academicJournals

Vol. 13(20), pp. 2004-2009, 14 May, 2014
DOI: 10.5897/AJB2013.13540
Article Number: EF04CE244578
ISSN 1684-5315

http://www.academicjournals.org/AJB

African Journal of Biotechnology

Full Length Research Paper

Genetic variability assessment of accessions of *Corchorus olitorius* L. using sodium dodecyl sulphate polyacrylamide gel electrophoresis

Chinyere Chioma Isuosuo and Florence Ifeoma Akaneme*

Department of Plant Science and Biotechnology, University of Nigeria, Nsukka, Nigeria.

Received 07 December, 2013; Accepted 8 May, 2014

Corchorus olitorius **is one of the neglected indigenous leafy vegetables in Nigeria. Majority of the accessions of this species stored at the National Centre for Genetic Resources and Biotechnology (NACGRAB) Moor Plantation, Ibadan, Nigeria, have only been characterized morphologically. To provide further information on the extent of genetic diversity, this study was initiated to assess the genetic variabilities among 14 accessions of the species using sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). Seven polypeptide bands were obtained ranging from 9.73 to 88.79 KDa thus depicting variations in the banding patterns of the accessions. The unweighted pair group method with arithmetic mean algorithm (UPGMA) dendrogram grouped the 14 accessions into two clusters and five groups with 50% of the accessions in one group. Accession 3 which was obtained from Lagos state was observed to be a very distant relative of the other accessions and so could be combined in a breeding programme with any of the others.**

Key words: Genetic, variability, *Corchorus olitorius,* accessions.

INTRODUCTION

The genus *Corchorus* has undergone many taxonomic revisions. It was originally placed in the family Tiliaceae, subsequently in the family Malvaceae but it has now been placed in the family Sparrmaniaceae (Heywood et al., 2007). *Corchorus olitorius* otherwise known as Bush Okro or Jew's mallow has been found to be very useful in the following industries among others: interior decoration, accessories sector of apparel and textile industry, footwear industry, pulp and paper industry as a source of non-wood fibrous material (Khan, 2008). The leaves are generally rich in protein, β-carotene, iron, calcium, vitamin B, vitamin C and folic acid (Sinha et al., 2011; Mavengahama et al., 2013) and they form part of the meals of people of Asia, Middle East and parts of Africa (Fondio and Grubben, 2011; Sinha et al., 2011). Adebooye et al. (2003) listed the species as one of the seven highly valued indigenous leafy vegetables (ILV) in Nigeria. Certain problems, however, limit its production

*Corresponding author. E-mail: ifeoma.akaneme@gmail.com. Tel: (234) 803 6698 201.

Abbreviations: SDS-PAGE, Sodium dodecyl sulphate polyacrylamide gel electrophoresis; **UPGMA,** unweighted pair group method with arithmetic mean algorithm.

and improvement (Fondio and Grubben, 2011). These include absence of improved seeds, impermeability of seed coat and flower drop after emasculation.

The authors called for the generation of improved cultivars and improved seeds. Any crop improvement programme, however, can only succeed on the strength of the genetic diversity available to breeders (Keatinge et al., 2008). Estimation of genetic diversity involves germplasm collection, characterization and evaluation. Some of the local landraces of *C. olitorius* which are maintained at National Institute for Horticultural Research (NIHORT), Ibadan Nigeria have been characterized morphologically, based on variations in leaf shapes. Opabode and Adebayo (2005), however, are of the opinion that the genetic improvement of ILV's including *C. olitorius* urgently requires the application of biotechnological techniques, such as molecular breeding.

Due to the cost of using molecular markers for germplasm characterization, many authors have advocated for the use of biochemical markers such as (protein or isozyme) particularly Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE technology) as a reliable tool for economic characterization of germplasm (Iqbal et al., 2005; Stoyanova and Bolla, 2010; Kakaei and Kahrizi, 2011). SDS-PAGE, a technique for analyzing protein contents, provides information on the level of relatedness of accessions of a species on the basis of similarity or dissimilarity of protein bands of the different accessions (Ejele and Osuagwu, 2003). Singh et al. (2004) also endorsed the use of protein markers for germplasm characterizations and pointed out that since storage proteins are the third- hand copy of DNA, they reflect the genetic make-up of the plant and as such could be used to distinguish genetically different varieties or accessions of a species.

Little or no molecular studies have been performed to estimate the diversity of *C. olitorius* in Nigeria (Ogunkanmi et al., 2010). Most genetic diversity studies had been focused on vegetative and physiological characteristics. In order to fully achieve the objectives of Raw Materials Research and Development Council (RMRDC, 2007) of Nigeria, which include the boosting of the production of jute fiber for textile and wearing apparel, there is need to characterize further the various accessions of *C. olitorius* being kept in the seed banks of the National Centre for Genetic Resources and Biotechnology (NACGRAB), Ibadan, Nigeria and National Institute for Horticultural Research in Nigeria (NIHORT).

This study was therefore, undertaken to evaluate the genetic variability among fourteen accessions of *C. olitorius* obtained from NACGRAB with the aid of SDS-PAGE.

MATERIALS AND METHODS

The *C. olitorius* accessions which were characterized in this study are shown Table 1. The method of Laemmli (1970) was used in carrying out the SDS-PAGE. During the process of protein extraction, 20 seeds of the respective accessions were macerated with mortar and pestle. The resulting powder (0.2 g) were homogenized thoroughly using a vortex with an extraction buffer containing 0.5 M Tris-HCl (pH 6.8), 2.5% sodium dodecyl sulphate (SDS), 10% glycerol and 5% 2-mercaptoethanol. The respective samples were centrifuged at 10,000 rpm for 5 min. The supernatants (500 µl) were collected in separate vials. Sodium dodecyl sulphate polyacrylamide gel electrophoresis was carried out using 4% stacking gel and 12% resolving or separating gel. The runs were performed 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 monitor the movement of protein molecules in the gel. The gels were run at 90 V for 2 h in an Ominipac mini- vertical gel apparatus using promega protein as a standard marker. They were gently removed and washed with 500 ml of the gel fixing solution and subsequently covered with 400 mls of Coomassie blue stain at room temperature for 3 to 4 h and were gently agitated. The coomassie stains were removed after staining by covering gels with 250 mls of the destaining solution. The destaining solution was changed severally until the protein bands were seen clearly without background staining of the gel.

Table 1. *C. olitorius* accessions and their states of origin.

S/N	Accession number	State of Origin
A_1	NG/AA/SEP/09/173	Osun
A_2	NG/TO/AUG/09/008	Ogun
A_3	NG/AO/AUG/09/003	Lagos
A_4	NG/OE/10/002	-
A_5	NG/MR/MAY/09/004	Ogun
A_6	NG/SA/DEC/07/0403	Niger
A_7	NG/SA/DEC/07/0402	Niger
A_8	NG/AO/MAY/09/018	Osun
A_9	NG/SA/07/189	Osun
A_{10}	NG/OA/JUN/09/001	Oyo
A_{11}	NG/SA/JAN/09/142	Niger
A_{12}	NG/OCT/09/001	-
A_{13}	NG/AO/MAY/09/013	-
A_{14}	NG/SA/07/203	Ondo

Banding patterns of the 14 accessions were examined and photographed. Each band was considered as a character and presence or absence was coded for analysis. In order to estimate genetic diversity, a dendrogram (a tree - like diagram that shows the degree of relatedness among organisms) was constructed using numerical taxonomic and multivariate analysis system software (NTSYS-pc) version 2.2.

RESULTS AND DISCUSSION

The electrophorogram of the protein bands are shown in Figures 1 and 2. Seven polypeptide bands ranging from 9.73 to 88.79 KDa were recognized among the 14 accessions screened in this study (Figures 1 and 2, Table 2). The bands showed variability on the basis of intensity and presence/absence of any of them among the accessions. There were no bands 2, 4 and 6 in accessions 1 to 7 and no bands 1, 3 and 7 in accessions 8 to 14 (Table 2). The protein band 3 was present in accessions 3 and 5

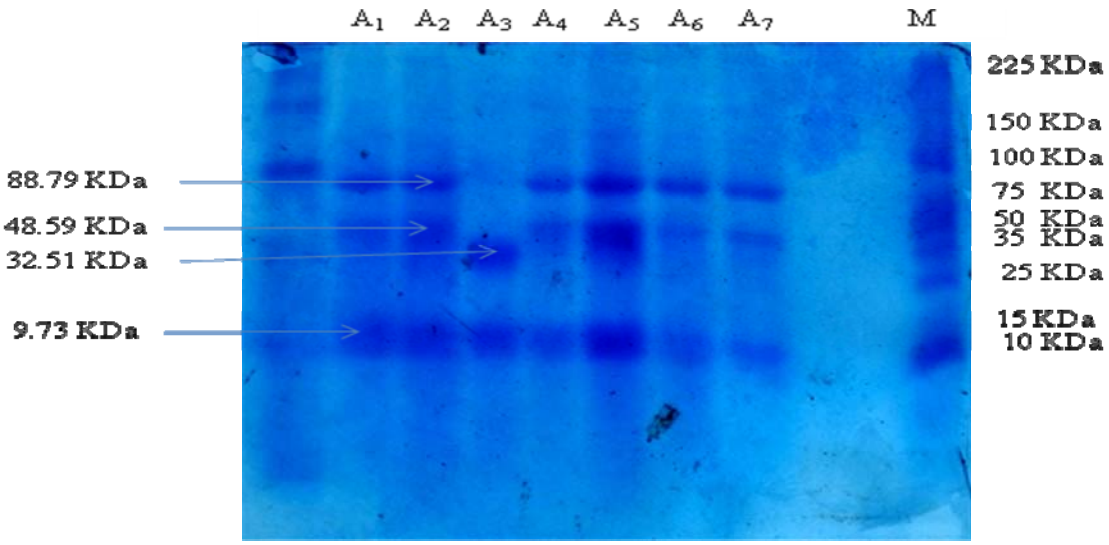

Figure 1. SDS - PAGE electrophorogram of the seed protein for accessions 1 to 7 of *Corchorus olitorius.* M is the Promega standard molecular protein marker.

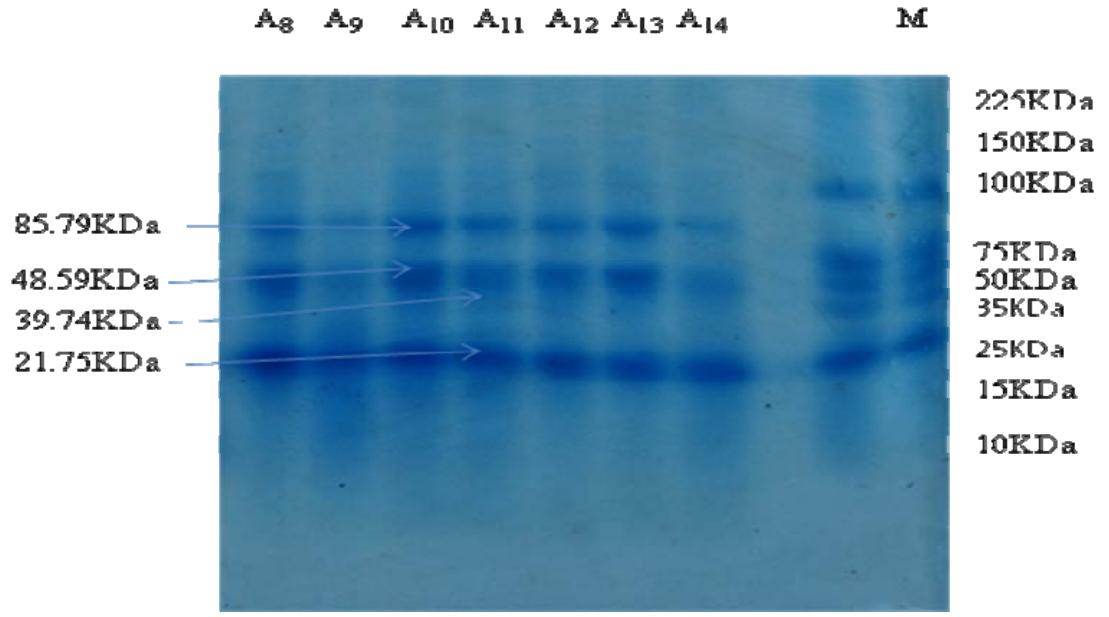

Figure 2. SDS - PAGE electrophorogram of the seed protein for accessions 8 to 14 of *Corchorus olitorius.* M is the Promega standard molecular protein marker.

only. Band 5 (48.59 KDa) was present in all accessions except accession 3 and the degrees of intensities in the 13 accessions differed (Table 2).

The banding patterns across the accessions were moderately dissimilar indicating moderate heterogeneity of the seed storage proteins. This corroborates the report of Hossain et al. (2002) that genetic variability in *C. olitorius* is limited due to self-pollination. Self pollination generally generates offsprings that closely resemble the parents genetically. Some investigators (Soetan and Fafunsho, 2009; 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 distinc-tiveness of the plants. Mehlhorn (2008) observed that protein migration differences correspond to amino acid composition differences which in turn correspond to the differences in gene sequences although according to him, silent point mutations cannot be detected. Inspite of this, he concluded that protein electrophoresis gives the first indication of the existence of genetically different populations or species.

Table 2. Intensities of bands present in each *Corchorus olitorius* accession.

Band number	Molecular weight	A_1	A_2	A_3	A_4	A_5	A_6	A_7	A_8	A_9	A_{10}	A_{11}	A_{12}	A_{13}	A_{14}
1	9.73	++++	++++	+++	+++	++++	+++	++	0	0	0	0	0	0	0
2	21.75	0	0	0	0	0	0	0	+++	++	++	++	+++	+++	++++
3	32..51	0	0	+++	0	++	0	0	0	0	0	0	0	0	0
4	39.74	0	0	0	0	0	0	0	0	0	+	+	0	+	0
5	48.59	+++	+++	0	++	++++	+	+	+++	+++	++++	+++	+++	+++	++++
6	85.79	0	0	0	0	0	0	0	++	+	+++	++	++	++	+
7	88.79	++	++	0	++	+++	++	++	0	0	0	0	0	0	0

+, Band present; 0, band absent.

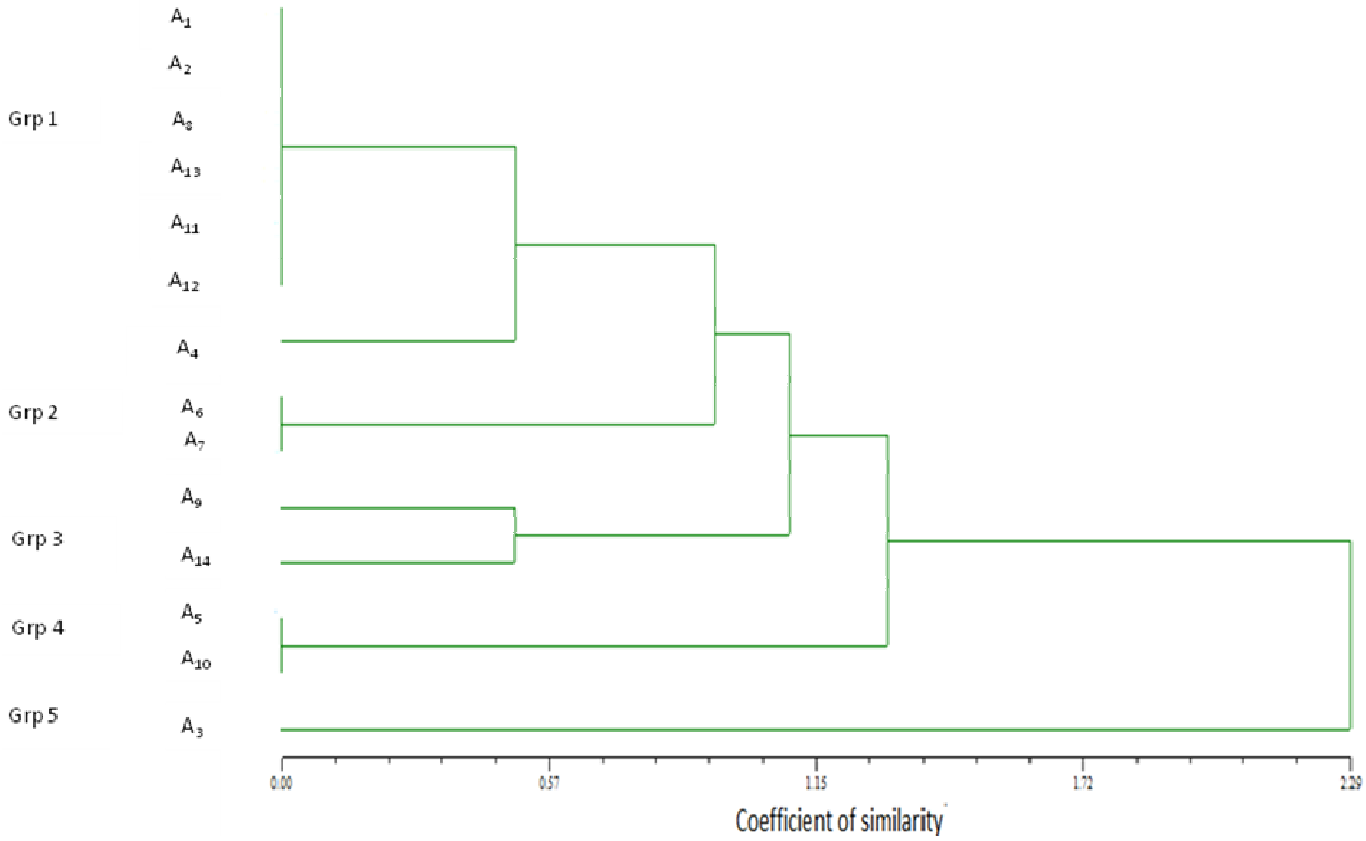

Figure 3. Dendrogram showing the relationship among the 14 accessions of *C. olitorius*.

Amersham BioSciences (1999) opined that the position a protein occupies along the separation lane gives a good approximation of its size and after staining, the band intensity is a rough indicator of the amount present in the sample. Uniform or similar banding patterns observed in some accessions could be due to these proteins being conserved (Ullah et al., 2009). Torkpo et al. (2006) noted that differences in bands which could possibly be associated with economically important traits such as diseases resistance, nematode, parasitic weeds and drought resistance should be a viable research option.

The unweighted pair group method with arithmetic mean algorithm (UPGMA) clustering method based on Jaccard's similarity coefficient separated the 14 accessions into two clusters and five groups at an UPGMA distance coefficient range of 0.48 to 2.29 (Figure 3). Groups 1 to 4 formed the 1st cluster at similarity coefficient of 1.32 while group 5 formed the second cluster at a coefficient of 2.29. Clustering observed in the dendrogram could indicate close genetic proximity or relatedness. Accessions 6, 7 and 11 originated from Niger state but while accessions 6 and 7 were found in group 2, accession 11 was found in group 1. The accessions from

Osun State (A_1&A_8; A_9) were also found in two different groups: group 1 (A_1 & A_8) and Group 3 (A_9) - that were distant from each other. Ogun state was the home of accessions A_2 and A_5 which were part of groups 1 and 4, respectively. The groups (1 and 4) were really distant from each other. It can be deduced from Figure 3 that all the accessions in groups 1 and 5 had the least similarity and maximum distance. Accessions 9 and 14 may be closely related even though they were collected from Osun and Ondo states, respectively. Relative closeness (Yi et al., 2008) could be due to the fact that there is no cross boundary check among divisions or states and seed exchange between farmers may disseminate plants from one region to the other. This could be an indication that these accessions may have been moved from one state to the other, therefore, implying that the seeds may be the same genetically. Accession 3 originated from Lagos State and it was observed to be an independent group and not related closely to other accessions. Accessions on different groups could create wider variation when crossed because they are dissimilar genetically. Maity et al. (2009) had earlier noted that based on distance between accessions of different clusters, contrasting parents may be identified and used in the crossing programme for generating wider variability for selection and crop improvement.

Conclusion

In conclusion, seed protein electrophoresis was able to show the differences and relationship among the 14 accessions of *C. olitorius* evaluated in this study. This result agrees with the report of Patra and Chawla (2010) that electrophoretic analysis of total soluble proteins is widely recognized as a technique for cultivar identification. Thus, it can be deduced from the present study that accession 3 (A_3) can be combined in a breeding programme with the other accessions because it is a very distant relative, while accessions A_1, A_2, A_8, A_{13}, A_{11} and A_{12} may not be used together in a breeding progamme since they appear to be duplications of the same material, just as A_6 and A_7; A_5 and A_{10} appear to be duplications of each other, respectively.

ACKNOWLEDGEMENTS

The authors wish to thank the National Centre for Genetic Resources and Biotechnology (NACGRAB) Ibadan, Nigeria for providing the seeds of the accessions used in this work.

Conflict of Interests

The authors wish to declare that there are no conflicts of interest with respect to this article.

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