AcademicPres

Available online: www.notulaebiologicae.ro

Print ISSN 2067-3205; Electronic 2067-3264

Not Sci Biol, 2015, 7(2):177-183. DOI: 10.15835/nsb.7.2.9504

Notulae Scientia Biologicae

SDS-PAGE Characterisation of Crude Seed and Leaf Proteins in *Corchorus* Species

Christiana Adeyinka AJALA*, Joseph Akintade MORAKINYO

*University of Ilorin, Faculty of Life Sciences, Department of Plant Biology, P.M.B 1515 Ilorin, Kwara State, Nigeria; dristianaadeyinka@gmail.com (*corresponding author); morakinyoja@yahoo.com*

Abstract

Crude protein separation was carried out for *Corchorus incisifolious*, *Corchorus aestuans*, *Corchorus tridens* and *Corchorus olitorious* using Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS-PAGE). Plants were collected both from wild and cultivated sites and samples included leaves and seeds for the electrophoretic study. Distinct polymorphism in electrophoretic banding patterns of seed and leaf proteins following SDS- PAGE was observed in the four *Corchorus* species studied. Forty- two polypeptide bands were observed in the seed and a total of eleven polypeptide bands were observed in the leaves of the *Corchorus* species studied. The electrophoretic study revealed protein bands with various intensities ranging from high, to low and faint. The results showed that there was variation in both the seed and leaf proteins of the *Corchorus* species studied. A dendrogram constructed based on the Single Linkage Cluster Analysis (SLCA) clustering method revealed three major clusters for seeds. Cluster I consisted of *C. incisifolious* and *C. aestuans*, cluster II consisted of *C. tridens*, while cluster III consisted of *C. olitorious*. The leaf protein extracts were grouped into two clusters, cluster one containing *C. incisifolious* and *C. aestuans*, while the other contained *C. tridens* and *C. olitorious*.

Keywords: dendrogram, electrophoretic separation, genetic diversity, protein polymorphism, SLCA analysis

Introduction

Jute mallow or Jew's mallow sometimes referred to as Egypt spinach, or West African sorrel, or bush okra, due to the nature of the immature fruit, is a fibrous flowering plant of the genus *Corchorus* L. belonging to the family *Malvaceae* formerly *Tiliaceae* and of recent *Sparrmanniaceae*. *Corchorus* has been classified into a number of families including *Capparaceae, Cistaceae, Papaveraceae* and *Tiliaceae* (Whitlock *et al.*, 2003). It consists of about 40-100 species of which 30 grow in Africa (Makinde *et al.*, 2009). It is one of the major fibre crops in the world, especially in Indian subcontinent, alongside cotton (Basu *et al.*, 2004). Several species of *Corchorus* are used as a vegetable, of which *Corchorus olitorius* is most frequently cultivated as vegetable in Nigeria. The genus consists of annual or short-lived perennials (Benor *et al.*, 2010) distributed in tropical, subtropical and warm temperate regions of the world (Edmonds, 1990), and is represented by the two cultivated jute species, vix, *C. capsularis* L. (the white jute) and *C. olitorius* L. (the tossa jute/Jew's mallow). It exists both as wild and cultivated leafy vegetable; the cultivated species includes *Corchorus incisifolious, Corchorus olitorious* L. and *Corchorus capsularis* which are higher in fibre and are affected by diseases and pests, while the wild type includes *Corchorus pseudoolitorious*, *Corchorus fasicularis*, *Corchorus tridens*, *Corchorus aestuans* etc. The species found in Nigeria include *Corchorus aestuans*, *Corchorus tridens, Corchorus incisifolious* and *Corchorus olitorius*. These four species are well distributed in the country and are popularly called Ewedu in the South Western region.

Although the centre of diversity of *Corchorus* appears to be in Africa (Mahapatra and Saha, 2008), the origin and phylogeny of this genus still remain contended (Benor *et al.*, 2010), with little information about the genetic and evolutionary relationship between wild *Corchorus* spp. and the cultivated species (Basu *et al.*, 2004; Roy *et al.*, 2006). While the Indo-Burma region, including South China, is the centre of origin of *C. capsularis*, Africa is the centre of origin for *C. olitorius* (Roy *et al.*, 2006). These two species constitute an important crop of the South East Asian countries and Brazil, providing environmental-friendly (biodegradable and renewable) lingo-cellulose fibre.

The theoretical basis for the use of proteins in evaluating phylogenetic and taxonomic relationship has been documented in literature cited by Yaakov *et al.* (1974). The significant role that gel electrophoresis of protein can play in systematics' has also been stressed. Gottlieb (1971) observed that variation in banding pattern could be equated to variations in genes coding for various proteins. A number of works have been carried out that utilized protein analysis in delimiting taxa as exemplified by the researches carried out by Morakinyo and Olorode (1988), Akpabio (1988), Akinwusi and Illoh (1995) and Folorunso and Olorode (2002).

SDS-PAGE is a more reliable tool and accurate technique to discriminate between cultivars (Salwa *et al.*, 2005). It can also be used to purify protein fractions and characterize lectins in *Corchorus olitorious* leaves (Khan *et al.*, 2008). SDS-PAGE, a low

Received: 08 Jan 2014. Received in revised form: 04 June 2015. Accepted: 06 June 2015. Published online: 21 June 2015.

Table 1. Collection locations of the studied *Corchorus* species

S/N	Name of species	Voucher no	Collection
1	*Corchorus incisifolious*	UIH 001/154	New Botanical garden, University of Ilorin, Kwara State.
2	*Corchorus aestuans*	UIH 002/129	Opposite Ladoke Akintola University (Lautech), Ogbomoso, Oyo State.
3	*Corchorus tridens*	UIH 003/113	New Botanical garden, University of Ilorin, Kwara State.
4	*Corchorus olitorious*	UIH 001/154	New Botanical garden, University of Ilorin, Kwara State.

cost and relatively simple technique, has been used effectively to decipher genetic diversity among/between genotypes in different plant species (Cooke, 1984; Chandra, 2008; Mukherjee and Datta, 2008).

Seed protein analysis by SDS- PAGE has also proved to be an effective way of revealing the differences and similarities among taxa. The high stability of the seed protein profile and its additive nature make seed protein electrophoresis a powerful tool in elucidating the origin and the evolution of cultivated plants (Ladizinsky and Hymowitz, 1979).

The aims of this study are therefore to characterize the four species of *Corchorus* using crude protein profiling and to assess species delineation in the genus, using these crude protein profiles.

Materials and methods

The experimental materials consisted of two cultivated species namely *Corchorus olitorius* and *Corchorus incisifolious,* locally called Agbadu and Yaya respectively, and two wild species namely *Corchorus aestuans* and *Corchorus tridens*. Seeds of *Corchorus aestuans* were collected from already established plants at a location opposite to Ladoke Akintola University (Lautech), at Ogbomoso in Oyo state, while seeds of the other species were collected from already established plants at the botanical garden, University of Ilorin, Ilorin, Kwara State; seeds were identified at the Herbarium Unit from the University of Ilorin (Table 1).

This study was carried out in the Biotechnology Laboratory, Department of Animal Science, Obafemi Awolowo University, Ile-ife, Osun State.

SDS-PAGE was used for protein separation. Seeds were obtained from matured fruits of the species collected from the locations shown in Table 1 and crude proteins were extracted from them.

Electrophoretic study of the protein variations in the seeds and leaves of the *Corchorus* species were carried out using 12% polyacrylamide gel. The species were screened for total protein banding pattern by using a modified method of Laemmli (1970) described by Aguegia *et al.* (1994), Omitogun *et al.* (1999) and Torkpo *et al.* (2006).

Dried seeds of each variety were separately grounded in porcelain mortar and 0.3 g, 0.4 g, 1.6 g and 2 g of the ground samples were weighed into labelled test tubes. 1.05 ml, 1.4 ml, 5.6 ml and 7 ml of 0.6 M NaCl (extraction buffer) was added to each sample respectively in the test tubes and covered. These were stored for about 12 hours. The samples were then centrifuged for 10 mins at 3,000 revolutions per minutes (rpm). The resulting supernatants from each sample contained fat; 0.5 ml of toluene was added to each sample's supernatants at first and centrifuged at 3,000 rpm for 10 mins to defat it. The supernatants from each sample were still seen to contain fat, and 1 ml of toluene was further added to each supernatant subsequently, and was centrifuged again at 3,000 rpm for 10 mins, till no traces of fat were found in the samples.

The resultant supernatant from each sample with no fat was sipped from the test tube using a micro pipette into Eppendorf vials and each vial was labelled as appropriate and kept in the freezer.

The supernatant was then subjected to SDS-PAGE (Sodium Dodecyl Sulphate Poly Acrylamide Gel Electrophoresis). The gel consists of two portions, the separating gel and the stacking gel. The gel plate was set up before the mixing of the gel in a thin spacer. After the gel has been prepared, 45 µl each of *Corchorus incisifolious, Corchorus tridens* and *Corchorus olitorious* extract were mixed with 15 µl of sample buffer, and 30 µl of *Corchorus aestuans* were mixed with 10 µl of sample buffer (maintaining extract-buffer ratio 1:3). Each sample was denatured for 4 mins at 95 °C and then allowed to cool for 1 hour. 10 µl of each sample were loaded in the designated well in the following order: *Corchorus incisifolious, Corchorus aestuans, Corchorus tridens* and *Corchorus olitorious*. Running buffer was added into the electrophoretic chamber. The gel was allowed to run at 150 min volts for 1 hour.

After the electrophoretic separation, the gels were gently removed from the apparatus and put in a staining solution (Comassie brilliant blue) overnight. The gels were then destained in destaining solution until they were completely clear. After staining and destaining, the gels were stored by leaving them inside water in order to prevent the protein bands from disappearing. Data were obtained from the electrophoretic runs by scoring for the presence (1) and absence (0) of bands in the gels. Photographs of the gels were taken and schematic diagrams were drawn.

Relative mobility (Rm) values were calculated for the bands using the formula:

Relative mobility (Rm) = (distance travelled by a band / total length of gel) × length of band on paper.

Single Linkage Cluster Analysis (SCLA) was carried out on the data using Paleontological Statistics (PAST).

Sokal and Sneath's (1963) coefficient of similarity was used to show the level of similarity of protein profiles in the species:

Sokal and Sneath coefficient of similarity (Cs) = (a/a+b+c) ×100

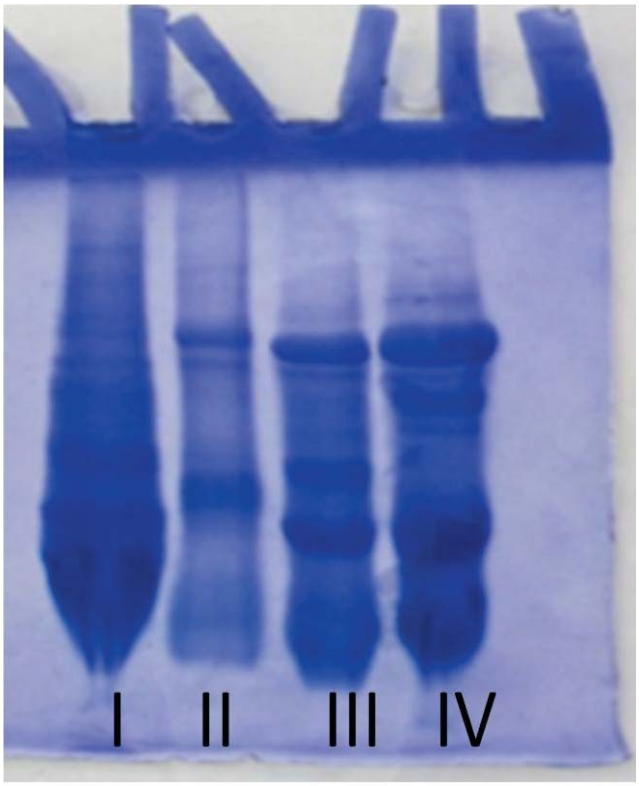

Plate 1. Electrophoregrams of the SDS-PAGE of seed protein of the four *Corchorus* species; Pattern of protein distribution in the seeds of *Corchorus* spp. studied, where I is *C. incisifolious*, II is *C. aestuans*, III is *C.tridens* and IV is *C. olitorious*

Where a = number of bands common to any two species; b = number of bands present in species 1 and not in 2; c = number of bands present in species 2 and not in 1.

Results and discussions

The gels for the seed protein electrophoresis of the four species of *Corchorus* studied are presented in Plate 1, and the schematic diagrams are shown in Fig. 1.

Table 2. Relative mobility values of seed protein in different bands (in cm)

Band no/sample	*C. incisifolious*	*C. aestuans*	*C. tridens*	*C. olitorious*
1	0.005	0.005	0.3	0.3
2	0.2	0.1	0.5	0.4
3	0.5	0.4	0.8	0.6
4	0.6	1.3	1	0.8
5	0.8	1.5	1.5	1
6	1	4.5	1.9	1.5
7	1.3	8.5	2.5	1.9
8	1.9	-	2.8	2.5
9	3.2	-	3.2	2.8
10	4.3	-	3.9	3.2
11	8.5	-	5.7	3.5
12	-	-	8.5	5.7
13	-	-	-	8.5

There are both qualitative and quantitative variations regarding quantity (number), position and staining intensity of the bands. Interspecific and species specific bands were recorded.

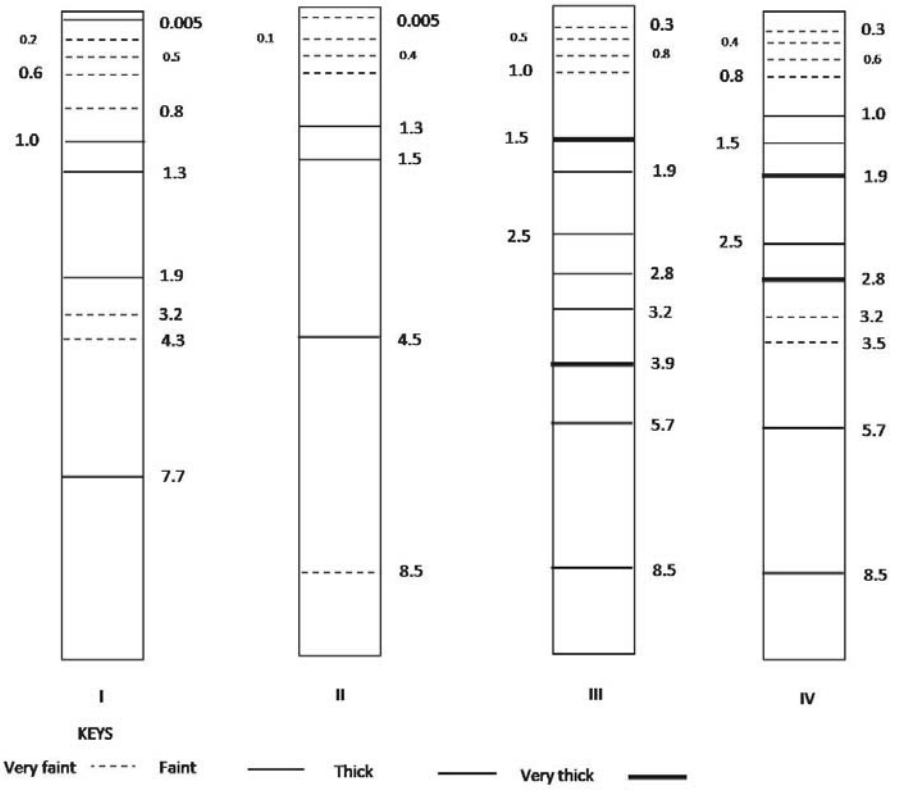

Fig. 1. Schematic diagrams of the electrophoregrams of seed proteins of *Corchorus* spp. studied, where I is *C. incisifolious*, II is *C. aestuans*, III is *C. tridens* and IV is *C. olitorious*

Table 3. Protein band distribution in seeds of the four species of *Corchorus* studied

Species	Slow bands (0-3.0 cm)	Intermediate bands (3.1-6.0 cm)	Fast bands (6.1-9.0 cm)	Total no of bands/species	Unique bands
C. incisifolious	8	2	0	10	3
C.aestuans	5	1	1	7	2
C. tridens	8	3	1	12	1
C. olitorious	9	3	1	13	1
Total	30	9	3	42	

Table 4. Sokal and Sneath's similarity indices based on seeds of *Corchorus* spp. using the relative mobility (Rm) values in (%)

Species	*C. incisifolious*	*C. aestuans*	*C. tridens*	*C. olitorious*
C. incisifolious	-	5.9	23.53	26.3
C. aestuans	5.9	-	11.76	17.64
C. tridens	23.53	11.76	-	66.67
C. olitorious	26.3	17.64	66.67	-

Table 5. Protein band distribution in leaves of the four species of *Corchorus* spp. studied

Species	Slow bands (0-3.0 cm)	Intermediate bands (3.1-6.0 cm)	Fast bands (6.1-9.0 cm)	Total no of bands/species	Unique bands
C. incisifolious	2	0	0	2	0
C. aestuans	2	0	2	4	1
C. tridens	2	0	1	3	0
C. olitorious	2	0	1	2	0
Total	8	0	4	11	

Table 6. Relative mobility (Rm) values of leaf protein in different bands (in cm)

Band no/ species	*C. incisifolious*	*C. aestuans*	*C. tridens*	*C. olitorious*
1	0.6	0.6	0.6	0.6
2	1.8	0.8	1.8	1.8
3	-	7.6	7.6	7.6
4	-	12.7	-	-

Table 7. Sokal and Sneath's similarity index for *Corchorus* spp. of leaves based on the relative mobility (Rm) values in (%)

Species	*C. incisifolious*	*C. aestuans*	*C. tridens*	*C. olitorious*
C. incisifolious	-	50	66.7	66.7
C. aestuans	50	-	75	75
C. tridens	66.7	75	-	100
C. olitorious	66.7	75	100	-

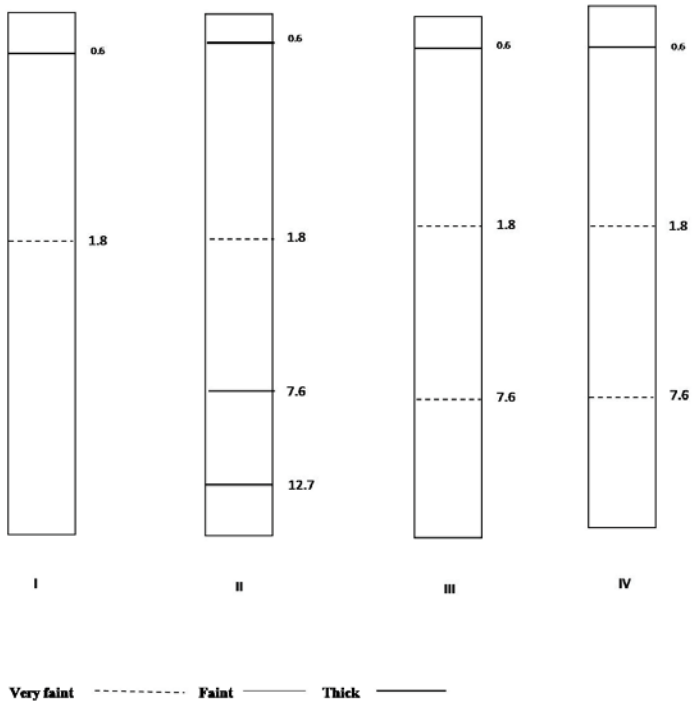

Fig. 2. Schematic diagram of stained protein bands recognized after gel electrophoresis of leaf proteins of *Corchorus* species studied, where I is *C. incisifolious*, II is *C. aestuans*, III is *C. tridens* and IV is *C. olitorious*

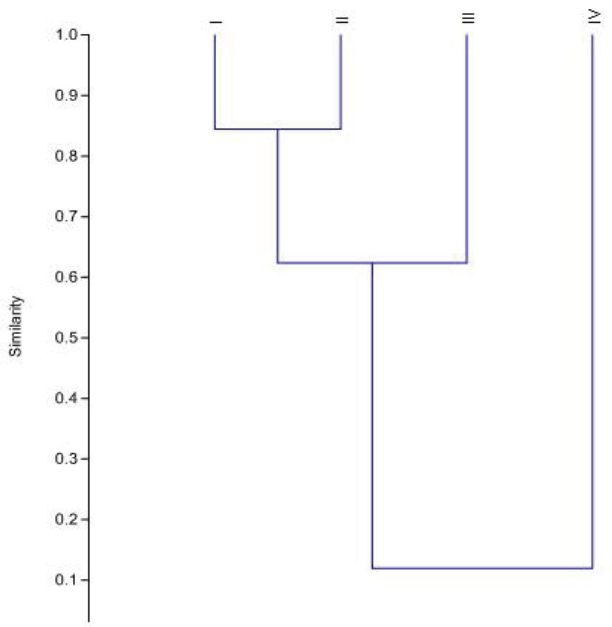

Fig. 3. Single linkage cluster analysis (SLCA) dendrogram of relative mobility (Rm) values for seed protein in the four species of *Corchorus* studied, where I is *C. incisifolious*, II is *C. aestuans*, III is *C. tridens* and IV is *C. olitorious*

Plate 1 shows distinct electrophoretic banding patterns and led to the detection of a total of forty-two (42) bands (Table 3). Some protein bands in each species of *Corchorus* studied were species-specific, as there were no two species with completely identical profile.

The results further showed that three bands (7.1%) were fast bands, nine (21.4%) were intermediate in movement, while thirty bands (71.4%) were slow moving protein bands (Table 3). Interspecific bands were widespread among the species as observed in the bands at 0.005 (present in 2 species), 0.3 (present in 2 species), 0.5 (present in 2 species), 0.6 (present in 2 species), 0.8 (present in 3 species), 1.0 (present in 3 species),1.3 (2 species), 1.5 (3 species), 1.9 (3 species), 2.5 (2 species), 2.8 (2 species), 3.2 (3 species), 5.7 (2 species) and 8.5(3 species). Unique bands occur at 0.1, 0.2, 3.5, 3.9, 4.3, 4.5 and 7.7 (Fig. 1).

Sokal and Sneath's (1963) coefficient of similarity revealed a generally low level of similarity (5.9%- 66.7%) among the seed protein bands of the four species studied (Table 4). The highest coefficient of similarity occurred between *C. tridens* and *C. olitorious*.

The Single Linkage Cluster Analysis (SLCA) dendrogram of the relative mobility (Rm) values of seed protein bands is presented in Table 2. The SLCA diagram showed that the 4 species separated into three main groups, with *C. incisifolious* and *C. aestuans* in the first main cluster, *C. tridens* on the second cluster and *C. olitorious* on the third one, indicating that *C. incisifolious* and *C. aestuans* are more closely related, while *C. incisifolious* and *C. tridens* are more distantly related to *C. olitorious*. From Table 4, it can be deduced that *C. tridens* is closest to *C. olitorious*, followed by *C. incisifolious* and then by *C. aestuans* using the percentage relative mobility values.

The schematic diagram of the leaf protein electrophoresis of the four species of *Corchorus* studied was shown in Fig. 2. There are both qualitative and quantitative variations as regards number, position and intensity of bands stained. Interspecific bands were also recorded.

SDS-PAGE of leaf protein of the four species showed distinct electrophoretic banding patterns that led to the detection of a total of eleven (11) bands (Table 5). The protein band from the leaves of the four species of *Corchorus* appeared generic as they are present in all species except for absence of bands at 7.6 in *C. incisifolious* and presence of a band at 12.7 in *C. aestuans*, which is the only specific band in the leaf crude proteins. The results further showed that eight bands (72.2%) were slow bands, there were no intermediate bands and four bands (36.4%) were fast moving protein bands.

From Table 7 data calculation, it could be noted that *C. tridens* is closest to *C. olitorious*, followed by *C. aestuans* and then by *C. incisifolious*. This is slightly different from the similarities deduced from the seed electrophoregrams in Table 4.

Sokal and Sneath's (1963) coefficient of similarity revealed a generally high level of similarity in the leaf protein bands of the four species studied which ranged from 50% to 100% (Table 7). The highest coefficient of similarity occurred between *C. tridens* and *C. olitorious*. The Single Linkage Cluster Analysis (SLCA) dendrogram of the relative mobility (Rm) values of leaf protein bands are presented in Table 6. The SLCA diagram (Fig. 3) shows that the four species separated into *C. incisifolious* and *C. aestuans* on the first main cluster and *C. tridens* and *C. olitorious* on the second main cluster. *C. incisifolious* and *C. aestuans* are closely related, while *C. tridens* and *C. olitorious* are distantly related to one another and to *C. incisifolious* and *C. aestuans* respectively.

SDS-PAGE is a dependable method for determining the presence of soluble proteins. The results from this work showed variation in the pattern of electrophoretic mobility of proteins. Protein variation is an indication of protein polymorphism and thus phenotypic variation which forms the basis of separation of individuals in a particular population into different taxa. Proteins are considered to be direct products of genes and can be taken as markers of these genes (Ladizinsky, 1983). As such, protein can be taken as additional means for characterising systematic categories. Ladizinsky (1983) reported that seed protein profile often shows genetic affinities within a taxon or between different biological entities and that seed protein profile is species specific. Electrophoretic analysis of the seed storage proteins had direct relationship with the genetic background of the proteins that reveal genetic diversity. Such analysis can be used to certify the genetic makeup of germplasm (Iqbal *et al.*, 2005; Javaid *et al.*, 2004).

The present investigation revealed similarities in the overall polypeptide profile of the seed proteins from the seeds of the four species of *Corchorus* studied. This uniformity of the seed protein agreed with the findings of Ladizinsky and Alder (1975) and Ahmad and Slinkard (1992), who examined different cultivars of chicken pea and concluded that seed protein was a very conservative trait.

Similarly, Raymond *et al.* (1991) and De Vries (1996) also reported similar electrophoretic patterns of protein among the cultivars of sunflower and lettuce respectively. Ladizinsky and Hymowita (1979) also stated that taxonomic categories below the species level, despite morphological and ecological differences, still possess basically the same seed protein profiles.

The presence of interspecific bands at the same distances from the anode among the species reflects to a large extent, a measure of affinity which also agrees with the idea of Daas and Nybom (1967) that the concept of biochemical distance among species of known genetic relationship depicts affinity. According to Hubby and Lewontin (1966) and Gottlieb (1971), when a particular electrophoretic band appears in all individuals examined in a population, it is assumed that the gene coding the enzyme does not vary. Generic bands occurred at 0.6, 1.8, 7.6. This assumption can be used to label the band 0.6, 1.8, 7.6 with varying degree of intensity in the species, with *Corchorus aestuans* having the most intense of the generic bands, since this band tends to prove that the species are from the same parental stock.

The presence of common bands in as seen in Table 6 evidenced the common origin of *Corchorus* species and suggested that genes for these bands are conserved. These may be adaptive genes which have evolved, become dispersed, and fixed in the species over evolutionary time. Gotlieb (1971) observed that the presence of common bands (eg: in Figs. 1 and 2) in a group of taxa reflects evolutionary relationship. Electrophoresis of the seed grouped the species into three main groups. *Corchorus incisifolious* and *Corchorus aestuans* belong to the same cluster, while *Corchorus tridens* and *Corchorus olitorious* belong to different clusters forming the second and the third clusters. For the leaf, *Corchorus incisifolious* and *Corchorus aestuans* were still grouped into the same cluster, while *Corchorus tridens* and *Corchorus olitorious* were grouped in the same cluster, all forming two main clusters for the leaf electrophoresis.

Electrophoresis of the seed and leaf proteins appeared to demonstrate the close relationship of the *Corchorus* species studied. The results obtained from both the seed and leaf electrophoresis showed that *C. incisifolious* and *C. aestuans* are the closest, followed by *C.tridens* and *C. olitorious*, as shown in Fig. 3.

Conclusions

The relationship and distinctiveness among Nigerian *Corchorus* species have been demostrated using protein profiling. The diversity of protein bands are indicative of genetic diversity and may be useful in delineation of *Corchorus* species. There is a strong correlation between protein band patterns and species diversity among the species. The clustering scores among the species suggested that there is a strong relationship among the species. Electrophoresis of seed proteins justifies the inclusion of the species studied in the same genus and their specific delineation. The presence of genetic diversity is important for improving any crop plant. The four *Corchorus* species examined in this study showed their level of relatedness, enhancing the possibility of genes exchange through artificial hybridisation in the future.

References

Aguegia A, Fatokun CA, Han SK (1994). Leaf protein analysis of ten Cocoyam (*Xanthosoma sagitifolium* (L.) Schott and *Colocasia esculenta* (L) Schott geneotypes. Proc of the Fifth Symposium ISTRC-AB pp 348-353.

Ahmad F, Slinkard AE (1992). Genetic relationship in the genus *Cicer* L., as revealed by polyacrylamide gel electrophoresis of seed storage proteins. Theor Applied Genetics 84(5-6):688-692.

Akinwusi O, Illoh HC (1995). Crude protein electrophoresis of seeds of some species of Hibiscus. Nigerian Journal of Botany 8:71-76.

Akpabio KE (1998). Crude protein electrophoresis of seeds eight species of *Crotalaria* L. Nigerian Journal of Botany 1:106-110.

Basu A, Ghosh M, Meyer R, Powell W, Basak SL, Sen SK (2004). Analysis of genetic diversity in cultivated jute determined by means of SSR markers and AFLP profiling. Crop Sci 44(2):678-685.

Benor S, Blattner FR, Demissew S, Hammer K (2010). Collection and ethnobotanical investigation of *Corchorus* species in Ethiopia: potential leafy vegetables for dry regions. Genetic Resources and Crop Evolution 57(2):293-306.

Chandra A (2008). Genetic relationship among accession of *Stylosanthes hamata* based on seed proteins profile. Indian J Genrt Plant Breed 68:208-211.

Cooke RJ (1984). The characterisation and identification of crop cultivars by electrophoresis. Electrophoresis 5(2):59-72.

Daas H, Nybom N (1967). The relationship between *Brassica nigra*, *B. campestris*, *B. oleracea* and their amphidiploid hybrids studied by chemotaxonomy. Canadian Journal of Genetics and Cytology 9(4):880-890.

De Vries IM (1996). Characterisation and identification of *Lactuca sativa* cultivars and wild relatives with SDS-Electrophoresis. *Lactuca* sect. *Lactuca*, *Compositae*. Grace 43:193-202.

Edmonds JM (1990). Herbarium survey of African *Corchorus* L. species. Systematic and ecogeographic studies on crop genepools 4. International Board for Plant Genetic Resources, Rome.

Folorunso AE, Olorode O (2002). Electrophoresis of crude protein of seeds of some Genera of Annonaceae. Nigerian Journal of Horticultural Science 7:6-9.

Gotlieb LD (1971). Gel electrophoresis: new approach to study of evolution. Bioscience 21(18):939-944.

Hubby JL, Lewontin RC (1966). A molecular approach to the study of genic heterozygosity in natural population. I. The number of alleles at different loci in *Drosophilia pseudoobscura*. Genetics 54(2):577-594.

Iqbal SH, Ghafoor A, Ayub N (2005). Relationship between SDS-PAGE markers and Ascochyta blight in chickpea. Pakistan Journal of Botany 37(1):87-95.

Javaid A, Ghafoor A, Anwar R (2004). Seed storage protein electrophoresis in groundnut for evaluating genetic diversity. Pakistan Journal of Botany 36(1):25-30.

Khan MMH, Mijanur Rahman ATM, Salim Uddin MD, Shahanaz K, Pervin F, Absar N (2008). Purification and characterisation of lectins from jute (*Corchorus olitorius*) leaves. Journal of the Chinese Chemical Society 55(5):1171-1177.

Ladizinsky G (1983). Study of evolutionary problems by means of seed protein electrophoresis. In: Seed proteins: Biochemistry, genetics and nutritive value, Werner G, Herman PM (Eds). Martins NHOff/DR W Junk Publishers. The Hague/Boston/London.

Ladizinsky G, Adler A (1975). The origin of chickpea as indicated by seed protein electrophoresis. Israel Journal of Botany 24:183-189.

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

Laemmli UK (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227(5259):680-685.

Mahapatra AK, Saha A (2008). Genetic resources of jute and allied fibre crops. In: P.G. Karmakar SK, Hazra T, Ramasubramanian RK, Mandal MK, Sinha, Sen HS (Eds) Jute and allied fibre updates'. Central Research Institute for Jute and Allied Fibres, Kolkata pp 18-37.

Makinde SCO, Oluwole OS, Ojekale BA, Olufeyimi SR (2009). Effects of intrapopulation competition on morphological and agronomic characters of jute plant (*Corchorus olitorious* L.). Afri J Biotechnol 8:2195-2201.

Morakinyo JA, Olorode O (1988). Cytogenetic studies in *Sorghum bicolour* (L.) Moench Cytologia 53(4):653-658.

Mukherjee M, Datta AK (2008). Evaluation of genetic diversity in five species of *Ocimum* by SDS-PAGE. Indian J Genet Plant Breed 68:212-214.

Omitogun OG, Jackal L, Thottappilly G (1999). Isolation of insecticidal lectin enriched extracts from african yam bean *(Sphenostylis sternocarpon)* and other legume species. Entomol Exp Appl 90:301-311.

Raymond J, Inquello V, Azanza JL (1991). The seed proteins of sunflower: comparative studies of cultivars flower. Phytochem 30(9):2849-2856.

Roy A, Bandyopadhyay A, Mahapatra AK, Ghosh SK, Singh NK, Bansal KC, Koundal KR, Mohapatra T (2006). Evaluation of genetic diversity in jute *(Corchorus species)* using STMS, ISSR and RAPD markers. Plant Breed 125(3):292-297.

Salwa MA, Hanan HL (2005). Germination and protein patterns of some genotypes of two species of jute as affected by NACl stress. Pakistan Journal of Biological Sciences 8(2):227-234.

Sokal RR, Sneath PHA (1963). Numerical Taxonomy. Freeman WH (Eds). San Fransisco.

Torkpo EY, Danquah EY, Offei SK, Blay ET (2006). Esterase, total protein and seed storage protein diversity in Okra (*Abelmoschus esculentus* (L.) Moench) West Afr J Appl Ecol 9:177-183.

Whitlock BA, Karol KG, Alverson WS (2003). Here chloroplast DNA sequences confirm the placement of the Enigmatic *Oceanopapaver* within *Corchorus* (Grewiodeae: Malvaceaes l, Formely Tiliaceae). International Journal of Plant Sciences 164:35-41.

Yaakov S, Waines JG, Ayala RP (1974). Single-disc electrophoresis of maize, wheat and sorghum. Bulletin of the Torrey Botanical Club 101(4):207-210.