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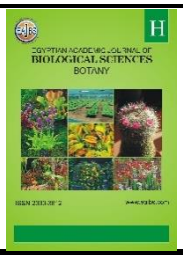

Inter-Specific Variation in SDS-PAGE Electrophograms of Total Leaf Proteins in Some Species of Subtribe Cassiinae

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ABSTRACT

The study employs the SDS-PAGE electrophoretic techniques evaluate the taxonomic implications of some species in the subt Cassiinae (Caesalpinaceae) using total leaf proteins. The aim was to as the genetic variation and relationship among the 15 species of subt Cassiinae through electrophoretic studies of their leaf proteins. Total proteins were extracted and separated on 12% polyacrylamide gels us standard protocols. Young leaves (0.8g) of the plants were washed v distilled water and macerated with sterile mortar and pestle in 0 Phosphate Buffer-Saline (PBS) containing 0.4M NaCl at PH 8.0. Res obtained revealed that protein pattern was taxon-specific as no two spe have the same banding pattern. Distant polymorphism in electrophor banding patterns of the leaf was observed through a total of forty-one (polypeptide bands. Variation existed not only in the number of bands also in the intensity of bands in the leaf samples studied. The coefficient similarity range between 0.076 – 0.845. The hierarchical cluster anal (dendrogram) for the 15 species revealed two major clusters. The first gr are *Senna spectabilis*, *S. alata*, *S. hirsuta*, *Chamaecrista rotundifolia*, *mimosoides*, *S. biflora* and the second group are *S. podocarpa*, *S. sopher occidentalis*, *S. obtusifolia*, *Cassia italica*, *S. siamea*, *C. singueana*, *sieberiana* and *C. fistula*. An artificial key for the studied species of subt Cassiinae based on their band relationships is also provided. Therefore, number and intensity of bands are additional characters that can be used species delimitation in subtribe Cassiinae.

INTRODUCTION

Protein electrophoresis has been utilized successfully to study hereditary of various plants and in its germplasm for its usage in crop breeding programmes (Javid *et al.*, 2004). Oladipo and Illoh (2012) employed the technique in the infrageneric delimitations of the genus *Jatropha* and concluded that the differences observed in the protein profiles of the taxa studied are indicative of hereditary diversity and very useful in the taxonomic delimitations of the members of the genus.

The protein profiling of germplasm and utilization of hereditary markers have been broadly and successfully used to determine the taxonomic and evolutionary aspects of several plants (Nisar *et al.*, 2007; Hameed *et al*., 2009, Oladipo and Illoh, 2012). Among all biochemical techniques, Sodium Dodecyl Sulfate Polyacrylamide Gel electrophoresis (SDS-PAGE) is widely utilized due to its legitimacy and simplicity for describing the genetic structure of plants germplasm (Javid *et al*., 2004). SDS-PAGE is practically a reliable method because seed storage proteins are largely independent of environmental fluctuation (Nisar *et al.,* 2007; Hameed *et al.,* 2009). Characterization of germplasm using biochemical fingerprinting has special attention; SDS-PAGE is most economical simple and extensively utilized biochemical technique for analysis of genetic structure of germplasm (Iqbal *et al.,* 2005).

The number of electrophoretic bands could be an expression of genetic trait controlling nuclear genes and it could be used in plant taxonomy and evolution (Kolawole, 2017). Emre *et al.* (2007) reported the use of electrophoretic analysis of seed proteins in the evaluation of genetic variability and systematic problems in some species of *Lathyrus* L. grown in Turkey. Azeez and Morakinyo (2004) on the Electrophorectic characterization of crude leaf proteins in *Lycopersicon* and *Trichosanthes* cultivars reported that the presence of common protein bands among species might be evidence of common evolutionary origin.

Genus *Cassia* consists of annual or perennial herbs, shrubs and trees that have been differentiated based on the number of leaflets, fertile and sterile stamens in single flower and glands present on the leaves (Deshmukh *et al.*, 2014). Irwin and Barneby (1981, 1982) raised the genus *Cassia* L. *sensu lato* to the level of subtribe and elevated previous subgenera to generic rank viz. *Senna* Mill and *Chamaecrista* Moench under the subtribe Cassiinae and tribe *Cassieae* Bronn ex Irwin and Barneby of Caesalpiniaceae. The taxonomic treatment of the genus *Cassia* L. *senso lat.* has been done in some countries, namely Malaysia (De Wit, 1955); Pakistan (Ali and Quraishi; 1967) and Nigeria (Saheed and Illoh; 2010). Ogundipe *et al*. (2009) and Saheed and Illoh (2010) reviewed the genus using leaf epidermal characters while Soladoye *et al*. (2010) employed leaf morphological characters in delimiting taxa.

This study therefore, compares the electrophoretic pattern of protein bands distribution and identifies interspecific variations that exist among the studied species and their taxonomic implications.

MATERIALS AND METHODS

Plant Collection:

The specimens for the present study were collected from 6 States in Nigeria (Table 1 and Figure 1) shows the distribution of the studied species of Cassiinae in Nigeria.

Mature plant specimens from the field were used for the study. The fresh specimens were collected in open vegetation, from roadsides and bushy areas in various parts of Nigeria. Upon collection of fresh plants, voucher specimens were prepared according to the established protocol of Soladoye *et al.* (2010) and modified by Kolawole *et al.* (2016) and were deposited in the Forest Herbarium, Ibadan and voucher number were given.

Protein Fingerprinting Using SDS Gel Electrophoresis for Leaf Proteins:

Electrophoretic study of the protein variations from the leaves of the taxa studied was carried out at the Bioscience Centre of the International Institute of Tropical Agriculture, IITA, and Ibadan using 12% polyacrylamide gels.

The species were screened for total protein banding patterns by using a modified method of Laemli (1970) as described (Aguegia *et al*. 1994, Omitogun *et al.* 1999 and Tokpo *et al*. 2006). Young leaves (0.8 g) of the plants were washed with distilled water and macerated with sterile mortar and pestle in 0.8% phosphate buffer-saline (PBS) containing 0.4 M NaCl at pH 8.0. The extract was centrifuged at 5000 rpm for 10 minutes, 15 µl of each supernatant was electrophoresed in 12% polyacrylamide-bisacarylamide gel. Gels were stained with 0.3% Coomassie Brilliant blue for 18 hours. Destaining was in a mixture of methanol, acetic acid and distilled water (1.3v/v). This was done overnight in order to visualize the protein bands for subsequent scoring. The gels were scanned with Gel doc scanner and the images stored for scoring.

Table 1: List of species studied with site of collection and herbarium voucher number

Species	Places of Collection/Locality	Voucher number
Senna alata (L.) Roxb.	Sekona village, Ede South LGA, Ede, Osun State.	FHI 109787
Senna podocarpa (Guill. and Per.) Lock	U I Botanical Nursery, Dept. of Botany, U I, Ibadan, Oyo State.	FHI 109870
Senna sophera (L.) Roxb.	Along Agah Ganmo Road, Ifelodun LGA, Kwara State.	FHI 109785
Senna hirsuta (L.) Irwin & Barneby	Infront of FUOYE main gate, Oye Ekiti, Ekiti State	FHI 109869
Senna occidentalis (L.) Link.	Gaa Immam Area, Along Ajase-Ipo Road, Ilorin, Kwara State	FHI 109866
Senna obtusifolia (L.) Irwin & Barneby	Along Pindinga- Kashere road,Akko LGA, Gombe State	FHI 109790
Senna spectabilis (DC.) Irwin and Barneby	Infront of LAUTECH Senate Building, Ogbomoso, Oyo State.	FHI 109965
Senna siamea (Lam.) Irwin and Barneby	Behind Federal High Court, GRA, Bauchi State.	FHI 110012
Senna biflora Linn.	Infront of Olu of Akoda Palace, Akoda, Ede, Osun State	FHI 109867
Cassia fistula Linn.	Beside Block 10, UNILORIN Main Campus, Ilorin, Kwara State.	FHI 109792
Cassia italica Mill.	GSU campus, Gombe Metropolis, Gombe State	FHI 109966
Cassia singueana (Del.) Lock.	Along Kashere –Alhaleri road, Kashere, Gombe State.	FHI 109965
Cassia sieberiana DC.	Beside Sports Centre, OAU Campus, Ile Ife, Osun State.	FHI 109967
Chamaecrista mimosoides (L.) Greene	Ori Eru Village, Asa LGA, Kwara State.	FHI 109868
Chamaecrista rotundifolia (Pers.) Greene	Tunfure Cattle village, Along Airport road, Gombe, Gombe State.	FHI 109788

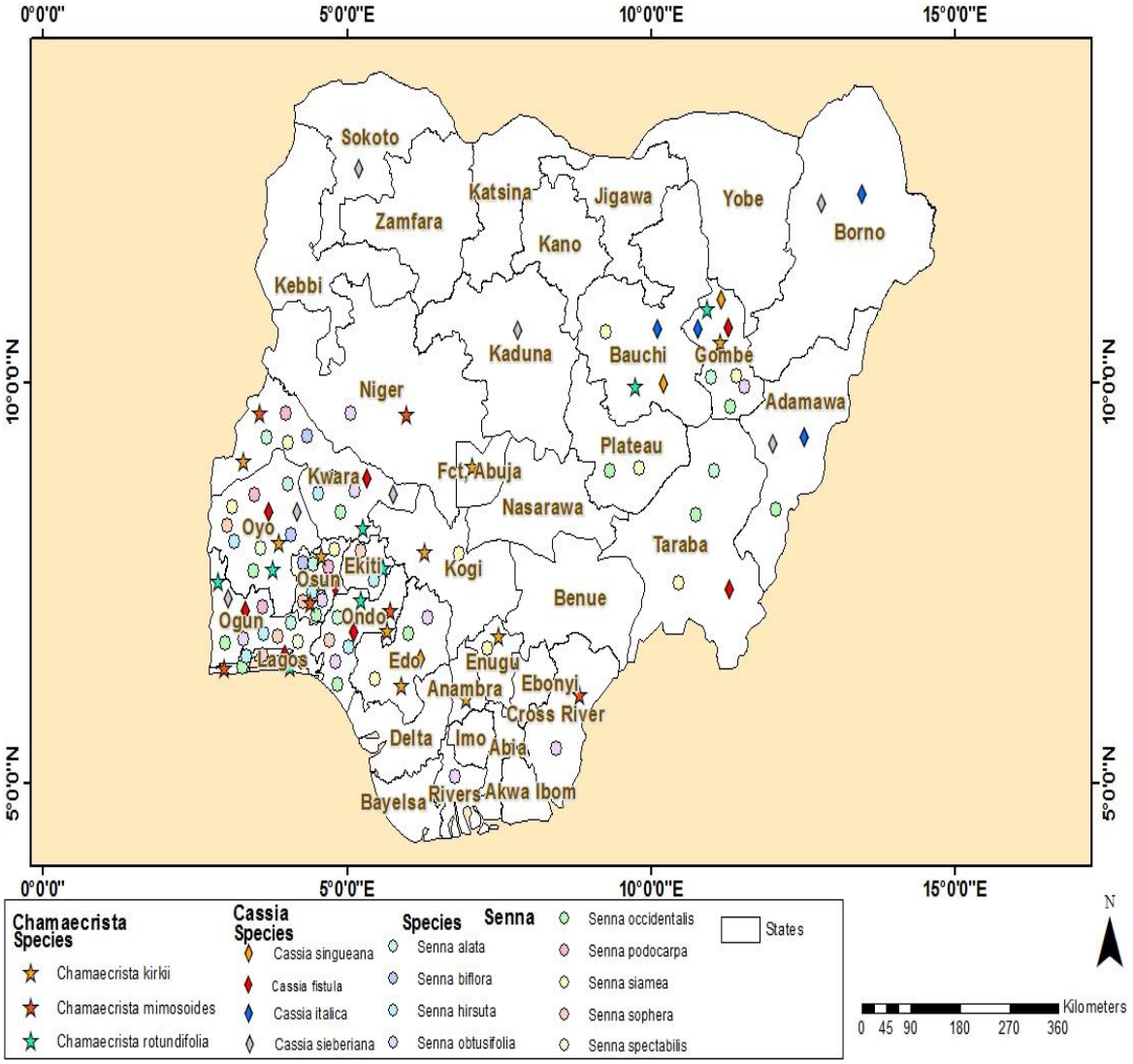

Fig. 1: Locations of the studied species of subtribe Cassiinae in Nigeria

Data Analysis:

To avoid ambiguity in the data, only consistent protein bands between 10,000 and 170,000 kb were considered for data recording. Bands clearly visible in at least one species were scored 1 for the present, 0 for absent and entered in a binary matrix.

The similarity Index proposed by Nei and Lei (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 of species a;

N_b = number of bands in species b;

A dendrogram (hierarchical cluster) was constructed using the unweighted pair group method (UPGMA). All computations were done using SPSS V20 windows software.

RESULTS

The patterns of the protein distribution in the studied species of Cassinae and their diagrammatic representation are shown in Figures 2 and 3 A and B respectively. A close examination of band distribution shows that no two species have the same banding patterns. Marked differences were recorded for numbers, the combination of bands and

intensity of bands between the studied species of subtribe Cassiinae. An artificial key was formulated and used in the identification of the studied species using the band relationships of the leaf protein (Table 2). The relationship between all the species of subtribe Cassiinae studied on the basis of protein bands distribution is also shown in Table 3. The band ranges from one to four (Figures 3 A and B) namely faint bands, faint thick bands, thick bands and very thick bands. Protein band distribution in the studied species of subtribe Cassiinae. The results revealed that the total number of bands is 41, fast-moving bands is 10 bands (24.4%), 24 bands (58.5%) are slow moving bands and intermediate bands are 7 which amount to 17.1% of the total bands. Most of the bands were found to be slow moving bands (0 to 2cm), followed by fast moving bands (5.1 to 10cm) followed by intermediate moving bands (2.1 to 5cm) respectively. *C. italica*, *S. biflora*, *S. alata* and *S. sophera* possess the highest number of bands (4) while the least number of bands (1) was observed in *Chamaecrista mimosoides*.

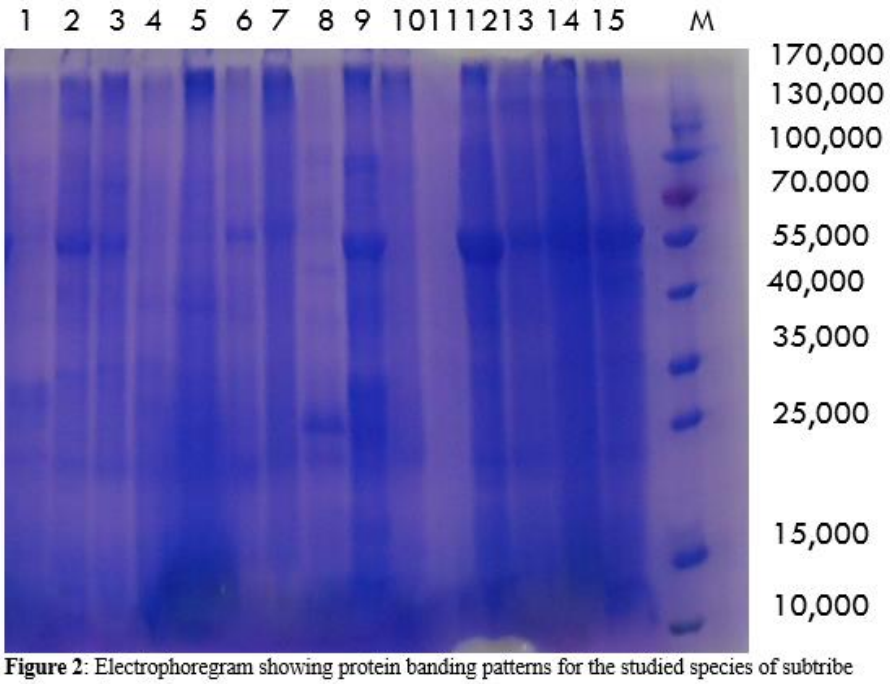

Figure 2: Electrophoregram showing protein banding patterns for the studied species of subtribe Cassiinae.

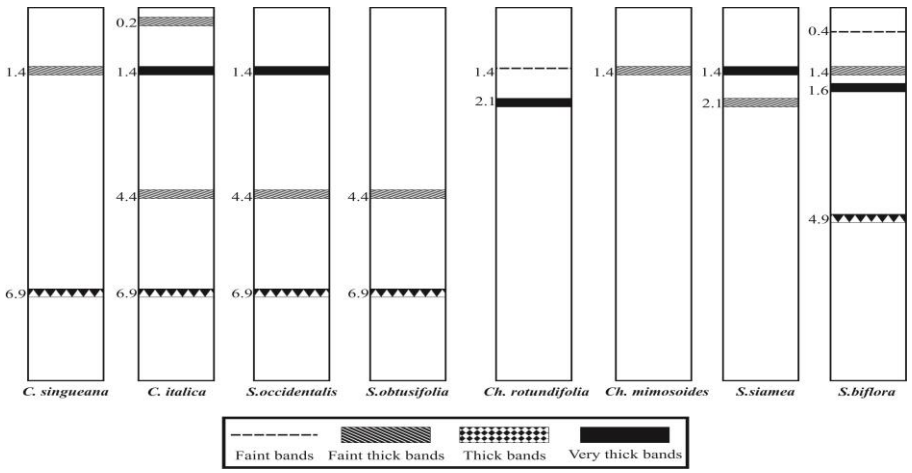

Fig. 3 A: Schematic diagrams of leaf protein distribution pattern of studied species of subtribe Cassiinae.

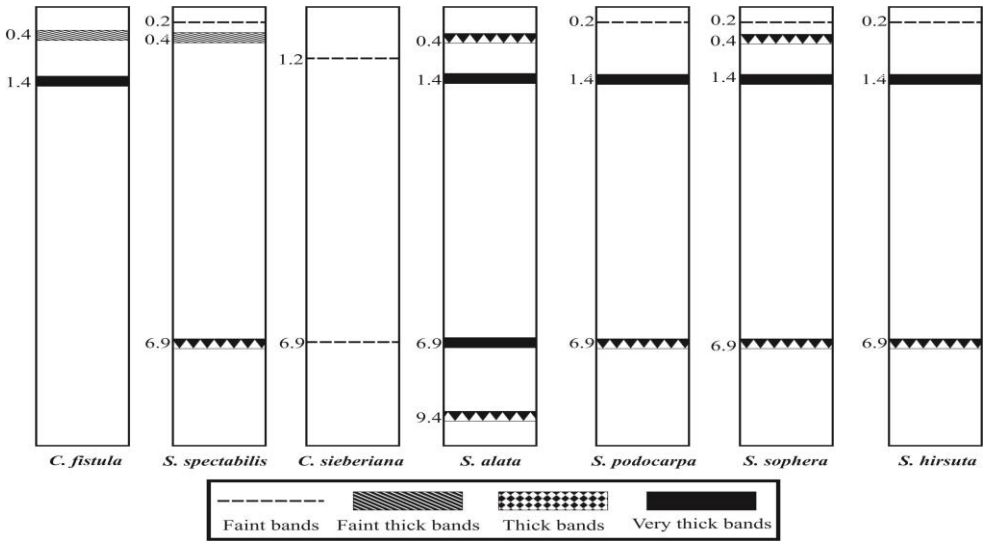

Fig. 3 B: Schematic diagrams of leaf protein distribution pattern of studied species of subtribe Cassiinae

Table 2: An artificial key for the studied species of subtribe Cassiinae based on their band relationships.

Bands relationships	**Species of Cassiinae**
Cassiinae species with more than two (2) bands	*C. italica, S. occidentalis, S. biflora, S. spectabilis, S. alata, S. podocarpa, S. sophera, S. hirsuta*
Thick band present at 1.4 cm, and total bands is four (4).	*S. biflora*
Band absent at 1.4 cm and total band is three (3).	*S. spectabilis*
Very thick bands present at 1.4 cm	*C. italica, S. occidentalis, S. alata, S. podocarpa, S. sophera, S. hirsute*
Total number of bands in gel is four (4)	*C. italica, S. alata, S. Sophera*
Two very thick bands present in gel and thick band present at 9.4 cm	*S. alata*
Faint thick band present at 9.4 cm	*C. italica*
Bands present at 0.2 cm and 0.4 cm	*S. sophera*
Total number of bands in gel is three (3)	*S. occidentalis, S. podocarpa, S. hirsuta*
Band absent at 0.2 cm	*S. occidentalis*
Very thick band present at 1.4 cm	*S. podocarpa*
Thick bands present at 6.9 cm	*S. hirsute*
Cassiinae species with two (2) bands or less in their gel	*C. singueana, S. obtusifolia, Ch. rotundifolia, Ch. mimosoides, S. siamea, C. fistula, C. sieberiana*
Total number of band in gel is one (1)	*Ch. mimosoides*
Total number of band in gel is two (2)	*C. singueana, S. obtusifolia, Ch. rotundifolia, S. siamea, C. fistula, C. sieberiana*
Faint band present at 1.4cm	*Ch. rotundifolia*
Two faint bands present at 1.2 and 6.9 cm	*C. sieberiana*
Thick band present at 6.9 cm	*C. singueana and S. obtusifolia*
Faint thick band present at 1.4 cm	*C. singueana*
Faint thick band present at 4.4 cm	*S. obtusifolia*
Very thick band present at 1.4 cm	*S. siamea and C. fistula*
Faint thick band present at 0.4 cm	*C. fistula*
Faint thick band present at 2.1 cm	*S. siamea*

Table 3: Protein bands distribution in the studied species of subtribe Cassiinae.

Names of species	Total number of bands	Higher band (5 – 10cm)	Intermediate band (2.1 – 5cm)	Lower band (0 – 2cm)
Cassia singueana	2	1	-	1
Cassia italica	4	1	1	2
Senna occidentalis	3	1	1	1
Senna obtusifolia	2	1	1	-
Chamaecrista rotundifolia	2	-	1	1
Chamaecrista mimosoides	1	-	-	1
Siamea siamea	2	-	1	1
Senna biflora	4	-	1	3
Cassia fistula	2	-	-	2
Senna spectabilis	3	-	1	2
Cassia sieberiana	2	1	-	1
Senna alata	4	2	-	2
Senna podocarpa	3	1	-	2
Senna sophera	4	1	-	3
Senna hirsuta	3	1	-	2
Total	41	10	7	24

In Table 4, the similarity indices for the leaf proteins of the studied species of subtribe Cassiinae shows that the highest similarity coefficient of 0.845 was observed between *Cassia singueana* and *Cassia italica*, *Senna occidentalis* and *Senna obtusifolia*; *Senna siamea* and *Cassia italica*, *Senna occidentalis*, *Senna obtusifolia*. The least similarity coefficients of 0.076 were observed between *Senna hirsuta* and *Senna siamea* and between *Senna hirsuta* and *Senna podocarpa*.

The dendrogram grouped the fifteen studied species of the subtribe Cassiinae using the leaf protein into 2 main clusters (Figure 4). The first cluster comprises six (6) species while the second cluster is represented by nine (9) species. Cluster I comprises of six species namely *S. spectabilis*, *S. alata*, *S. hirsuta*, *Ch. rotundifolia*, *Ch. mimosoides* and *S. biflora* while Cluster II is subdivided into 3 sub-clusters. Sub-cluster A comprises of seven species namely *S. podocarpa*, *S. occidentalis*, *S. obtusifolia*, *C. italica*, *S. siamea* and *C. singueana* while sub-cluster B and C are *C. sieberiana* and *C. fistula* respectively.

Table 4: Similarity Index for the leaf proteins of the studied species of subtribe Cassiinae.

Plant Species	*C. singueana*	*C. italica*	*S. occidentalis*	*S. obtusifolia*	*Ch. rotundifolia*	*Ch. mimosoides*	*S. siamea*	*S. biflora*	*C. fistula*	*S. spectabilis*	*C. sieberiana*	*S. alata*	*S. podocarpa*	*S. sophera*	*S. hirsuta*
C. singueana	1.000														
C. italic	0.845	1.000													
S. occidentalis	0.845	1.000	1.000												
S. obtusifolia	0.845	1.000	1.000	1.000											
Ch. rotundifolia	0.000	0.000	0.000	0.000	1.000										
Ch. mimosoides	0.378	0.447	0.447	0.447	0.000	1.000									
S. siamea	0.657	0.845	0.845	0.845	0.000	0.378	1.000								
S. biflora	0.098	0.192	0.192	0.192	0.000	0.258	0.098	1.000							
C. fistula	0.598	0.707	0.707	0.707	0.000	0.632	0.598	0.408	1.000						
S. spectabilis	0.255	0.302	0.302	0.302	0.000	0.674	0.255	0.522	0.426	1.000					
C. sieberiana	0.598	0.707	0.707	0.707	0.000	0.158	0.598	0.000	0.250	0.426	1.000				
S. alata	0.255	0.302	0.302	0.302	0.000	0.674	0.255	0.522	0.426	1.000	0.426	1.000			
S. podocarpa	0.657	0.845	0.845	0.845	0.000	0.378	0.657	0.098	0.598	0.255	0.598	0.255	1.000		
S. sophera	0.478	0.707	0.707	0.707	0.000	0.316	0.837	0.000	0.500	0.213	0.500	0.213	0.837	1.000	
S. hirsute	0.076	0.000	0.000	0.000	0.000	0.400	0.076	0.258	0.158	0.674	0.158	0.674	0.076	0.158	1.000

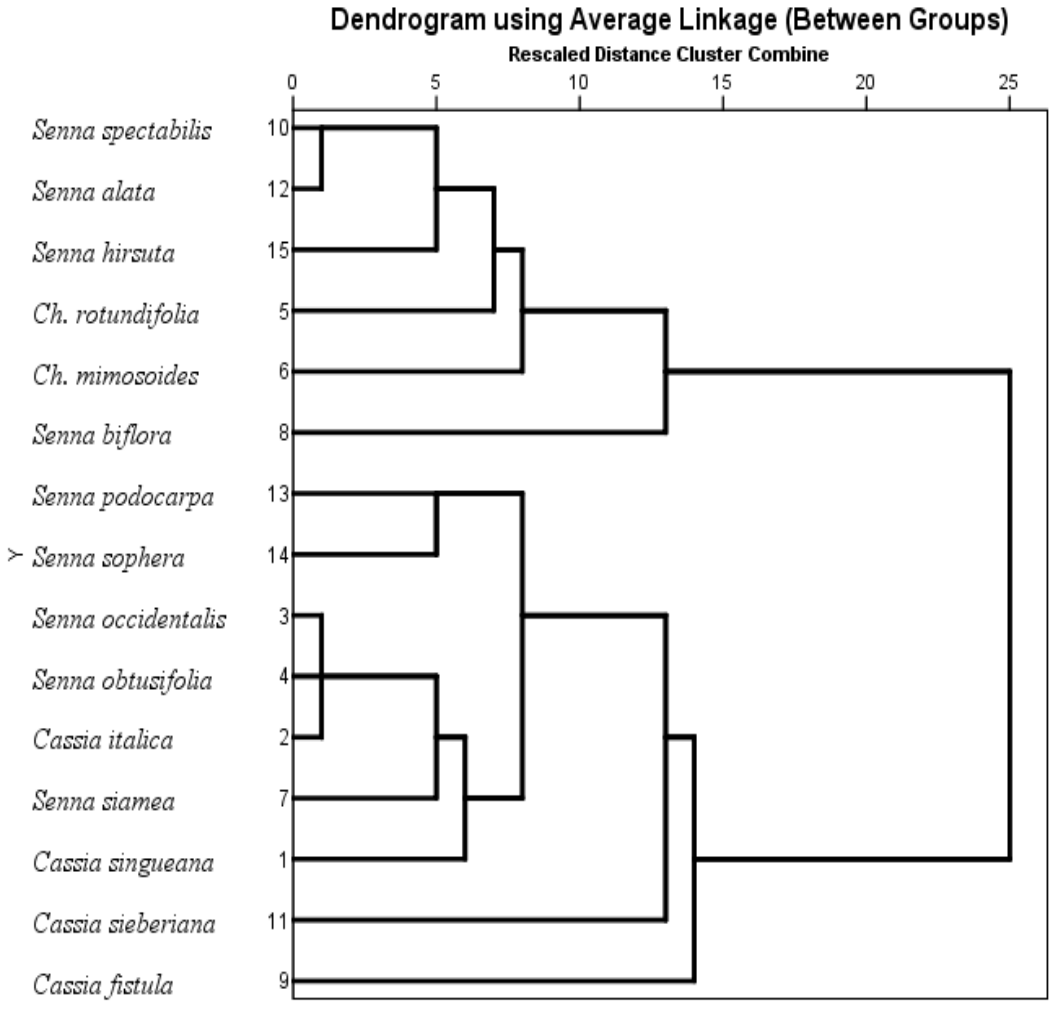

Fig. 4: Dendrogram using Average Linkage between groups of leaf proteins of the studied species of subtribe Cassiina

DISCUSSION

The significance of electrophoretic evidence in plant systematics has been discussed in detail by many researchers especially on *Jatropha* (Oladipo and Illoh, 2012), *Ludwigia* (Folorunso and Adelalu, 2015), *Corchorus* (Ajala and Morakinyo, 2015) and varieties of *Abelmoschus esculentus* (Abdulrahaman *et al.*, 2015).

Electrophoretic analysis of leaf proteins has a direct relationship to the genetic background of the proteins that reveal genetic diversity, and such analysis can be used to certify the genetic makeup of germplasm (Javid *et al.*, 2004 and Iqbal *et al.*, 2005). The results show that no two species of Cassiinae studied have the same number and intensity of protein bands. This reveals that the protein-banding pattern may be a reflection of their morphological characters. Protein variation in the species of Cassiinae studied is an indication of protein polymorphism. This depicts the genetic divergence in them and at the same time forms the basis of the separation of individuals in a particular population into different groups (Olatunji and Morakinyo, 2015; Folorunso and Adelalu, 2015).

The variation observed in the distribution of protein bands in the species of subtribe Cassiinae studied is species specific. This corroborates the report of Olson (1967) that biogenetic relationships can be best revealed by quantitative results emanating from chemotaxonomic methods. In addition, Sadia *et al.* (2009) opined that landraces are a useful source of genetic differences and the greater the chance of a landrace possessing gene combinations of interest to plant breeders. The degree of variation observed in the protein profiles of the taxa is indicative of genetic divergence among them. The band at 6.9 cm appeared in all the species of *Senna* studied, which is an indication that band 6.9 cm can be tapped is a generic band because of its presence in the *Senna* species but was absent from the two species of *Chamaecrista.* This supports the earlier findings of Akinwusi and Illoh (1995) that when the band appears in all individuals in a population, it is assumed that the gene that codes the enzyme of protein does not vary.

Conclusion:

In the dendrogram constructed using leaf proteins parameters, the two *Chamaecrista* species clustered mostly with *Senna* species, also in the second sub-cluster the two species of *Cassia* clustered together. The differences observed in the protein profiles of the taxa studied are indicative of genetic diversity and thus, may be useful in the taxonomic delimitation of members of subtribe Cassiinae. The results of the leaf protein electrophoresis demonstrate close relationship and distinctness of the taxa studied and could, therefore, be important in inter and infra-generic delimitations.

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Competing Interests:

The authors declare that they have no financial or personal relationships, which may have inappropriately influenced them in writing this article.

Authors' Contributions:

KOS initiated the project; KOS and AAA are responsible for the project design. KOS carried out electrophoretic study while AAA provided the background study of the subtribe. Both KOS and AAA jointly write the results, discussion and conclusion together.

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