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Seed protein electrophoresis of some members of the family fabaceae

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Seed storage protein profiles of 10 members of the family Fabaceae were assessed using sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). Total seed storage protein of the studied plants resolved on 10% SDS polyacrylamide gels showed variations in their banding pattern. Results of SDS-PAGE pattern also revealed a band common to all the plant species studied which suggests that this band could be tagged as generic band among members of Fabaceae. The maximum genetic affinity of 0.93 was observed between *Vigna subterranea* and *Arachis hypogaea*, while minimum genetic affinity of 0.32 was observed between *Senna siamea* and *Albizia lebbeck* which further reveal wide genetic diversity among the studied plant species. This observation also suggested that *V. subterranea* (Bambara groundnut) and *A. hypogaea* (groundnut) are genetically very close and should be put together taxonomically. Nineteen (19) major bands were recorded and only *S. siamea* had two specific bands which indicate that these two bands could be used to distinguish this species from other legumes considered in this study. It could be concluded that SDS-protein electrophoresis is an important tool for genetic analysis and this protocol has revealed a considerable amount of genetic diversity among the 10 studied plant species for their discrimination.

Key words: Fabaceae, sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE), storage protein, genetic affinity, electrophoresis.

INTRODUCTION

Legumes are among the three largest families of flowering plants. The group is the third-largest land plant family, after Orchidaceae and Asteraceae, with 730 genera and over 19,400 species comprising herbs, shrubs, trees and climbers. Members if this family include a number of important agricultural crops like *Glycine max* (soybean), *Phaseolus vulgaris* (beans), *Pisum sativum* (pea), *Cicer arietinum* (chickpeas), *Medicago sativa* (alfalfa), *Arachis hypogaea* (groundnut), *Ceratonia siliqua* (carob), and *Glycyrrhiza glabra* (licorice).

Legumes are useful as human and animal food, as wood and soil-improving components of agricultural and agroforestry system. This large family is divided into three subfamilies - the Mimosoideae, Caesalpinoideae and Faboideae. Javaid et al. (2004) reported that the diversity observed in protein profiles and seed storage proteins have

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potential for species classification and also serves as marker for interspecific hybridization procedure. Thus, this technique could better be used to establish interspecific diversity and phylogenetic or evolutionary relationships among various species. Suranto (2002) reported that protein electrophoresis has been regarded to be the most useful tool for resolving systematic problems in situations where morphological characters are felt inadequate to make taxonomic decisions.

Increasingly, electrophoresis has become an additional tool to unravel taxonomic and phylogenetic problems (Azeez and Morakinyo, 2004). Most applications of electrophoretic techniques in plant classifications use gel medium supports. This has resulted from the reliability of data produced by gel electrophoresis, which have been accepted widely, particularly in studies of plant population genetics (Mohammed et al., 2006; Sadia et al., 2009, Atoyebi et al. 2014). Omonhinmin and Ogunbodede (2013) opined that because of the high level of inter-specific diversity among members of the Fabaceae family, there is need to review the traditional taxonomic position of the family. Several studies have been conducted on other families like Aspidiaceae and Athyriaceae (Dhir et al*.,* 1975), Aracaceae (Mohammed et al*.,* 2006), Cucurbitaceae (Yadav, 2008) and Solanaceae (Bhat and Kudesia 2011) using SDS-PAGE method but such studies on Fabaceae family is still scanty.

According to Bruneau et al*.* (2008), sub-family Caesalpinioideae forms a basal grade from which a monophyletic Faboideae and Mimosoideae arise. In the report of Van den Bosch and Stacey (2003), most authors supported the monophyletic origin of members in the Fabaceae family while several recently published floristic accounts still refer to legumes as having a polyphyletic origin leading to their grouping into three sub-families often referred to as sub-families: Caesalpinioideae*,* Mimosoideae and Faboideae. Bruneau et al*.* (2008) opined that the family *Fabaceae* is long overdue for an extensive taxonomic re-appraisal. The present study was therefore undertaken to assess the systematic relationships among some selected species of Fabaceae using protein electrophoretic method and possibly assess the mode of evolution of the selected legumes from the three sub-families.

MATERIALS AND METHODS

Collection of samples

Seeds of 10 members of the family Fabaceae (Table 1) were collected in May 2013 and stored for six months to get their seed storage protein. Two of the 10 studied plant species were under the sub-family Mimosoideae, three under Caesalpinioideae and five under Faboideae. Proper identification of each plant species used was carried out at the Department of Biological Sciences of Kogi State University, Anyigba.

SDS gel electrophoresis of seed proteins

Seeds from each sample were dried in an oven before homogenizing with an extraction buffer containing 0.05 aM Tris-HCl (pH7.4) 4°C. Bromophenol blue was added to the sample buffer as a tracking dye to watch the movement of proteins in the gel. The homogenate was centrifuged at 10,000 r.p.m for 15 min at 4°C and the supernatant was used for electrophoresis. Twelve percent (12%) SDS-gel was used for the run following the Discontinuous Electrophoretic method of Leammli (1970). The sigma® maker used to trace the bands contains 13 proteins ranging from 6,500 to 205,000 kb.

Data analysis

To avoid ambiguity in data, only consistent protein band between 6,500 and 205,000 kb were considered for data recording. Bands clearly visible in at least one species were scored 1 for present, 0 for absent and entered in binary matrix.

The similarity index proposed by Nei and Li (1979) was used to locate the degree of similarity (S_{ab}), between two cultivars a and b according to the formula:

$$S_{ab} = 2N_{ab} / (N_a+N_b)$$

Where, N_{ab}= number of bands common to both species a and b; N_a = number of bands in species a;
N_b = number of bands in species b; a dendogram (hierarchical cluster) was constructed using the unweighted pair group method average (UPGMA). All computations were done using SPSS V21 window software.

RESULTS

The patterns of protein in the 10 studied members of Fabaceae is shown in Plate 1. A close examination of the bands revealed that the studied plants shows differences in their banding patterns with marked difference in the numbers and intensities of the bands. A total of 19 protein bands were observed among the 10 studied legume species while band number 8 is the only band common to the studied plant species (Figure 1).

The total number of bands and number of unique bands in Table 2 shows that accession number 6 (*A. lebbeck*) possessed the highest number of bands (16) while the least number of band (3) was observed in accession number 2 (*Senna siamea*). Table 3 shows that the higher similarity coefficient of 0.93 was observed between accession number 9 (*V. subterranea)* and accession number 10 (*A. hypogaea*) while the least similarity of 0.32 was observed between accession number 2 (*S. siamea*) and accession number 6 (*Albizia lebbeck*).

The hierarchical cluster analysis for the 10 studied plant species (Figure 2) grouped the plants into two major clusters. The first cluster comprised of four species while six species occupied the 2nd cluster. The four species under the first cluster were accession numbers 3 (*Parkia biglobosa*), 6 (*Albizia lebbeck*), 9 (*Vigna subterranean*) and 10 (*Arachis hypogaea*) while the six species under the second cluster were 1 (*C. pulcherrima)*, 2 (*S. siamea*), 4 (*P. vulgaris*), 5 (*D. regia*), 7 (*V. unguiculata*) and 8 (*Glycine max*). Accessions 9 (*Vigna subterranean*) and 10 (*Arachis hypogaea*) showed the highest similarity among the studied plant species.

Table 1. Description of the 10 species of *Fabaceae* Studied.

Accession number	Common name	Location of collection	Habit of the plant	Scientific name	Sub-family
1	Pride of Barbados	Anyigba	Tree	*Caesalpinia pulcherrima*	Caesalpinioideae
2	Cassia tree	Anyigba	Tree	*Senna siamea*	Caesalpinioideae
3	Locust bean tree	Anyigba	Tree	*Parkia biglobosa*	Mimosoideae
4	Common beans	Anyigba market	Herb	*Phaseolus vulgaris*	Faboideae
5	Flamboyant plant	Anyigba	Tree	*Delonix regia*	Caesalpinioideae
6	Siris tree	Anyigba	Tree	*Albizia lebbeck*	Mimosoideae
7	Cowpea	Anyigba market	Herb	*Vigna unguiculata*	Faboideae
8	Soybeans	Anyigba market	Herb	*Glycine max*	Faboideae
9	Bambara nut	Anyigba market	Herb	*Vigna subterranea*	Faboideae
10	Groundnut	Anyigba market	Herb	*Arachis hypogaea*	Faboideae

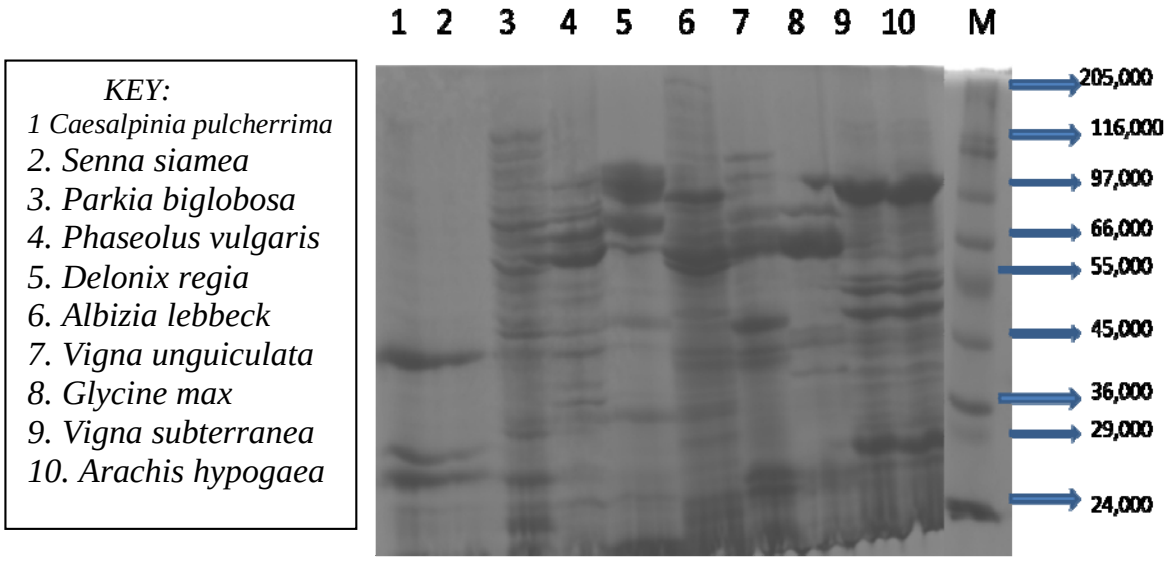

Figure 1. Electrophoregram showing protein banding patterns for the 10 legume plant species studied.

DISCUSSION

Protein electrophoresis is a better tool for the identification of genetic diversity and tracing evolutionary processes in plants than morphological markers (Omonhinmin and Ogunbodede, 2013; Natarajan, 2014). Seed protein electrophoresis according to Berber and Yaşar (2011) is increasingly being utilized as an additional approach for species identification and as a useful tool for solving evolutionary problems in plants. Ehsapour et al. (2010) and Sinha et al. (2012) attributed this to the fact that proteins stored in the seeds are highly independent of environmental factors. Alege et al. (2013) and Yatung et al. (2014) opined that the discrepancy between morphological and protein profile is due to the impact of the environment on the former.

The 10 plant species under the Fabaceae family studied revealed that no two plants share the same protein banding patterns which indicates that genetic diversities exist among the plant species. The presence of a common band (band number 8) among the 10 plant species suggests their close genetic affinity and common ancestry.

This band is coded for by a gene that has become fixed in different species under the Fabaceae family over evolutionary time. This is in agreement with the finding of Azeez and Morakinyo (2004) that the presence of common bands in *Lycopersicon* and *Trichosanthes* species depicts their common evolutionary origin. Also, Akinwusi and Illoh (1995) attributed the appea-rance of a common band in all individual in a population to the fact that the gene coding for the enzyme or protein does not vary.

S. siamea had two unique bands (bands number 18 and 19) which suggest that these two bands can be tagged as species specific bands for the identification of this

Table 2. The total number of bands and the unique bands among the 10 studied plants.

Accession number	Plant species	Total number of bands	Bands specific to each species
1	*Caesalpinia pulcherrima*	7	Nil
2	*Senna siamea*	3	Nil
3	*Parkia biglobosa*	15	Nil
4	*Phaseolus vulgaris*	11	Nil
5	*Delonix regia*	7	Nil
6	*Albizia lebbeck*	16	2
7	*Vigna unguiculata*	9	Nil
8	*Glycine max*	6	Nil
9	*Vigna subterranea*	14	Nil
10	*Arachis hypogaea*	14	Nil

Table 3. Similarity index for the 10 species of Fabaceae studied.

Accession number	1	2	3	4	5	6	7	8	9	10
1	-									
2	0.60	-								
3	0.64	0.33	-							
4	0.56	0.44	0.77	-						
5	0.58	0.40	0.64	0.78	-					
6	0.60	0.32	0.84	0.67	0.52	-				
7	0.50	0.52	0.67	0.80	0.75	0.64	-			
8	0.62	0.44	0.48	0.59	0.62	0.55	0.80	-		
9	0.48	0.35	0.83	0.72	0.48	0.73	0.61	0.50	-	
10	0.57	0.35	0.83	0.72	0.48	0.73	0.61	0.50	0.93	-

1, *Caesalpinia pulcherrima*; 2, *Senna siamea*; 3, *Parkia biglobosa*; 4, *Phaseolus vulgaris*; 5, *Delonix regia*; 6, *Albizia lebbeck*; 7, *Vigna unguiculata*; 8, *Glycine max*; 9, *Vigna subterranean*; 10, *Arachis hypogaea.*

plant. This report is in line with the findings of Mohammed et al. (2006) on members of Aracaceae.

The similarity coefficient range of 0.32 to 0.93 indicates that genetic diversity exists within the family Fabaceae for their systematics. The highest similarity observed between 9 (*Vigna subterranea)* and 10 (*Arachis hypogaea*) is a clear indication that they are phylogenetically related than every other species studied. In contrary, the least similarity observed between 2 (*S. siamea*) and 6 (*A. lebbeck*) suggests that they evolved along different evolutionary trends. This therefore justified their placement under different sub-families, Mimosoideae and Caesalpinioideae, respectively.

The hierarchical cluster analysis for the 10 studied plant species showed two major clusters. This suggests that the origin of Fabaceae may be along more than one evolutionary line. This is further supported by the fact that all the members of sub-family Mimisoideae (that is, *Parkia biglobosa* and *A. lebbeck*) considered in this study clustered together in the first group while the three members of the Caesalpinioideae (*C. pulcherrima*, *S. siamea* and 5 *Delonix regia*) studied remained together in the second group. This is an indication that the Mimosoideae and Caesalpinioideae lines may be the two lines of evolution in the family Fabaceae. The scattering of the members of the sub-family Faboidae between the two clusters supports origin through two evolutionary lines. This observation in a way contradicts the earlier report of Bruneau et al. (2008) that the sub-family Caesalpinioideae forms a basal grade from which a monophyletic Faboideae and Mimosoideae arise. Cluster analysis and similarity matrix revealed very close genetic similarity between the two accessions with underground pods; that is, accessions 9 (*V. subterranea*) and 10 (*A. hypogaea*), Bambara groundnut and groundnut, respectively. This strongly suggests their placement under the same genus taxonomically while the remaining eight members studied should retain their genera.

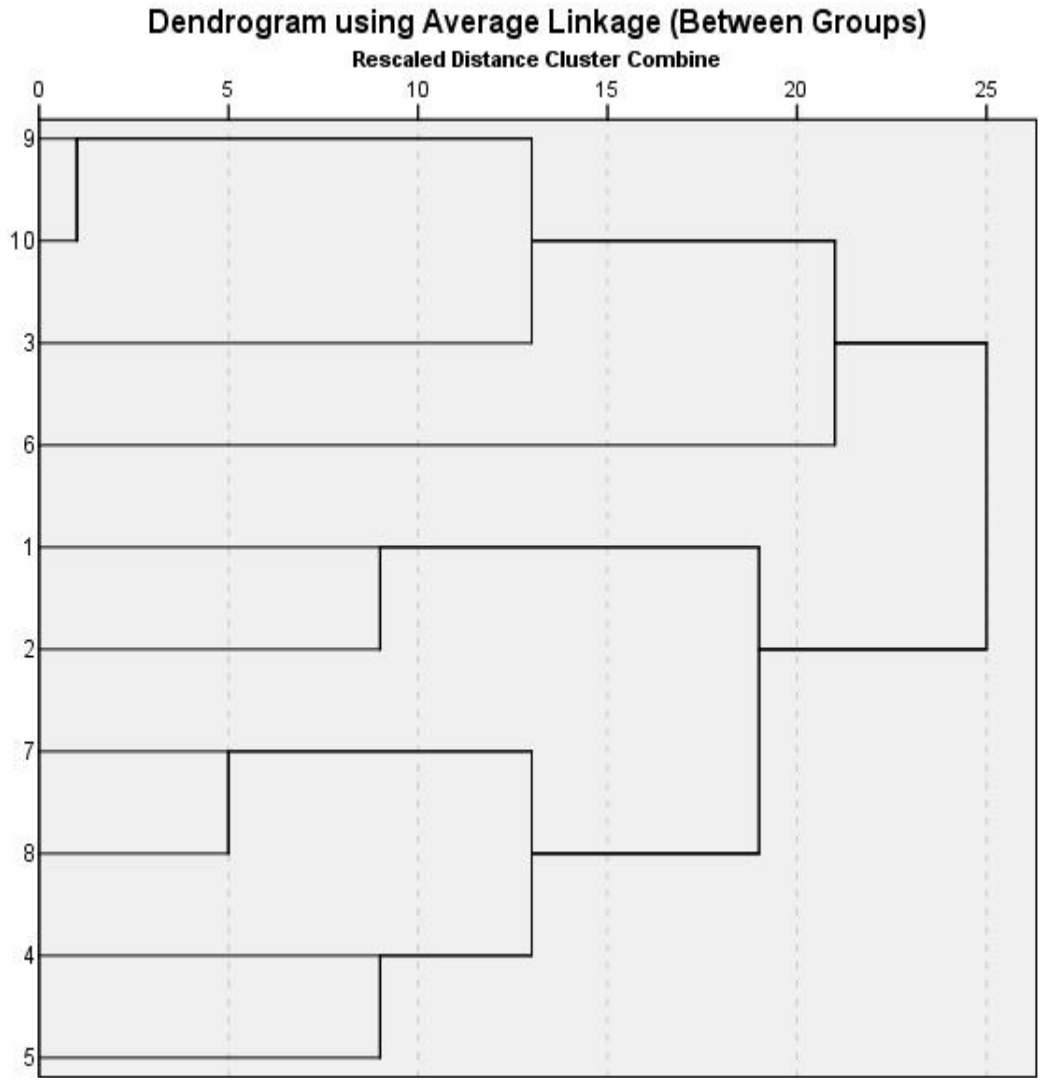

Figure 2. Hierarchical cluster analysis for the 10 legumes studied. 1, *Caesalpinia pulcherrima*; 2, *Senna siamea*; 3, *Parkia biglobosa*; 4, *Phaseolus vulgaris*; 5, *Delonix regia*; 6, *Albizia lebbeck*; 7, *Vigna unguiculata*; 8, *Glycine max*; 9, *Vigna subterranean*; 10, *Arachis hypogaea.*

CONCLUSION AND RECOMMENDATION

The objectives of this study which were to assess the genetic similarities among the 10 selected legumes, re-appraise their taxonomic position and unravel their possible mode of evolution have been achieved. The study suggests evolution of Mimosoideae and Caesalpinioideae along two lines from which members of the Faboidae originated. Also, it was observed that *V. subterranea* (Bambara groundnut) and *A. hypogaea* (groundnut) are genetically very close and should be put under the same genus. It is therefore recommended that other techniques especially molecular markers like random amplified polymorphic DNA (RAPD) should be employed to compliment the findings of this study. Also, a larger number of genera under the Fabaceae should be considered.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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