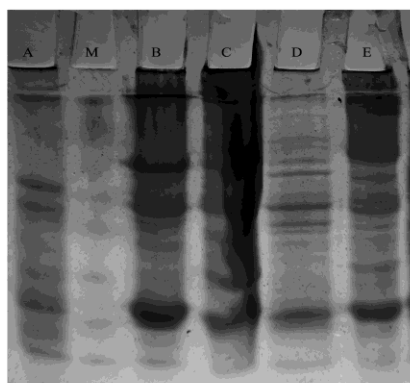


Fig. 1 . SDS Protein profiles of [A] *B. variegata* [M] Marker [B] *B. acuminata* [C] *B. purpurea* [D] *B. racemosa* [E] *B. tomentosa*

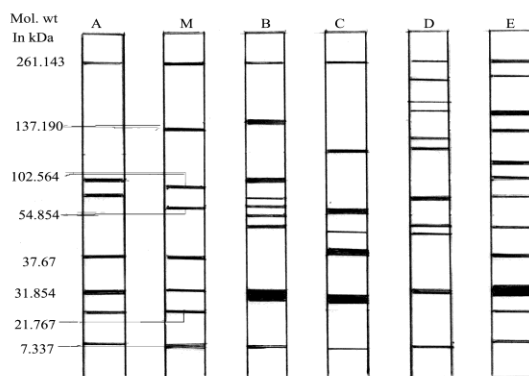


Fig. 2. Diagrammatic representation of SDS Protein profile of seed of [A] *B. variegata* [M] Marker; [B] *B. acuminata*; [C] *B. purpurea*; [D] *B. racemosa*; [E] *B. tomentosa*