Int. J. Mendel, Vol. 35(1-2), 2018 & 36(1-2), 2019 35-40 ISSN0970-9649

SDS-PAGE OF SEED PROTEINS IN SOME MEMBERS OF *Caesalpinia* Linn. spp.

Om Prakash* and Meenakshi Singh*

Key words : *Caesalpinia*, Caesalpinieae, SDS-PAGE, Electrophoresis, Phylogeny.

SDS-PAGE of seed protein profiles were in three species including four taxa of *Caesalpinia* viz. *C. bonduc* , *C. coriaria*, *C. pulcherrima* var. *flava* and *C. pulcherrima* var. *pulcherrima*. Taxonomic relationships between the four taxa were discussed in the light of their morphological and phytochemical criteria. SDS-PAGE data have revealed that *C. pulcherrima* var. flava and *C. pulcherrima* var. *pulcherrima* share maximum bands in common out of recorded protein bands. This relatively high number of common recorded bands was indicative of their common origin. *C. pulcherrima* var. *flava* showed intermediate between *C. bonduc* and *C. coriaria*. Lowest number of common protein bands in between, *C. coriaria* and *C. pulcherrima* var. *pulcherrima* may be correlated with primitiveness and woodiness among all the species under consideration.

INTRODUCTION

The genus *Caesalpinia* L. belongs to family *Caesalpinia*ceae, tribe Caesalpinieae is a pantropical genus of trees, shrubs and prickly climbers comparising of about 150 species distributed through out the world (Polhill and Vidal,1981).Sanjappa(1992) recorded twenty species of *Caesalpinia* including both wild and cultivated from India. Earlier, Baker (1878) reported ten species from Indian sub-continent. The genus *Caesalpinia* L. with seven species found in the Botany of Bihar and Orissa (Haines,1921-25) are widely distributed throughout Bihar. Three species of *Caesalpinia* (four taxa) have been identified from Patna district, viz. *C. bonduc*, *C. coriaria*, *C. pulcherrima var. flava* and *C. pulcherrima var. pulcherrima*. Many species are important as ornamental, medicinal or timber producing (Burkart,1952). The genus is widespread and diverse characterised by a distinctive morphology. Taxonomic relationship of these taxa; have attracted the attention of taxonomists not only because of their classification is limited to a very few characters (Lersten and Curtis,1994; Rudall et al.,1994) but also because of the nuclear boundaries and the confusion in nomenclature between them (Kit et al., 1994; Shehata ,1997).

Many taxonomic studies have been carried out to discuss relationship of the *Caesalpinia* at specific level using different criteria. Nageshwar et al. (1984) and Prabha Choudhary and Choudhary (1987) analysed the phytochemical structures among a number of species and pointed out close relationship between *C. pulcherrima* and each of *C. sepiaria* and *Delonix regia*.

Seed protein banding patterns as revealed by polyacrylamide gel electrophoresis in the presence of sodium dodecyle sulfate (SDS-PAGE) have provided a valid source of taxonomic evidence for addressing taxonomic relationships at both the generic and specific levels (Ladizinsky and Hymowitz, 1997; Cook,1984; Badr,1995). Variations in SDS-PAGE of seed protein profiles have successfully been used to differentiate between species in number of genera, for example *Vigna* (Paino et al.,1993), *Trifolium* (Badr,1995), *Phaseolus* (Schmit et al.,1996) and *Lathyrus* (El-Shanshoury,1997).

On the other hand, morphological characters can help in solving taxonomic problems and must not be ignored in reconstructing plant relationship and phylogeny (Werff and Endress,1991; Donoghue and Sanderson,1992). Morphological criteria were used to reassess the relationships among various plant families and genera eg. Rohrer et al., (1991) and Robertson et al., (1992) on the Rosaceae, Kadereit et al., (1994) on the Papaveraceae and Sun and Chung, (1986); Rohwer, (1994) on the Lauraceae.

In the present study about the taxonomic relationship among the taxa of genus *Caesalpinia* on the basis of seed protein profile (SDS-PAGE) and its relationship with morphological characters has been worked out.

MATERIALS AND METHODS

For studying the SDS-PAGE, seeds of the examined taxa of *Caesalpinia* were collected from various localities of Patna district. To extract seed proteins each with an equal weight of pure, clean, sterile fine sand and powdered using mortar and pestle and protein from seeds were extracted in protein solubilization solution (0.5% M Tris-HCl, pH=6.8, 10% glycerol, 2% SDS, 0.5% 2-mercaptoethanol and 0.1% bromophenol blue) then transferred to Eppendorf tube and centrifuged 30 seconds. The supernatant was transferred to new tubes placed into a boiling water bath for 4 minutes. The extract was cooled and loaded on each gel tube. Electrophoresis was carried out at 20 mA current for 3-4 hours till the tracking dye reaches the bottom of gel. After electrophoresis the gels were taken out of the tubes for the detection of protein bands.

***Plant Biosystematics and Molecular Biology Lab.**
Dept. Of Botany, Patna Science College, P.U, Patna-5, Bihar, India.

Staining of the protein band was performed by dipping the gel into the plates containing Comassie Brilliant Blue for 24 hours. Destaining was carried in 10% acetic acid. The bands were examined in white light transilluminator and gel was photographed.

Table-1 Some morphological characters of the taxa studied of *Caesalpinia*.

S. No.	Parameters	C. bonduc	C. coriaria	C. pulcherrima var. flava	C. pulcherrima var. pulcherrima
1.	Habit	Shrub (Scandant)	Small sized tree	Shrub	Shrub
2.	Stem	Armed	Unarmed	Armed	Armed
3.	Leaf				
	Type	Compound-Bipinnate	Compound-Bipinnate	Compound-Bipinnate	Compound-Bipinnate
	Size (Leaflet)	2-5.8 x 1.2-2.7 cm	3-9 x 1-2 mm	1-2.5 x 0.5-1.2 cm	1-2.5 x 0.5-1.2 cm
	Shape	Oblong-lanceolate	Oblique-oblong	Oblong-ovate	Oblong-ovate
	Surface Texture	Glabrous	Glabrous	Glabrous	Glabrous
	Apex	Obtuse to sub-acute	Round-truncate	Mucronulate	Mucronulate
	Base	Rounded and unequal	Pulvinous	Pulvinous	Pulvinous
	Margin	Entire to undulate	Entire	Smooth	Smooth
4.	Stipule	Compound foliacious	Acicular	Linear	Linear
5.	Inflorescence	Supra-axillary	Supra-axillary to terminal	Axillary	Axillary
6.	Flower				
	Colour	Yellow	Pale-greenish white	Yellow	Crimson-red
	Size	1.3 cm across	0.8-1 cm across	3 cm across	3 cm across
	Pod size	5-8 x 3.5-4.5 cm	3-6 x1.9-2 cm	7-9 x 1.5-1.7 cm	7-9 x 1.5-1.7 cm
	Pod shape	Oblong covered with prickles	Ovate-oblong	Oblong	Oblong
	Pod joints	----	At 3-6 points	8-10 points	8-10 points
7.	Seed				
	Colour	Greenish to ash grey	Brown	Brown or Black	Brown or Black
	Size	1.5x2.0 cm	4-5x2.5-3 mm	9-10x6 mm	9-10x6 cm
	Shape	Globular	Oblong	Oblong	Oblong
	No. of seed/pod	01-Feb	03-Jun	08-Oct	08-Oct

RESULTS AND DISCUSSION

The studies of seed protein through electrophoretic techniques of various species of *Brassica* and *Sinapis* (Vaughan *et al.*,1966; Vaughan and Waite,1967 a, 1967 b) have thrown a considerable light on the taxonomic status and indicated possible phylogenetic relationship. Vaughan and Denford (1968) found that among the three species of *Brassica* every one could be distinguished from others by a unique band in their seed protein profile.

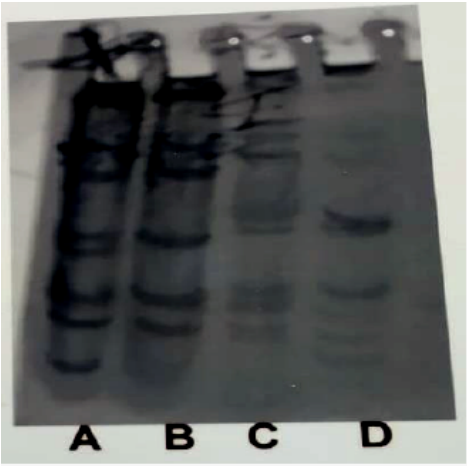

Fig. 1

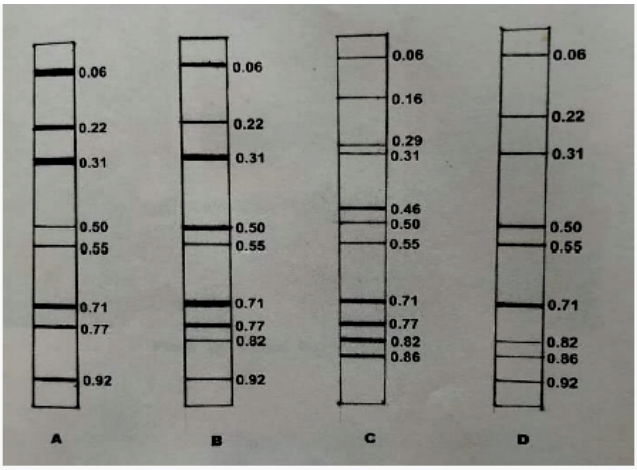

Fig. 2

Fig. 1: Photograph of seed protein banding profile using SDS-PAGE, Fig. 2: Drawing of seed protein profile in each of the taxa studied (A. *C. pulcherrima var. pulcherrima*, B. *C. pulcherrima var. flava*, C. C. *coriaria*, D. C. *bonduc*)

Fox et al., (1964) in a comparison of soluble proteins of a few species of Leguminosae found that protein band patterns of species within a genus resembled one another more closely then the species belonging to different genera. Deshborough and Piloquin(1966) from the study of protein band data, obtained with proteins extracted from various species of *Solanum* concluded that the patterns were specific for each species. Similar conclusions were drawn in the same genus by Edmonds and Gildwell (1977) while studying on different ploidy level and Sarkar and Bose (1984) using single seed protein in the characterization of rice varieties. Johnson and Hall (1966) in a report on electrophoretic studies on wheat proteins state that "homology between the bands of different species based on similarity in migration velocity, provides a criterion of genetic affinity from which evolutionary realtionship may be inferred." Besides these findings, seed proteins have extensively been studied from taxonomic point of view in number of plants like A*vena* (Jain and Singh,1979), Cowpea (Khan et al.,1980), *Phlox* (Levin and Schaal,1970), *Vicia* (Ladizinsky,1975), *Triticum* and *Aegilops* (Caldwell and Kasarda,1978) etc.

The high stability of seed protein profile and its additive nature make seed protein electrophoresis a powerful tool in elucidating the origin and evolution of cultivated plants (Ladizinsky and Hymowitz,1979). A cultivated plant and its wild progenitor form a common gene pool (Harlen and deWet,1971) and can be considered from genetic point of view as a member of the same species. Similarity between the protein profile of wild species and their cultivated counterparts has been reported in cotton (Johnson and Thein,1970; Cherry *et al.,* 1970; Johnson, 1975),Soybean(Mies and Hymowitz, 1975; Savoy 1977) and Peanut(Cherry,1975).

The pattern of the total protein content present in three species (four taxa) of *Caesalpinia* show some variation among them (Figs 1 and 2). Differences arise not only in intensity of bands but also in nature of bands. The Rf value between different species of *Caesalpinia* ranged from 0.06 to 0.92. The value depicts the mobility of the protein on gel surface. The minimum Rf value 0.06 is same in all four taxa while maximum Rf 0.92 is seen in *C. bonduc*, *C. pulcherrima var. flava* and *C. pulcherrima* var. *pulcherrima*. Bands 1 (Rf=0.06), 5(Rf=0.31), 7(Rf=0.50), 8(Rf=0.55), and 9(Rf=0.71) are exactly alike in all the four taxa. Band 10(Rf=0.77) are common to *C. coriaria*, *C. pulcherrima var. flava* and *C. pulcherrima* var. *pulcherrima*. Band 13(Rf=0.92) is common to the *C. bonduc*, *C. pulcherrima var. flava* and *C. pulcherrima* var. *pulcherrim*a. Band 12(Rf=0.86) is common to *C. bonduc* and *C. coriaria* whereas band 4(Rf=0.29) and band 6(Rf=0.46) are confined to single species *C. coriaria*, (Table- 2). Common bands are however taxonomically irrelevant.

Table-2. Bands distribution among different taxa of *Caesalpinia*

Band No.	Rf value	C. pulcherrima var. pulcherrima	C. pulcherrima var. flava	C. coriaria	C. bonduc
1	0.06	+	+	+	+
2	0.16	–	–	+	–
3	0.22	+	+	–	+
4	0.29	–	–	+	–
5	0.31	+	+	+	+
6	0.46	–	–	+	–
7	0.5	+	+	+	+
8	0.55	+	+	+	+
9	0.71	+	+	+	+
10	0.77	+	+	+	–
11	0.82	–	+	+	+
12	0.86	–	–	+	+
13	0.92	+	+	–	+

Table-3 Percentage similarity between two taxa of Caesalpinia.

S.No.	Species × Species	Percentage similarity
1	C1 × C2	88.88%
2	C1 × C3	46.15%
3	C1 × C4	70.00%
4	C2 × C3	53.84%
5	C2 × C4	80.00%
6	C2 × C4	53.84%

Where, *C1= C. pulcherrima var. pulcherrima, C2= C. pulcherrima var. flava , C3= C. coriaria, C4= C. bonduc.*

A total of 11 bands are detected in *C. coriaria*, while 9 bands in *C. bonduc* and *C. pulcherrima var. flava* and 8 bands in *C. pulcherrima* var. pulcherrima. Bands similarity also varied between any two species of genus *Caesalpinia*. Maximum 8 bands are similar between *C. pulcherrima var. flava* and *C. pulcherrima* var. pulcherrima while minimum 6 bands are common between *C. coriaria* and *C. pulcherrima* var. pulcherrima. Bands 2(Rf=0.16), 4(Rf=0.29) and 6(Rf=0.46) are specific to *C. coriaria*. Morphological characters of *C. coriaria* solid,cylindrical without armed stem, compound oblique-oblong leaf with rounded-truncate apex and pulvinous base whereas both varieties of *C. pulcherrima* could be morphologically differentiated by possessing compound oblique-oblong leaves with mucronulate apex and pulvinous base. *C. bonduc* is scandant shrub having oblong-lanceolate leaf with obtuse to sub-acute apex, rounded unequal base and compound foliaceous stipule (Table-1). Thus further research is still needed on this taxon in particular to elucidate its relationship with the other taxa included in *Caesalpinia*.

REFERENCES

Badr, A. (1995). Electrophoretic studies of seed proteins in relation to the chromosomal criteria and relationships of some taxa of *Trifolium*. Taxon 44:183-191.

Bruneau, A., Forest, F., Herendeen, P. S., Kligaard, B. B. and Lewis, G. P., (2001): Phylogenetics relationship in the Caesalpinioideae (Leguminosae) as inferred from Chloroplast trnL. Intron Sequences. Systematic Botany, 26 (3): 487-514.

Burkart, A. (1952). Las Leguminosas argentinas, Silvestres y cultivadas. Acme Agency, Buenos Aires.

Cherry, J. Siegel C. I., Margulies S.I. Donner, M. (1970). Pharyngeal localization of symptoms of gastroesophageal reflux. Ann. Otol Rhinol Laryngol 79 : 912-914.

Cook, R. T. (1984). The characterization and identification of crop cultivars by electrophoresis Electrophoresis (Japan) 5:59-72.

Desborough, S. L. and Piloquin, S.T. (1966). Esterase Isozymes from *Solanum tuberosum*. Phytochemistry, 6 : 989-994.

Donoghue, M.J. and Sanderson, M.J. (1992). The Suitability of Molecular and Morphological Evidence in Reconstructing Plant Phylogeny In "A molecular Systematics of Plants Soltis,P.S. Soltis, D.E, and Doyle J.J. Editors Chapman and Hall New York Pp:340-368.

Edmonds, J. M. and Gildwell, S. I. M. (1977). Acrylamide gel electrophoresis of Seed Proteins from *Solanum* (Section *Solanum*) species. Plant Syst. Evol., 127,277-91.

El-Shanshoury, A.R (1997). The use of seed proteins revealed by SDS-PAGE in taxonomy and phylogeny on some *Lathyrus*. Biol. Plant 39:553-559.

Fox, D. T., Thurman, D. A. and Boulter, D. (1964). Studies on the Proteins of Seeds of the Leguminosae 1. Albumins. Phytochemistry, 3: 417- 19.

Haines, H. H. (1921-1925) The Botany of Bihar and Orissa. Adland and Son and West Newman. Ltd., London.

Harlan, J. R. and De Wet, J.M.J. (1971). Towards a Rational Classification of Cultivated Plants. Taxon, 20: 509-517.

Jain, S. K. and Singh, R. S. (1979). Population Biology of *Avena* VII. Allozyme variation in Relation to the Genome Analysis. Bot. Gaz., 140 (3): 356-362.

Johnson, B. L. and Hall, O. (1965), Am. J. Bot. 52.1965,506.

Johnson, B. L. and Hall, O. (1966): Electrophoretic Studies of Species Relationships in *Triticum*. Acta Agr. Workers Proc, 64: 278-279.

Johnson, B. L., Thein M. M. (1970). Assessment of evolutionary affinities in *Gossypium* by protein electrophoresis. Am. J. Bot 57 :1081-1092

Johnson, B. L. (1975). *Gossypium palmeri* and a polyphyletic Origin the New World Cotton. Bull. Torrey Bot. Club, 102,340-349.

Kadereit, J.W.; Blattner, F.R.; Jork, K and Schwarzbach, A. (1994). Phylogenetic analysis of the Papaveraceae SI (incl. Fumariaceae, Hypecoaceae and *Pteridophyllum*) based on morphological characters. Bot. Jahrb. Syst. 116:361-390.

Khan, Md. R. I., Gatehouse, J.A. and Boulter, D. (1980). The Seed Protein of Cowpea (*Vigna unguiculata*. Walp) J. Exptl. Botany, 31 (125), 1599-1611.

Kit ,G.C.; Lewis, G. P.; Sprent. J. I and Mickey, D. (1994). Chemotaxonomy of seed non protein amino acids in *Caesalpinia* S.l. In: Sprent.,R. and Mickey, D. (Eds.), Advances in legume systematics, Part 5. Pp 101-105. Royal Bot. Gard. , Kew.

Ladizinsky, G. (1975). Seed protein electrophoresis of wild and cultivated species of the section Faba of *Vicia*. Euphytica 24:785-788.

Ladizinsky, G. and Hymowitz, T. (1979) Seed protein electrophoresis in taxonomic and evolutionary studies. Theo. App. Genet. 54:145-151.

Lersten, N. R. and Curtis, J. D. (1994). Leaf anatomy in *Caesalpinia* and *Hoffimanseggia* (Leguminosae: Caesalpinioideae) with emphasis on secretory structures. PL. Sys. Evol. 192:231-255.

Mies, D. W. and Hymowitz, T. (1973). Comparative Electrophoretic Studies of Trypsin Inhibitors in Seeds of the Genus *Glycine*. Bot. Gaz., 134: 121-123.

Nageshwar, G.,; Radhakrishniah, M and Narayan, L. (1984). Chemotaxonomy of *Caesalpinia*. Curr. Sc., 53: 813-814.

Piano, D; Urzo, M,; Pedalino, M. Grillo, S.; Rao, R.; Tucci, M.; Urzo-M.P.; Ng-NQ and Monti, L.M. (1990). Variability in major seed proteins in different Vigna species. Cowpea genetic resources, 1: 90-110 (Niger).

Polhill, R. M. and Vidal, J. E. (1981). Tribe 1. *Caesalpinia*eae. In Polhill, R. M. and Raven, P. H., (Eds.): Advances in legume systematics, Part 2, Pp. 81-95 Royal Bot. Gard., Kew.

Prabha-Choudhary and Choudhary, S. S (1987). Phytochemical relationship among some members of *Caesalpinia*ceae. Plant Phys. and Biochem. ,14:220-226.

Robertson, K.R., Phipps, J.B. and Rohrer, J.R. (1992). Summary of leaves in the genera of Maloideae (Rosaceae). Ann. Missouri Bot. Gard. 79: 81-94.

Rohrer, J.R., Robertson, K.R. and Phipps, J.B., (1991): Variation In Structure Among Fruits Of Maloideae (Rosaceae) Amer. Jour. Bot. 78(12)1617-1635.

Rohwer, J.G.(1994). A note on the evolution of the stamens in the Laurales, with emphasis on the Lauraceae. Botanica. Acta (107): 2, Pp: 103-110.

Rudall, P.J.; Myers, G. and Lewis, G. P. (1994) Floral secretory structures in *Caesalpinia* s. l. and related genera. In: Ferguson, I.K. and Tucker, S., (Eds.), Advances in Legume systematics, Part 6, Pp 41-52. Royal Bot. Gard., Kew.

Sanjappa. (1992). Legumes of India. Bishen Singh Mahendra Pal Singh. Dehra Dun India, pp. 9-14.

Schmit, V. ; Debouck, S.G. and Baudoin, J. P. (1996). Biogeographical and molecular observations of *Phaseolus glabellus* (Fabaceae: Phaseolinae) and its taxonomic status. Taxon 45: 493-501.

Shehata, A. A. (1997). Morphology and Embryology of *Caesalpinia gilliesii*, *C. pulcherrima* and *Delonix regia* (Leguminosae: Caesalpinioideae). J. Union Arab Biol., Cairo, 4: 197-217.

Sun, B. Y. and Chung , Y.H. (1986). Taxonomic studies on the Lauraceae in Korea: morphology of inflorescences. Korean J. Bot. 29(4) 329-340.

Werff, H. and Endress, P.K. (1991). *Gamanthera* (Lauraceae). A new genus from Costa Rica. Ana. Missouri Bot. Gard. 78: 401-408.