

Molecular Characterisation of Some Accessions of *Corchorus olitorius* L.

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ABSTRACT

Nigeria has several native leafy vegetables that have been neglected. *Corchorus olitorius* belongs to this group. Genetic diversity was studied in five accessions of the plant using sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS - PAGE). The aim was to determine the relationship (differences and similarities) among them. Sixteen polypeptide bands were obtained ranging from 12.29 KDa to 170 KDa indicating variations in the banding patterns of the accessions. The unweighted pair group method with arithmetic algorithm (UPGMA) dendrogram grouped the five accessions into three clusters with four groups showing 60% of the accessions in one group. The greatest similarity (96%) observed was between NGB00196 and NGB00194 while the lowest similarities (32%) NGB00191 and (40%) NGB00187. Adopting this technology can be useful in plant variety identification and registration of new plant varieties. NGB00191 was observed to be distantly related to the other accessions hence could be combined in a breeding programme.

Keywords: Diversity; *Corchorus olitorius*; Genetics; Electrophoresis, Protein.

INTRODUCTION

Corchorus olitorius known as ewedu (Yoruba), malafia, tungumuwa, lallo, ayoyo (Hausa); and Kerenkere, achingbara (Igbo) languages in Nigeria, molukhya in Arabic, is usually used as a leafy vegetable. The centre of origin of the plant is however believed to be Africa due to the presence of several mild and weedy relatives (Mbaye *et al.*, 2001). The genus *Corchorus*, with over 170 species in the Index Kewensis and probably with 50-60 species (Mahapatra and Saha 2008), has a classification problem (Mahapatra *et al.* 1998; Benor *et al.* 2010). The genus was traditionally classified within the family Tiliaceae (Purseglove 1968) but was later merged with the family Malvaceae within the core Malvales based on molecular evidence of the chloroplast genome (Alverson *et al.*, 1999; Whitlock *et al.*, 2003), as against the previously sole morphological evidence. However, it has been recently reclassified within the family Sparrmaniaceae (Heywood *et al.*, 2007).

To solve this problem, Sarkar *et al.* (2011) reassessed the nuclear genome sizes (ICDNA amounts) of the two cultivated jute species (*C. capsularis* L. and *C. olitorius* L.) in relation to their wild *Corchorus* relatives. Other workers had also contributed to this topical issue (Isuosuo and Akaneme, 2014). Meanwhile, proper identification of plant is the first essential step to its uses; a plant with identification problem has lost its potential power in all forms. *Corchorus olitorius* otherwise known as Bush okra or Jew's mallow is used in interior decoration. It is also used in the footwear and pulp industries where it acts as a non-wood fibrous material (Khan, 2008). It has carotene which helps in enhancing good eyesight, iron and calcium which are responsible for normal red blood cell, strong bones and teeth respectively as it contains varying proportion of Ca, Zn, Mn, K, Fe, β - carotene, vitamin B1, B2, C and E (Idris *et al.*, 2010, Sinha *et al.*, 2011). It is important for plant breeders to know the genetic diversity of a crop if crop improvement programs are to succeed (Keatinge *et al.*, 2008). This includes germplasm collection, characterization and evaluation (Isuosuo and Akaneme, 2014). Genetic diversity is one of the key

factors needed in the improvement of *Corchorus olitorius*. Genetic diversity is important in the selection of cultivar for plant improvement, this is because the estimates of genetic similarities and distances among genotypes are needed to select parent plants to be used in breeding programs (Becelaere *et al.*, 2005). A lot of studies have been carried out on the morphological characterisation of this plant (Adebo *et al.*, 2015; Ngomuo *et al.*, 2017) however the molecular information is scanty. Genetic diversity estimates made from morphological traits are unreliable due to the effect of the environment on them and those traits are usually limited (Maric *et al.*, 2004). The use of molecular markers in estimating genetic diversity are useful as they are not influenced by the environment, are abundant and previous pedigree information are not required (Bohn *et al.*, 1999).

The study of genetic diversity can be carried out using pedigree analysis, morphological traits or by use of molecular markers (Pejic *et al.*, 1998). Pedigree analysis when used in diversity tend to be inflated and hence unrealistic (Fufa *et al.*, 2005). But due to the cost of using molecular markers for germplasm characterization, many researchers have advocated for the use of protein of isozyme especially sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) which are used because of its simplicity and effectiveness in estimation of genetic diversity according to Ahmed *et al.* (2010) and as a reliable tool for economic characterization of germplasm (Iqbal *et al.*, 2005; Stoyanova and Bollar, 2010; Kakaei and Kahrizi, 2011). The molecular characterization of the Nigerian accessions of *C. olitorius* using SDS-PAGE is aimed at bringing out the genetic variation inherent in the accessions under study.

MATERIALS AND METHODS

Plant sample

Seeds of *Corchorus olitorius* accessions used in this study were obtained from National Centre for Genetic Resources and Biotechnology (NACGRAB), Ibadan, Nigeria. *Corchorus olitorius* accessions used in this

study include: NGB00187, NGB00191; NGB00194; NGB00196 and NGB00201.

SDS-PAGE Analysis

The genetic variability of seed storage-proteins was analysed using SDS- PAGE (Damania *et al.*, 1983). In the process of protein extraction, 10 seeds of each sample were ground to fine powder with mortar and pestle. A total of 400 µl sample buffer was added to 0.01g (10 mg) seed powder and mixed thoroughly by vortex in an Eppendorf tube (1.5ml) with a small glass rod. An extraction buffer which contains the following concentration: 0.5 M Tris-HCl (pH 6.8), 2.5% sodium dodecyl sulphate (SDS), 10% glycerol and 5% 2- mercaptoethanol was kept overnight at 40 °C. All samples were centrifuged at 13,000 rpm for 10 min. SDS- PAGE was carried out using a 4% stacking gel and 12% separating gel. They were run on a mini gel apparatus in Tris- glycine (pH 8.3) buffer. Bromophenol blue (BPB) was added to the sample buffer as tracking dye to maintain the movement of protein molecules in the gel. The gels were run at 90V for 2 hours in an omnipac mini-vertical gel apparatus using promega protein as a standard marker. After been ran, the gels were gently removed and washed with 500ml of the gel fixing solution and subsequently covered with 400ml of coomassie blue stain at room temperature for 3 to 4 hours and were gently agitated. The coomassie stains were removed after staining by covering gels with 250ml of the destaining solution (Laemi, 1970). The destaining solution was changed severally until the protein bands were clearly seen without background staining of the gel.

DATA ANALYSIS

The bands from the molecular studies were scored across the lanes. Their molecular weights were compared. The scoring of bands was carried out based on gel images. All the bands were treated as dominant markers and their homology were based on the level of migration on the gel. The bands were scored as present (1) or absent (0) across the genotypes and only reproducible bands in three amplification reactions were used in the final analysis. A

data matrix was constructed. Coefficient of similarity tree were produced by clustering the similarity data using the statistical software package PAST (Paleontological Statistics). Cluster analysis was performed by agglomerative technique using the Unweighted Pair Group Method with arithmetic averages (UPGMA). Dendrogram were constructed using the UPGMA algorithms in the MEGA 4.0 software. The intensities of bands are represented by cross.

RESULTS AND DISCUSSION

A total of 16 bands were generated in all the accessions ranging from 12.49 – 170 KDa. (Figure 1, Table 1). Bands 9 and 11 were present in accessions NGB00191, NGB00196 and NGB00194 though their molecular weight differs. The bands showed variability based on intensity and presence or absence of any of them among the accessions. Bands 1 and 2 were absent only in accession NGB00187, band 5 was present only in accession NGB00201, bands 3,6,8,12,14 and 15 were present in accession NGB00191 and band 4 is present in all accessions except accession NGB00191. Bands 7 are only present in accessions 3 and 5 (NGB00196 and NGB00194); band 10 was present only in accession 4 (NGB00187). Bands 13 and 16 were present in all accessions. NGB00191 had the highest number of bands with molecular weights ranging from 16.59 KDa to 62.50 KDa (Table 1).

The 5 accessions differed in terms of their degree of intensities (Table 2). The dendrogram generated using UPGMA grouped the accessions into three clusters with four groups at a similarity coefficient range of 0.32 to 0.96 (Fig 2). Accession 2 (NGB00191) at a genetic distance of 0.32 was observed to be a distant relative of accessions 3 (NGB 00196) and 4 (NGB00194). However, three of the accessions were shown to be closely related. Two accessions, NGB00194 and NGB00196 were clustered as same and had a similarity coefficient of 0.88, while accession NGB00201 is related to the duo at 0.54.

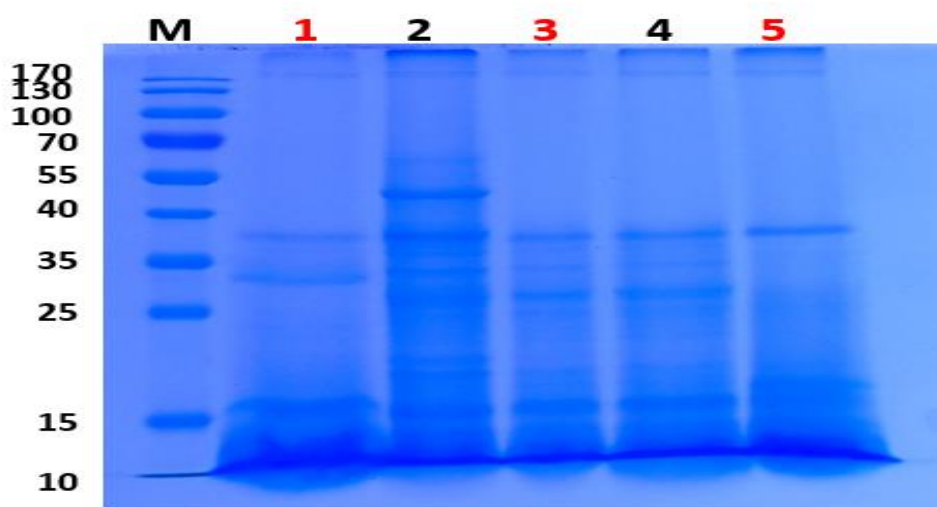


Figure 1: SDS-PAGE electrophorogram of the seed protein for accessions 1-5 of *Corchorus olitorius*. M is the standard protein marker. 1= NGB00187, 2 = NGB00191, 3 = NGB00196, 4 = NGB00194, 5 = NGB00201

Table 1: Molecular weight and Intensities of bands present in each *Corchorus olitorius* accessions

Bands number	molecular weight	NGB00187	NGB00191	NGB00196	NGB00194	NGB00201
1	12.49	0	++	++	++	+
2	14.5	0	++	++	++	+
3	16.59	0	+++	0	0	0
4	17	++++	0	++++	++++	++++
5	18.55	0	0	0	0	+++
6	19.5	0	+++	0	0	0
7	20	0	0	+	+	0
8	21	0	+++	0	0	0
9	30	0	++++	+++	++++	0
10	33	++++	0	0	0	0
11	34	0	++++	+++	++	0
12	36.5	0	+++	0	0	0
13	38.5	+++	++++	++++	++++	++++
14	47.5	0	++++	0	0	0
15	62.5	0	++	0	0	0
16	170	+	+++	+++	+++	+++

+,++,+++,++++Intensities of bands

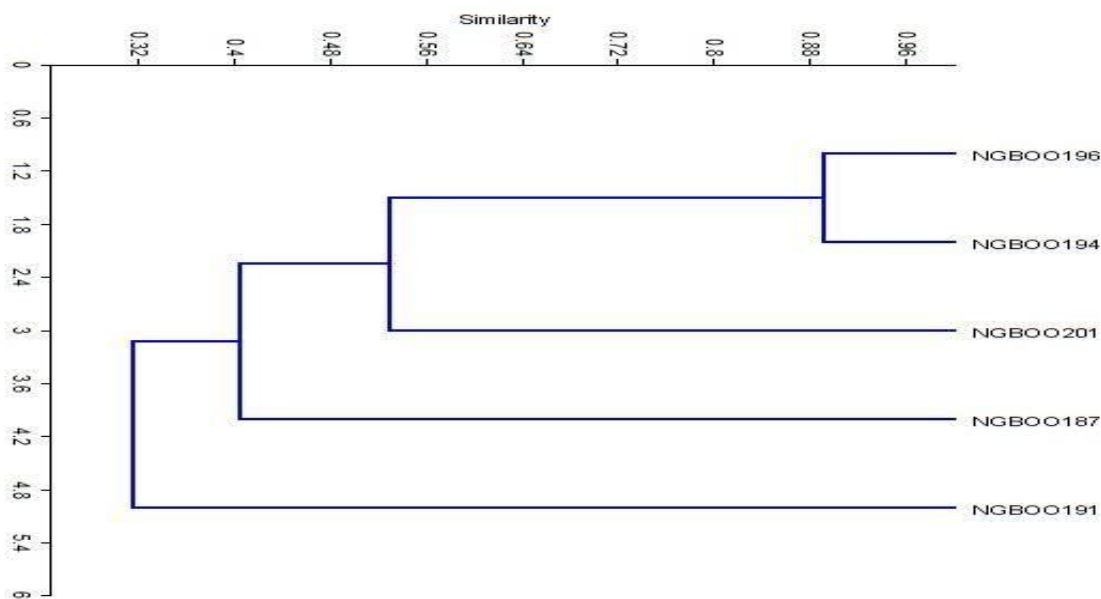


Figure 2: Dendrogram showing the relationship among the 5 accessions of *Corchorus olitorius* using SDS-PAGE. The banding patterns of the accessions were dissimilar indicating moderate heterogeneity of the seed storage proteins. This agrees with the work of Hossain *et al.* (2002) and Isuosuo and Akaneme (2014) that genetic variability in *C. olitorius* is low due to self pollination; self pollination generally generates offsprings that are genetically similar to the parents. Also based on the banding pattern, Soetan and Fafunsho (2009) and Vishwanth *et al.* (2011) noted that differences in bands could be used as basis for identification and to check for variation among accessions since the bands observed show the distinctiveness of the plant. The presence of bands 3,6,8,12,14 and 15 in accession NGB00191 is an

indication that protein is conserved in this region. Ullah *et al.* (2009) opined that uniform and similar banding pattern observed in certain accessions could be due to these proteins being conserved in the plant. In the present study bands 4 in accession NGB00187, NGB00196, NGB00194 and NGB00201 and band 13 which was present in all the accessions have high intensity, this is an indication of a high amount of protein in the accession under study. According to Amersham Biosciences (1999), the position occupied by a protein along the separation lane gives a fair estimation of its size and after staining, the band intensity is a guess on the amount present in the plant. SDS-PAGE electrophoresis of 14 accessions of *Corchorus olitorius* has been previously studied; however, the accessions were

different, but the result is correlated (Isuosuo and Akaneme, 2014).

The separation of the 5 accessions into 3 clusters and four groups as observed in the dendrogram indicates genetic diversity. Accessions NGB 00196 and NGB00194 were clustered as genetically same while NGB00201 was clustered as closely related to the duo. Relative similarity could be due to the fact that there is no cross-boundary check among divisions and seed exchange between farmers may disperse plants from one region to the other (Yi *et al.*, 2008). The dendrogram revealed low genetic diversity at protein level as most of the accessions are in the same cluster. Fufa *et al.* (2005) reported that the genetic diversity estimates based on seed storage protein were lowest because they were the major determinants of the end use quality, which is a highly desirable trait. Accession 2 (NGB00191) and accession 3 (NGB00187) were observed as independent groups and not closely related to other accessions. Accessions on other groups could establish wider variations when crossed because they are dissimilar genetically. It has been earlier noted by Maity *et al.* (2009) and supported by Siddiqui and Naz (2009) that contrasting parents may be identified based on distance between accessions of different cluster and can be used in the crossing programme for generating more variability for selection and crop improvement. Maity *et al.* (2009) also reported that species specific band(s) identified may be exploited for hybrid identification in breeding method.

CONCLUSION

In conclusion, this research revealed that seed storage protein electrophoresis is a useful marker in accession identification. Electrophoresis analysis of total soluble proteins is widely recognized as techniques for cultivar identification and pedigree analysis. This study was able to show the differences and similarities among the 5 accessions of *C. olitorius* evaluated. It can therefore be deduced that accession NGB00191 and NGB00187 can be combined with other accessions in a breeding programme because they are a very distant relative according to the clustering. Distantly related accessions when crossed give rise to offspring that are viable. It reduces the risk of inbreeding depression thereby giving rise to generation of plants with better yield when compared to the parent plant.

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