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Full Length Research Paper

Circumscription of the families within Leguminales as determined by cladistic analysis based on seed protein

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Comparative banding pattern of the seed protein of 17 taxa belonging to the three families Mimosaceae, Caesalpiniaceae and Fabaceae was studied with the aim of testing the proposed delimitation of the order Leguminales (Fabales) into the three families or subfamilies and to assess the phylogenetic relationships within the three families. Cluster and pairing affinity or similarity index analysis of the data from total protein grouped the 17 taxa into three discrete clusters based on their families. Considerable amount of homology was observed in the banding pattern between the different taxa.

Key words: Cluster analysis, seed protein, Mimosaceae, Caesalpiniaceae, Fabaceae.

INTRODUCTION

Leguminales (also called Leguminosae) is large, mostly treated as distinct order (Bhattacharya and Johri, 1998), consisting of three closely related families- Papilionaceae (Fabaceae), Caesalpiniaceae and Mimosaceae. These families share a number of morphological, anatomical and embryological characters, like racemose inflorescence, bisexual and actinomorphic or zygomorphic flowers with few to numerous basifixed stamens that dehisce longitudinally, monocarpellary, superior, unilocular ovary with marginal placentation and fruit being a dehisant or indehisant legume.

The close relationship of these families is emphasized by their consistent placement under the same order (Table 1). Although all the three families are abundant in tropics and subtropics of both hemispheres, Caesalpiniaceae and Mimosaceae are consistently recognized as distinct, from Mimosaceae are consistently recognized as distinct from Fabaceae being chiefly arborescent while Fabaceae being predominantly herbaceous. Takhtajan (1980) however, placed all the three under the same family. Fabaceae belonging to the order Fabales.

The status of the Leguminales (=Leguminosae) representing a family of 3 subfamilies Mimosaceae, Caesalpiniaceae and Fabaceae or Papilionaceae or an order embracing the 3 families, remains a disputed issue. Although the three have been placed separately as families or subfamilies under Leguminales (Mutchinson, 1959; Jones, 1955), Rosales [Engler (1909) revised by Melchior (1964)], Fabales (Cronquist, 1981; Dahlgren, 1983a; Stebbins, 1974) or Rutales (Throne, 1992) (Table 1), Bentham and Hooker (1965a), Rendle (1925), Wilber (1963), and Takhtajan (1980, 1987) have considered Leguminosae as a family of 3 subfamilies (Table 2). Following the International Code of Botanical Nomenclature, Jones (1955) proposed the ordinal name Leguminales while Stebbins (1974) and Dahlgren (1980a, 1983a) have further changed the name to Fabales based on the type Family Fabaceae.

The purpose of the present study is to re-assess the relationships within the Leguminales based on seed protein profile of 17 taxa (5 belonging to Mimosaceae, 6 to Caesalpiniaceae and 6 to Fabaceae) (Table 3). Similar cladistic analysis of Ericales based on studies of relationships among members of Epacridaceae, Empetraceae and Ericaceae has been done earlier by Crayan et al (1996), Kron (1996) and Powell et al. (1996). The aim of the present study was to test whether cladistic analysis of the seed protein data supports those classifications, where Leguminales has been subdivided

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Table 1. Classification of mimosaceae, caesalpiniaceae and fabaceae.

Taxonomist	Order	Family	Subfamily
Hutchinson (1959)	Leguminales	Mimosaceae Caesalpiniaceae Fabaceae	
Engler (1909) revised by Melchiar (1964)	Rosales	Leguminosae	Momosaceae Caesalpiniaceae Papilionaceae (Fabaceae)
Cronquist (1981)	Fabales	Mimosaceae Caesalpiniaceae Fabaceae	
Dahlgren (1983a)	Fabales	Mimosaceae Caesalpiniaceae Fabaceae	
Throne (1992)	Rutales	Fabaceae	Mimosoideae Caesalpinoideae Faboideae

Table 2. Taxonomic status of leguminosae.

Leguminosae as a family	Leguminosae as an order	Leguminales as an order	Fabales as an order
Benson (1970)	Brown (1814)	Hutchinson (1959, 1955)	Dahlgren (1983a)
Bentham and Hooker (1955a)	Lawrence (1951)	Jones (1955)	Stebbins (1974)
Rendle (1925)			
Takhtajan (1980, 1987)			
Wilber (1963)			

Table 3. Alphabetical list of taxa selected.

S/N	Species	Family
1.	*Acacia nilotica* Linn.	Mimosaceae
2.	*A. auriculiformis* A. Cunn. ex Benth	Mimosaceae
3.	*A. farnesiana* Willd	Mimosaceae
4.	*Albizzia lebbek* Benth	Mimosaceae
5.	*Cassia alata* Linn.	Caesalpiniaceae
6.	*C. fistula* Linn.	Caesalpiniaceae
7.	*C. occidentalis* Linn.	Caesalpiniaceae
8.	*C. siamea* Lamk.	Caesalpiniaceae
9.	*Cicer arietinum* Linn. [Chick pea]	Fabaceae
10.	*Cicer arietinum* Linn. [White Chick pea]	Fabaceae
11.	*Dalbergia sissoo* Roxb.	Fabaceae
12.	*Delonix regia* Reaf.	Caesalpiniaceae
13.	*Leucaena glauca* Benth	Mimosaceae
14.	*Peltophorum pterocarpum* (A.P. de Candolle) Backer ex K. Heyme	Caesalpiniaceae
15.	*Phaseolus vulgaris* Linn.	Fabaceae
16.	*Sesbania grandiflora* Pers.	Fabaceae
17.	*Tephrosia purpurea* Pers.	Fabaceae

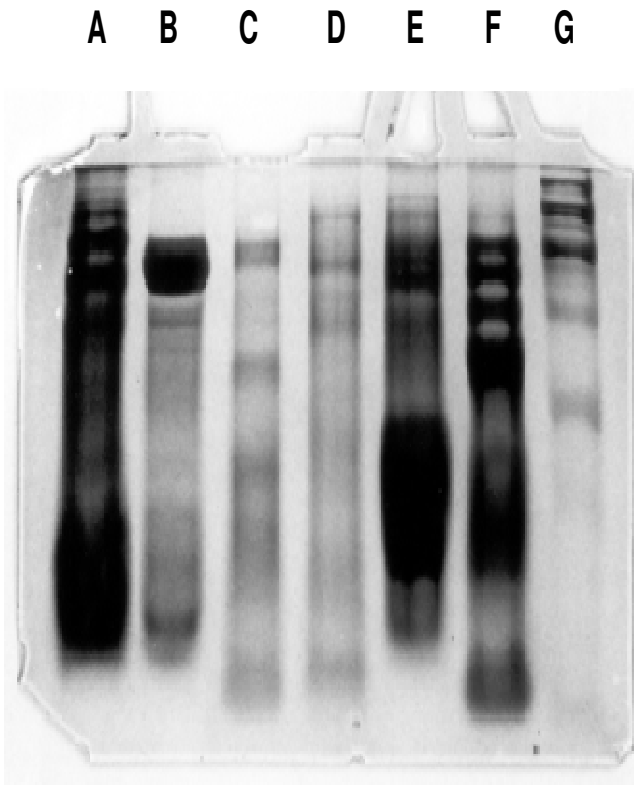

Figure 1. SDS-PAGE protein patterns of the seed extracts of 8 taxa of Caesalpiniaceae (A), *Cassia alata* (B), *C. fistula* (C), *C. occidentalis* (D), *C. siamea* (E), *Delonix regia* (F), *Peltophorum pterocarpum,* and (G) Marker protein.

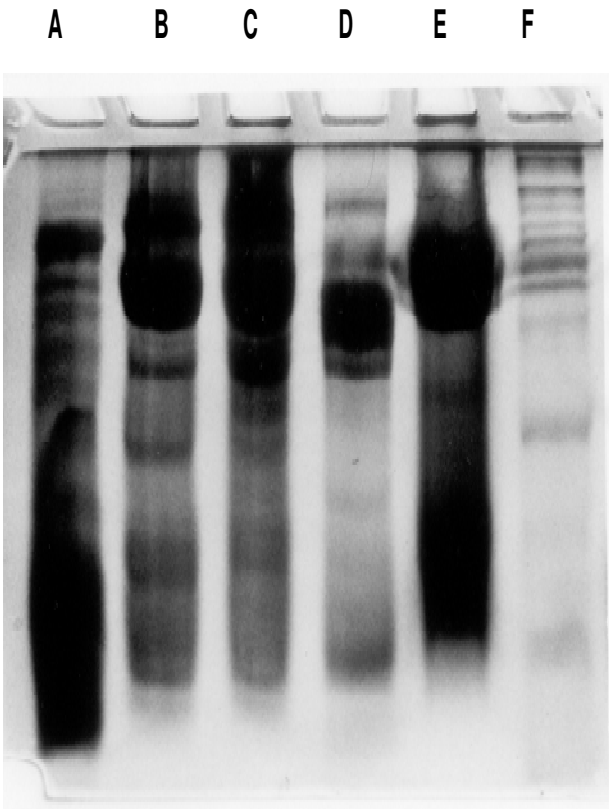

Figure 2. SDS-PAGE protein patterns of the seed extracts of 7 taxa of Mimosaceae (A), *Acacia nilotica* (B), *A. auriculiformis* (C), *A. farnesiana* (D) *Leucaena glauca* (E), *Albizzia lebbek,* and (F) Marker protein.

into 3 families or subfamilies- Mimosaceae, Caesalpiniaceae and Fabaceae.

MATERIALS AND METHODS

Seeds were collected from the mature pods from plants growing in different parts of west Bengal. The seeds were sterilized in 10%(v/v) chlorox and 0.1% (v/v) Tween 20 for 5 min (Mondal et al. 2000). After rinsing in sterilized distilled water for 30 min, the seeds were immersed in sterilized distilled water overnight and used for protein extraction.

Protein was extracted following the methods, Jensen and Lixue (1991). One gram of seed material (endosperm plus embryo) was ground with 10 ml Tris-glycine-buffer (0.01 M Tris, 0.08M glycine), pH 8.2 containing 2% NaCl for 30 min. The slurry was then centrifuged at 19,000 × g for 20 min at 10°C. The supernatant containing the soluble proteins was preserved and the extraction procedure was repeated twice with the pellet with double volume of the extraction, buffer and the supernatants pooled. The pellet was then used for the extraction of the insoluble storage protein by resuspending it in TGP buffer (0.01 M Tris, 0.08 M glycine) pH 8.2 + 2% NaCl and an equal volume of 62 mM Tris-HCl (pH 6.8), buffer containing 3.05% (w/v) SDS and 10.7% (W/v) glycerol and it boiled for 5 min. The supernatant was collected after centrifugation and the pooled supernatants were then used for sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE).

SDS-PAGE was done following the method of Laemmli (1970) using a 10% T mini-gel (8 × 7 cm gel). The gel was calibrated with marker mixture consisting of myosin, rabit muscle (M.W. 205 kDa), β-galactosidase, *Escherichia coli* (M.W. 116 kDa), phosphorylase b, rabbit muscle (M.W. 97.4 kDa), albumin bovine (M.W. 66 kDa), albumin egg (M.W. 45 kDa) and carbonic anhydrase, bovine erythrocytes (M.W. 29 kDa) obtained from Sigma Co., U.S.A. After electrophoresis, the gel was stained with 0.1% Coomassie Brilliant Blue R 250 and destained with methanol, acetic acid and water (4:1:5) mixture.

Pairing affinity or similarity index was calculated by the method described by Sokal and Sneath (1963) and Romero Lopes et al. (1979). Based on the results of electrophoretic analysis, the degree of pairing affinity (PA) was calculated by the formula.

$$PA = [(\text{Bands common to species A and B}) \times 100] / (\text{Total bands A and B})$$

A dendogram expressing the average linkage was computed using the cluster method- Unweighted Pair Group Method with Averages (UPGMA) relationship (Sneath and Sokal, 1973).

RESULTS AND DISCUSSION

The SDS-PAGE profile of the total protein (soluble and insoluble) of the seeds of the 17 taxa belonging to the three families shows a considerable amount of homology in their banding pattern (Figures 1- 4). Besides the common bands among the studied taxa, a 45 kDa legumin-

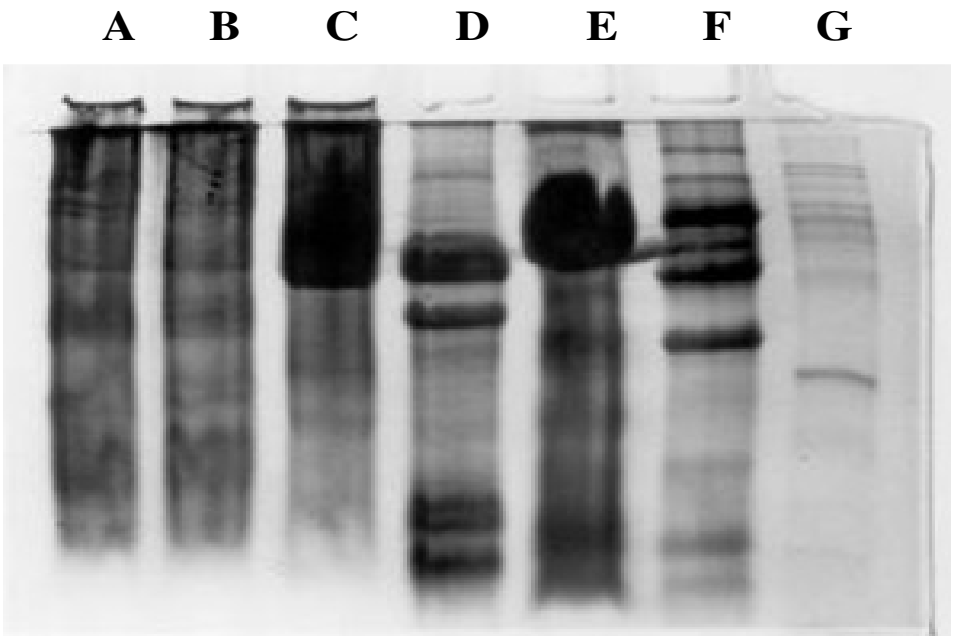

Figure 3. SDS-PAGE protein patterns of the seed extracts of 8 taxa of Fabaceae (A), Cicer arietinum [Chick pea] (B), Cicer arietinum [White Chick pea] (C), Phaseolus vulgaris (D), Tephrosia purpurea (E), Dalbergia sissoo (F) Sesbania grandiflora, and (G) Marker protein.

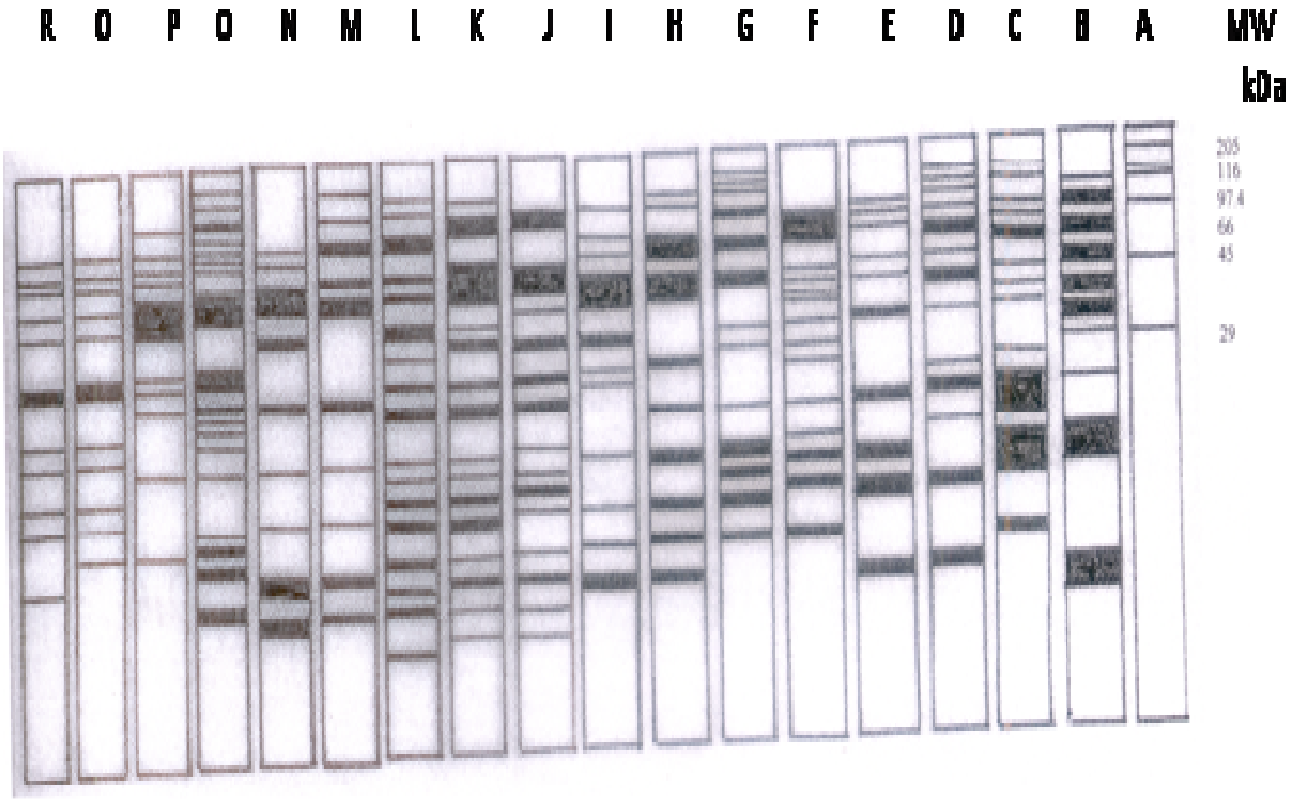

Figure 4. Diagrammatic representation of the SDS-PAGE pattern of the 17 taxa (A), Marker (B), *Peltophorum pterocarpum* (C), *Delonix regia* (D), *C. siamea* (E), *C. occidentalis* (F), *C. fistula* (G), *Cassia alata* (H), *Albizzia lebbek* (I), *Leucaena glauca* (J), *A. farnesiana* (K), *A. auriculiformis* (L), *Acacia nilotica* (M), *Sesbania grandiflora* (N), *Dalbergia sissoo* (O), *Tephrosia purpurea* (P), *Phaseolus vulgaris* (Q), *Cicer arietinum* [White Chick pea], (R) *Cicer arietinum* [Chick pea] .

Table 4. Pairing affinity values (%) of the 17 investigated taxa based on the electrophoretic patterns of seed protein.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1	100																
2	62.4	100															
3	57.6	70.6	100														
4	36.4	31.6	25.0	100													
5	25.0	36.8	36.8	35.4	100												
6	38.1	40.0	38.1	38.2	52.6	100											
7	36.8	38.9	41.2	36.8	34.3	33.3	100										
8	45.0	45.0	38.1	33.4	56.3	53.3	30.3	100									
9	33.3	35.0	38.0	28.3	35.0	28.4	26.4	29.2	100								
10	31.8	30.0	32.3	24.2	25.0	26.8	27.2	28.6	83.3	100							
11	28.6	38.9	35.0	28.3	29.4	26.8	24.8	24.9	37.2	35.4	100						
12	33.3	35.0	28.0	26.8	28.2	24.4	26.2	27.2	26.2	28.2	24.2	100					
13	47.6	40.0	48.2	49.8	33.3	32.2	33.3	36.2	32.2	33.3	31.4	34.8	100				
14	36.4	36.8	38.9	33.4	22.4	24.5	26.7	27.5	18.2	20.2	34.2	41.2	34.2	100			
15	28.2	21.0	22.2	22.3	23.2	26.2	23.2	26.2	44.4	46.4	48.2	34.2	28.2	26.2	100		
16	26.1	31.6	23.4	24.6	26.5	24.2	28.9	29.2	46.2	48.2	49.5	33.3	24.8	26.8	37.5	100	
17	33.3	29.2	29.2	29.2	24.2	28.6	26.3	28.4	48.2	46.1	31.7	36.2	26.2	24.2	40.2	47.4	100

1 to 17: Alphabetical list of taxa selected as indicated in Table 3.

like protein was found common in majority of the species. The pairing affinity index calculated on the basis of the electrophoric patterns of seed protein. Table 4 reveals highest percentage of similarity between species belonging to same genera (*Acacia, Cassia* and *Cicer*). Although there was considerable homology in the banding pattern between the three families, the interfamily pairing affinity was found to be higher than the intrafamily pairing affinity. A dendogram computed on the basis of the average linkage. Figure 5 shows the 17 taxa to be clustered into three discreate groups that are strictly according to their families with Caesalpiniaceae forming one cluster and Mimo-saceae and Fabaceae, the other two which clearly indicates that Caesalpiniaceae is more related to Mimosaceae than to Fabaceae. On the other hand, Fabaceae is more related to Mimo-saceae than to Caesalpiniaceae. Maximum amount of pairing affinity was observed between the two varieties of *Cicer* (83.3%) while *Pelto-phorum* was found to be most distantly related from *Cicer* (18.2% and 20.2%).

Conclusion

This study indicates that the seed protein data is phylogenetically informative in the assessment of the relationship among the three families. Since the three families are from three distinct groups, the molecular data of seed protein provide support to linkages among genera within the families. The result that was obtained strongly supports the classification of Engler (1909), Hutchinson (1959), Cronquist (1981), Dahlgren (1983a) and Throne (1992) who have subdivided Leguminales into the three families or subfamilies. Mimosaceae, Caesalpiniaceae and Fabaceae based on their morphology, anatomy embryology, etc., which also differ in their habit. Mimosaceae and Caesalpiniaceae which are chiefly arborescent and consistently distinct from Fabaceae, which are predominantly herbaceous, was found to be most closely related and Fabaceae was found to be distintly related to Caesalpiniaceae or Mimosaceae. Nevertheless, it is essential to corroborate relationships inferred from other molecular data sources like restriction fragment length polymorphism (RFLP) before being able to conclusively resolve the basal relationships in the family and as well as to test

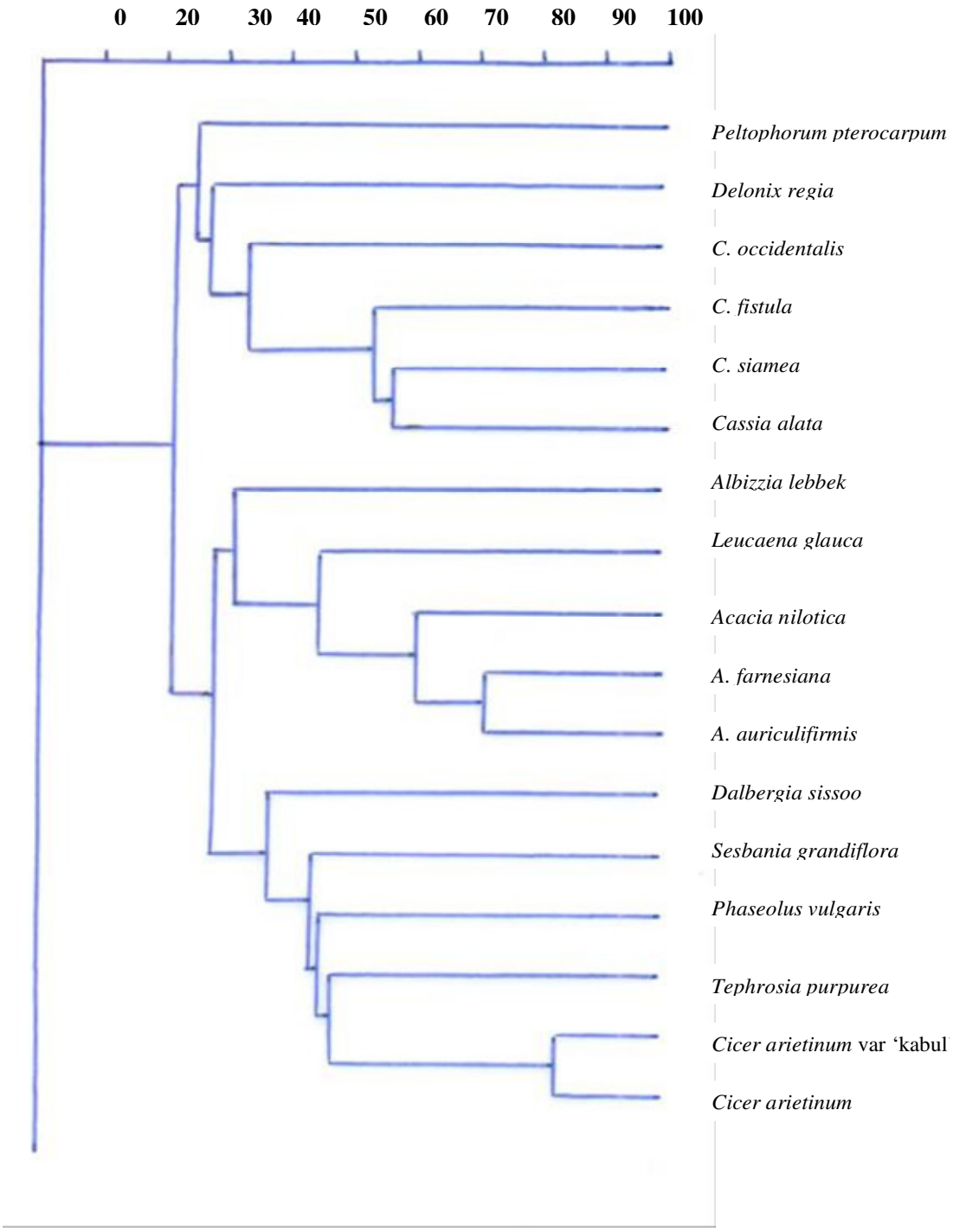

Figure 5. Dendogram representing the average linkage relationship among the 17 taxa of mimosaceae, caesalpiniaceae and Fabaceae shown by seed protein electrophoresis.

the pattern of relationships among the families.

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