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Cytological Diversity and Seed Storage Protein Profiling of most potent *Bauhinia* species

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Abstract: *To trace the interrelationships and for identification and characterisation of diversity among four species of medicinally important plant Bauhinia viz. B. acuminata, B. purpurea, B. racemosa, and B. variegata belonging to sub-family Caesalpiniaceae, cytological study with respect to chromosome number, somatic chromosome length of component arms of chromosome, T. F% and chromosome type and seed protein variation were investigated. Results showed that the three species have chromosome number 2n=28, the value of the total chromatin lengths was lowest (53.48µ) in B. racemosa and highest (57.32µ) in B. purpurea while in B. acuminata this value was 56.78µ suggesting a close relationship between B.purpurea and B. acuminata. The T.F% values of B. acuminata and B. purpurea stand close to each other. Beside the common bands among the studied taxa, 7.37 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 and 261.143 kDa protein was found common in all the four species. Maximum genetic affinities were observed between B. acuminata x B. purpurea (45.45%), while minimum between and B. racemosa and B. variegata(20%). On the molecular level, the present study gave the results with wide variations in their band numbers. Maximum number of protein bands (11 bands) was observed in B. racemosa, while minimum (7 bands) in B. purpurea and B. variegata.*

Key words: *Bauhinia, Karyotypes, Electrophoresis, Storage –protein*

I. Introduction :

Genus *Bauhinia* is the largest genera of the family Caesalpiniaceae which represents more than 300 species. It is the largest genus in the legume tribe Cercideae, sub tribe Bauhiniiae [1,2,3,]. *Bauhinia* genus consisting of trees, climbers and shrubs and is distributed in India [4]. It has great medicinal value.

The genus was originally named by Charles Plumier (1703), because the emarginated apical cone of leaves symbolized to him two Swiss botanist the (Bauhin brother, John, 1541-1613 and Caspar, 1560 -1624). Casper and Bauhin born in France but who lived and worked in Switzerland during the latter half of the 16th and the first part of the 17th Century. The name was accepted by Linneaus in 1753. In India, 15 species of the genus *Bauhinia* L. is reported [5] but the commonest are *B. variegata, B. purpurea, B. acuminata and B. tomentosa.* From Bihar and Orissa, 9 species have been reported in the 'Flora of Bihar and Orissa'[6].

Due to variation in their morphological structure, cytogenetic and the study of seed storage protein can be of great help in tracing interrelationships among the species. The basic chromosome number is one of the most widely used characters in Biosystematics. However, chromosome numbers are only of limited utility for tracing evolutionary relationship. Cytologically, the genus *Bauhinia* is characterized by a uniform chromosome number of 2n= 28, except *Bauhinia monandra* which has 42 chromosomes[7,8].

The seed storage protein analysis helps in identification and characterisation of diversity in crop varieties and also provides information on phylogenetic relationship of the accession [9,10,11]. 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[12, 13]. Seed protein patterns can also be used as a promising tool for distinguishing cultivars of particular crop species [14, 15]. The SDS-PAGE is considered to be a practical and reliable method for species identification [16].

Since in mature seeds, type and amount of proteins are more constant than other plant tissues [17] therefore, the SDS-PAGE pattern of seed storage proteins of selected species showed polymorphism on the basis of difference in protein intensity among genotypes.

To trace the interrelationships and for identification and characterisation of diversity among four species of medicinally important plant *Bauhinia* viz. *B. acuminata, B. purpurea, B. racemosa* Lam. and *B. variegata,* cytological study with respect to chromosome number, somatic chromosome length of component arms of chromosome, T.F% and chromosome type and seed protein variation were investigated by light microscopic study and SDS-PAGE technique respectively.

II. Materials and methods:

1.1 Cytological Studies: For studying the somatic chromosomes, seeds of selected species of *Bauhinia* were collected from various localities, were germinated on a moist filter paper in petridish and kept in an incubator at 27^0-29^0C. One to two cm long root tips of the germinating seeds were cut. They were thoroughly washed with a Camel-hair brush to remove the root cap. The root tips were given a pretreatment in saturated aqueous solution of para di-chlorobenzene for 2 hrs at 15^0C. After pretreatment, the roots were washed and fixed in acetic-alcohol mixture (1:3) and kept in refrigerator. In hot weather fixed materials were kept in refrigerator. After removing from fixative, the roots were thoroughly washed in water. Then they were gently warmed in 2% aceto-carmine repeatedly for 20-25 minutes. The materials were finally transferred to fresh aceto-carmine. The apical part of fresh root tips were cut off and squashed in 2 % acetocarmine. The chromosomes were separated by gentle tapping and squashing of the materials under the cover slip. Then the slide was sealed with paraffin wax for further studies.

2.1 Electrophoretic Study of Seed Storage Protein: Fresh mature seeds of selected species of *Bauhinia* were collected from different localities of Patna.

2.2 Protein Extraction: Protein was extraceted by method given by Jensen and Lixue [18]. Protein was extracted from overnight presoaked seeds in protein solubilization solution (62 m M 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 supernatent was transferred to a fresh tube and placed into a boiling water bath for 4 minutes.

2.3 SDS-PAGE: SDS-PAGE was done by method suggested by Lamelli [19]. 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 analysed 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) was 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).

2.3.1 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.

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

III. Results and Discussion:

The taxa under present investigation have been cytologically examined in details with respect to somatic chromosome length of component arms of chromosome, T.F% and chromosome type (Table 1). So far chromosome number is concerned, three species viz. *B. acuminata, B. purpurea, B. racemosa* and *B. variegata* have chromosome number 2n=28 (Plates 5-8, Fig. 1-4) which was a multiple of seven.

The studied *Bauhinia* species in general show a gross resemblance in the nature of the karyotype in rather short chromosomes with gradation in size but with no abrupt size difference in complements [9, 20]. The close scruitiny of the chromosome types worked out while preparing the karyotype and the karyotypic formulae of the species under reference: [*B. acuminata* - 1M (B) + 2 Sm (B) + 2 M (C)+ 9 Sm (C)], [*B. purpurea* - 3 Sm (B) + 3M (C) + 8 Sm (C)], [*B. racemosa* - 1M (B) + 2 Sm (B) + 7M (C) + 4Sm (C)], and [*B. variegata* – 1Sm (A) + 3 Sm (B) + 3 M (C) + 7 Sm (C)] amply reveals that B and C types of chromosomes are common to

all while A type of chromosomes are common to *B. tomentosa* and *B. variegata*. This is an indications of the fact that these five species represent quite a homogeneous and natural assemblage.

The studied *Bauhinia* species had small chromosomes and showed a length from 1.41µ to 3.01µ, which was almost in agreement with other explored species of the genus [9, 20]. Stebbins [21] regarded Caesalpinoideae as primitive within the family, because its species tend to have small chromosomes with relatively symmetrical karyotypes, a trend also found by Kumari and Bir [22] and our data.

Huziwara [23] has suggested that the lower T.F% value are indicative of highly asymmetrical and advanced karyotypes, while the higher T.F% value of the species indicative of symmetry of the karyotype as well as relatively primitive in nature. The present karyological studies coupled with minute karyotypic analysis bring to light that the highest T.F% value recorded in *B. racemosa* (49.92) indicated its highly asymmetrical karyotypes and relatively primitive nature while lowest T.F% value in *B. purpurea* (46.68) indicated its asymmetrical karyotypes and relatively advanced nature. However a near similarity in T.F% value of rest other species under reference **Table-1** indicated their development from common ancestor as a result of minor alteration in the representatives of the types were met with in different species which may considered as criteria for identification of these species.

The karyotype differences in the species of the genera included in the present investigation might have been brought about by the loss or gain in chromatin matter or by translocation and inversion resulting in the shift of position of the centromere or by altering the size of the chromosome. In the opinion of Singh and Roy [24] also, the translocation and inversion homozygotes are expected to be established easily and earlier in naturally self pollinated genera than in cross pollinated plants.

The range of total chromatin length in them varied and it has been observed to be 56.76 µ in *B. acuminata*, 57.66 µ in *B. purpurea*, 53.48 µ in *B. racemosa* and 56.98 µ in *B. variegata*. The total chromatin length in *B. purpurea* was minimum (53.32 µm) while total chromatin length in *B. racemosa* was maximum (57.66 µ), gives an indication that *B. purpurea* is advanced in nature and *B. racemosa* is primitive nature while remaining two species are intermediate between the two.

The difference in the total chromosome length in different species may be due to deletion or retention of the long heterochromatin segment. Darlington [25] recognized diminution in chromosome size as an established mechanism of evolution in plants. Therefore, it is quite possible that some of the species might have evolved either by diminution of chromosome size due to deletions or by increase in chromosome size to duplication. This amply suggest that translocations with duplication of segments in the chromosome arms have played definite roles at the diploid levels in the ancestral form followed by further chromosome doubling leading to the establishment of present divergent (2n=28). Karyotype studies provide valuable informations about the evolutionary trends and phylogenetic relationships of the species. The external morphology of the chromosomes is, of course well understood with the help of karyotype study. The chromatin length, value of T.F% and Total chromatin length (TCL) of three species under consideration are summarized in **Table –1**.

Seed storage protein was analyzed through SDS-PAGE using 10% Polyacrylamide gel. The pattern of the total protein content in four species of *Bauhinia* showed some variation among them(Plate 9, Fig.5). The Rf value between different species ranged from 0.08 to 0.91 (Table-2). The value depect 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 (Plate 9, Fig.5). Maximum number of protein bands (11 bands) was observed in *B. racemosa*, 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 four 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* 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-3). 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.,[26] for wheat varieties.

Collectively, Karyotype and seed storage protein profiling using SDS-PAGE have the potential to make a distribution between species. These parameters provide information about the phylogenetic relationship.

IV. Conclusion

Four *Bauhinia* species were used in order to elucidate their genetic diversity by using karyotypes and SDS-proteins. It could be concluded that the present results, species can differentiate among the studied *Bauhinia* species with their karyotypes, the value of total chromatin length (TCL) and T.F %. All of which have shown varying degree of overlapping closest relationship on all the earlier parameter employed in the present investigation. *Bauhinia purpurea, Bauhinia acuminata* and *B. variegata* showed close while *Bauhinia racemosa* stand far from these three.

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TABLE –1

Karyotypic variations in *Bauhinia* species

Species	Chromatin Length in µ	T.F %	TCL in µ
B. acuminata	1.81- 2.92	49.73	56.78
B. purpurea	1.41- 2.54	46.68	57.32
B.racemosa	1.78- 2.88	50.11	53.48
B. variegata	1.41- 3.01	48.40	55.52

TABLE-2

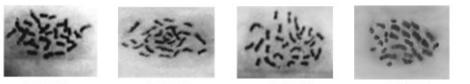
The Rf value of the bands that appeared on gel of *Bauhinia* species

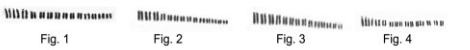
Band No.	Rf value	Mol. Wt. In KDa	Marker	*B. acuminata*	*B. purpurea*	*B. racemosa*	*B. variegata*
1	0.08	**261.143**	+	+	+	+	+
2	0.14	254.77		-	-	+	-
3	0.20	178.34		-	-	+	-
4	0.24	148.622		-	-	+	-
5	0.26	147.740	+	+	-	-	-

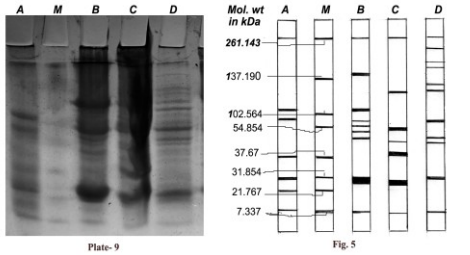
6	0.28	**137.190**		-	-	-	-
7	0.30	128.04		-	-	+	-
8	0.34	126.60		-	+	+	-
9	0.38	113.36		-	-	-	-
10	0.42	**102.564**		+	-	-	+
11	0.44	97.90	+	-	-	-	-
12	0.47	91.65		-	-	-	+
13	0.48	89.74		+	-	+	-
14	0.51	**54.854**	+	+	+	-	-
15	0.54	51.80		+	-	-	-
16	0.56	49.95		-	-	+	-
17	0.58	41.56		+	+	+	-
18	0.64	**37.67**	+	-	+	-	+
19	0.75	**31.85**	+	+	+	+	+
20	0.81	**21.767**	+	-	-	-	+
21	0.82	21.50		-	-	-	-
22	0.90	**7.337**	+	+	+	+	+

TABLE- 3
Percentage Similarity Index between *Bauhinia* Species

S. No.	Species x Species	Percentage Similarity
1	*B. acuminata* x *B. purpurea*	45.45 %
2	*B. acuminata* x *B. racemosa*	33.33 %
4	*B. acuminata* x *B. variegata*	33.33 %
5	*B. purpurea* x *B. racemosa*	38.46 %
6	*B. purpurea* x *B. variegata*	40.00 %
7	*B. racemosa* x *B. variegata*	20.00 %







Plates 1-4: Habit of Different *Bauhinia* species
Plates 5-8: Showing Metaphasic chromosomes (X1000)
Plate 5: *B. acuminata*; Plate 6: *B.purpurea*; Plate 7: *B. racemosa*; Plate 8: *B. variegata*
Fig. 1: *B. acuminata*; Fig. 2.: *B. purpurea*; Fig. 3: *B. racemosa*; Fig. 4: *B. variegata*
Plate 9: Showing Protein bands of
[A] *B.variegata*; [M] Marker; [B] *B. acuminata*; [C] *B. purpurea*; [D] *B. racemosa*
Fig. 5: Diagramatic representation of protein banding patterns of Bauhinia species.
[A] *B.variegata*; [M] Marker; [B] *B. acuminata*; [C] *B. purpurea*; [D] *B. racemosa*