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 Iliara, Kwara State, Nigeria; christianadeyinka@gmail.com (corresponding author); morakinyoj@yahoo.com

Abstract

Crude protein separation was carried out for Corchorus incisifolius, Corchorus aetumans, Corchorus tridens and Corchorus olitorius 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. incisifolius and C. aetumans, cluster II consisted of C. tridens, while cluster III consisted of C. olitorius. The leaf protein extracts were grouped into two clusters, cluster one containing C. incisifolius and C. aetumans, while the other contained C. tridens and C. olitorius.

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 a 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, viz. C. capsularis L. (the white jute) and C. aetumans L. (the tussa jute/Jew’s mallow). It exists both as wild and cultivated leafy vegetable; the cultivated species includes Corchorus incisifolius, Corchorus aetumans L. and Corchorus capsularis which are higher in fibre and are affected by diseases and pests, while the wild type includes Corchorus pseudolitorius, Corchorus fasciculatus, Corchorus tridens, Corchorus aetumans etc. The species found in Nigeria include Corchorus aetumans, Corchorus tridens, Corchorus incisifolius and Corchorus olitorius. These four species are well distributed in the country and are popularly called Eweedu 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 environmentally-friendly (biodegradable and renewable) ligno-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. Grothie (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), Akinnusi and Illo (1985) 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 olitorius leaves (Khan et al., 2008). SDS-PAGE, a low
cost and relatively simple technique, has been used effectively to
decipher genetic diversity among/between genotypes in different
plant species (Cooke, 1984; Chandira, 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
and the evolution of cultivated plants (Ladizinsky and
Hymowitz, 1979).

The aims of this study are therefore to characterize the
four species of C. orbiculatus 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 C. orbiculatus and C. incisifolius, locally called Agbadu and Yaya respectively, and
two wild species namely C. austroan and C. orbiculatus. Seeds of C. austroan 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 C. orbiculatus species were carried out
using 12% polyacrylamide gel. The species were screened for
total protein binding pattern by using a modified method of
Laemmli (1970) described by Agugia et al. (1994),
Omotogun 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 consisted 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 space. After the gel
has been prepared, 45 µl each of C. incisifolius, C. austroan
and C. orbiculatus extract were mixed with 15 µl of sample buffer, and 30 µl of C. orbiculatus
were mixed with 10 µl of sample buffer (maintaining extract-buffer ratio 1:3). Each sample was
denatured at 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: C. incisifolius, C. austroan, C. orbiculatus, C. orbiculatus. 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 vater 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) x length of band on paper

Single Linkage Cluster Analysis (SCLA) was carried
out on the data using Paleontologial Statistics (PAS 1).

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) =

\[ (a/a+b+c) \times 100 \]

### Table 1. Collection locations of the studied C. orbiculatus species

<table>
<thead>
<tr>
<th>S/N</th>
<th>Name of species</th>
<th>Voucher no</th>
<th>Collection</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>C. incisifolius</td>
<td>UJH 001/154</td>
<td>New Botanical garden, University of Ilorin, Kwara State.</td>
</tr>
<tr>
<td>2</td>
<td>C. austroan</td>
<td>UJH 002/129</td>
<td>Opposite Ladoke Akinlola University (Lautech), Ogbomoso, Oyo State.</td>
</tr>
<tr>
<td>3</td>
<td>C. orbiculatus</td>
<td>UJH 003/113</td>
<td>New Botanical garden, University of Ilorin, Kwara State.</td>
</tr>
<tr>
<td>4</td>
<td>C. orbiculatus</td>
<td>UJH 001/154</td>
<td>New Botanical garden, University of Ilorin, Kwara State.</td>
</tr>
</tbody>
</table>
Where \( n = \) 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>
<thead>
<tr>
<th>Band no/sample</th>
<th><em>C. incisifolius</em></th>
<th><em>C. aextum</em></th>
<th><em>C. tridens</em></th>
<th><em>C. olitorius</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.005</td>
<td>0.005</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>2</td>
<td>0.2</td>
<td>0.1</td>
<td>0.5</td>
<td>0.4</td>
</tr>
<tr>
<td>3</td>
<td>0.5</td>
<td>0.4</td>
<td>0.8</td>
<td>0.6</td>
</tr>
<tr>
<td>4</td>
<td>0.6</td>
<td>1.3</td>
<td>1</td>
<td>0.8</td>
</tr>
<tr>
<td>5</td>
<td>0.8</td>
<td>1.5</td>
<td>1.5</td>
<td>1</td>
</tr>
<tr>
<td>6</td>
<td>1</td>
<td>4.5</td>
<td>1.9</td>
<td>1.5</td>
</tr>
<tr>
<td>7</td>
<td>1.3</td>
<td>8.5</td>
<td>2.5</td>
<td>1.9</td>
</tr>
<tr>
<td>8</td>
<td>1.9</td>
<td>-</td>
<td>2.8</td>
<td>2.5</td>
</tr>
<tr>
<td>9</td>
<td>3.2</td>
<td>-</td>
<td>3.2</td>
<td>2.8</td>
</tr>
<tr>
<td>10</td>
<td>4.3</td>
<td>-</td>
<td>3.9</td>
<td>3.2</td>
</tr>
<tr>
<td>11</td>
<td>8.5</td>
<td>-</td>
<td>5.7</td>
<td>3.5</td>
</tr>
<tr>
<td>12</td>
<td>-</td>
<td>-</td>
<td>8.5</td>
<td>5.7</td>
</tr>
<tr>
<td>13</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>8.5</td>
</tr>
</tbody>
</table>

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

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

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**Fig. 1. Schematic diagrams of the electrophoreograms of seed proteins of *Corchorus* spp. studied, where I is *C. incisifolius*, II is *C. aextum*, III is *C. tridens* and IV is *C. olitorius***
Table 3. Protein band distribution in seeds of the four species of *Carthamus* studied

<table>
<thead>
<tr>
<th>Species</th>
<th>Slow bands (0-3.0 cm)</th>
<th>Intermediate bands (3.1-6.0 cm)</th>
<th>Fast bands (6.1-9.0 cm)</th>
<th>Total no of bands/species</th>
<th>Unique bands</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. incisifolius</em></td>
<td>8</td>
<td>2</td>
<td>0</td>
<td>10</td>
<td>3</td>
</tr>
<tr>
<td><em>C. aestuans</em></td>
<td>5</td>
<td>1</td>
<td>1</td>
<td>7</td>
<td>2</td>
</tr>
<tr>
<td><em>C. tridens</em></td>
<td>8</td>
<td>3</td>
<td>1</td>
<td>12</td>
<td>1</td>
</tr>
<tr>
<td><em>C. holitirius</em></td>
<td>9</td>
<td>3</td>
<td>1</td>
<td>13</td>
<td>1</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>30</strong></td>
<td><strong>9</strong></td>
<td><strong>3</strong></td>
<td><strong>42</strong></td>
<td><strong>42</strong></td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Species</th>
<th><em>C. incisifolius</em></th>
<th><em>C. aestuans</em></th>
<th><em>C. tridens</em></th>
<th><em>C. holitirius</em></th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. incisifolius</em></td>
<td>-</td>
<td>5.9</td>
<td>23.53</td>
<td>26.3</td>
</tr>
<tr>
<td><em>C. aestuans</em></td>
<td>5.9</td>
<td>-</td>
<td>11.76</td>
<td>17.64</td>
</tr>
<tr>
<td><em>C. tridens</em></td>
<td>23.53</td>
<td>11.76</td>
<td>-</td>
<td>66.67</td>
</tr>
<tr>
<td><em>C. holitirius</em></td>
<td>26.3</td>
<td>17.64</td>
<td>66.67</td>
<td>-</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Species</th>
<th>Slow bands (0-3.0 cm)</th>
<th>Intermediate bands (3.1-6.0 cm)</th>
<th>Fast bands (6.1-9.0 cm)</th>
<th>Total no of bands/species</th>
<th>Unique bands</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. incisifolius</em></td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td><em>C. aestuans</em></td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td><em>C. tridens</em></td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td><em>C. holitirius</em></td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>8</strong></td>
<td><strong>0</strong></td>
<td><strong>4</strong></td>
<td><strong>11</strong></td>
<td><strong>11</strong></td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Band no/species</th>
<th><em>C. incisifolius</em></th>
<th><em>C. aestuans</em></th>
<th><em>C. tridens</em></th>
<th><em>C. holitirius</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.6</td>
<td>0.6</td>
<td>0.6</td>
<td>0.6</td>
</tr>
<tr>
<td>2</td>
<td>1.8</td>
<td>0.8</td>
<td>1.8</td>
<td>1.8</td>
</tr>
<tr>
<td>3</td>
<td>-</td>
<td>7.6</td>
<td>7.6</td>
<td>7.6</td>
</tr>
<tr>
<td>4</td>
<td>-</td>
<td>12.7</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Species</th>
<th><em>C. incisifolius</em></th>
<th><em>C. aestuans</em></th>
<th><em>C. tridens</em></th>
<th><em>C. holitirius</em></th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. incisifolius</em></td>
<td>-</td>
<td>50</td>
<td>66.7</td>
<td>66.7</td>
</tr>
<tr>
<td><em>C. aestuans</em></td>
<td>50</td>
<td>-</td>
<td>75</td>
<td>75</td>
</tr>
<tr>
<td><em>C. tridens</em></td>
<td>66.7</td>
<td>75</td>
<td>-</td>
<td>100</td>
</tr>
<tr>
<td><em>C. holitirius</em></td>
<td>66.7</td>
<td>75</td>
<td>100</td>
<td>-</td>
</tr>
</tbody>
</table>

Fig. 2. Schematic diagram of stained protein bands recognized after gel electrophoresis of leaf proteins of *Carthamus* species studied, where I is *C. incisifolius*, II is *C. aestuans*, III is *C. tridens* and IV is *C. holitirius*
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 3). 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. incisifolius and presence of a band at 12.7 in C. aestivalis, 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 s closest to C. olitorius, followed by C. aestivalis and then by C. incisifolius. 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. olitorius. 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. incisifolius and C. aestivalis on the first main cluster and C. tridens and C. olitorius on the second main cluster. C. incisifolius and C. aestivalis are closely related, while C. tridens and C. olitorius are distantly related to one another and to C. incisifolius and C. aestivalis 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 (Ladzinsky, 1983). As such, protein can be taken as additional means for characterising systematic categories. Ladzinsky (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 Ladzinsky 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.

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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. incisifolius, II is C. aestivalis, III is C. tridens and IV is C. olitorius.
Similarly, Raymond et al. (1991) and De Vries (1996) also reported similar electrophoretic patterns of protein among the cultivars of sunflower and lettuce respectively. Ladzinsky and Hymanowa (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 a large extent, a measure of affinity which also agrees with the idea of Daas and Nyborn (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 Cordobus 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 Cordobus 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. Gottlieb (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 group the species into three main groups, Cordobus insisflosillus and Cordobus aestuans belong to the same cluster, while Cordobus tridens and Cordobus olitorius belong to different clusters forming the second and the third clusters. For the leaf, Cordobus insisflosillus and Cordobus aestuans were still grouped into the same cluster, while Cordobus tridens and Cordobus olitorius 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 Cordobus species studied. The results obtained from both the seed and leaf electrophoresis showed that C. insisflosillus and C. aestuans are the closest, followed by C. tridens and C. olitorius, as shown in Fig. 3.

Conclusions

The relationship and distinctiveness among Nigerian Cordobus species have been demonstrated using protein profiling. The diversity of protein bands are indicative of genetic diversity and may be useful in delineation of Cordobus 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 Cordobus species examined in this study showed their level of relatedness enhancing the possibility of genes exchange through artificial hybridisation in the future.

References


