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EGYPTIAN ACADEMIC JOURNAL OF
BIOLOGICAL SCIENCES
BOTANY



ISSN 2090-3812

www.eajbs.com

Vol. 5 No.1 (2014)

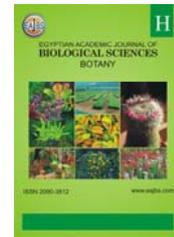
Egyptian Academic Journal of Biological Sciences is the official English language journal of the Egyptian Society for Biological Sciences ,Department of Entomology ,Faculty of Sciences Ain Shams University .

The Botany Journal publishes original research papers and reviews from any botanical discipline or from directly allied fields in ecology, behavioral biology, physiology, biochemistry, development, genetics, systematic, morphology, evolution, control of herbs, arachnids, and general botany.

www.eajbs.eg.net

Received : 12/4/2014

Accepted :25/9/2014



Characterization of three Okra [*Abelmoschus* (L.)] accessions using morphology and SDS-PAGE for the basis of conservation

Osawaru, M. E. * ; Ogwu, M. C. and Omologbe, J.

Plant Conservation Unit, Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Benin City, Nigeria

*edwinosawaru@yahoo.com

ABSTRACT

Okra [*Abelmoschus* (L.)] is native to Africa where it is an essential vegetable. It can be eaten raw, partially or well-cooked with wide range of edible parts. The taxonomy of the genus is regarded as complex. Hence, this study aims to characterize three Okra accession based on agro-morphological and biochemical characteristics. The Okra accessions were collected from National Center for Genetic Resources and Genetics (NACGRAB), Ibadan. Twenty five agro morphological characters were assessed including vegetative, quantitative and fruit characters such as main stem, stem colour, branching, stem base diameter, stem pubescence, leaf shape, leaf length, leaf width, petiole length, fruit colour, fruit shape and nature of fruit tip. SDS-PAGE was used to characterize soluble protein extracted from the Okra accessions. Result from the number of days to 50 % germination show that all the accessions recorded over 50 % germination from the fourth day. An assessment of stem and leaf vegetative character show that the stem colour ranged from green to red and red to purple, petiole and adaxial and abaxial surface of the lamina showed similar coloration. High level of variability was observed in the quantitative characters of the traits. The result of fruit character showed similarities between accessions NGAE-96-0011B and NGAE-96-0064C. It could not be obtained for accession NHGB-09-008A because it was yet to fruit during the period of study. Their biochemistry was also investigated using SDS-PAGE technique (using soluble protein profiling), the results showed that the proteins used as markers were effective in characterization of Okra in systematic studies. Visible and highly reproducible bands were produced. The indications of bands in the protein profiling showed more similarities among the *esculentus* species and distinguished them from the *caillei* species in the band compositions. The study suggest that two of the accessions are *esculentus* (NGAE-96-0064C and NGAE-96-0011B) while NHGB-09-008A is *caillei*. Therefore, it can be suggested that the variability observed in this study for all traits assessed were indicative of the differences in the genetic make-up of the cultivars considered.

Keywords: Okra (*Abelmoschus*), Characterization, SDS-PAGE, Taxonomy, Conservation

INTRODUCTION

Okra belongs to the family Malvaceae, genus *Abelmoschus*. It is native to tropical Africa where it serves as a staple vegetable crop (Indian Government, 2008; Kochhar, 1986; Hamon and van Stolen, 1989). This genus is represented by several species. In West Africa, Charrier (1984) reported two exclusively cultivated species. Osawaru *et al.* (2011) reported that the taxonomy of Okra is complex. It can be considered one of the most important and widely known vegetable crops grown throughout the tropic and sub tropics. This crop is suitable for cultivation as a garden crop as well as on large commercial farms.

In Nigeria, it is found in all political state (Akoroda, 1986; Cheddar and Fatokun, 1991) as well as in Ghana, Sudan and Cameroon (Schippers, 2000). The cultivated Okra is of two distinct species. The Common Okra, *Abelmoschus esculentus* (L) Moench and West African Okra *Abelmoschus caillei* (A.Chev) Stevels (Charrier, 1984). *Abelmoschus esculentus* is adapted to the “Sudano- sahelian” zone and the *A. caillei* is referred to as “Guinean” type of relation in its zone of cultivation (Charier, 1984; Cheddar and Fatokun 1991; Schippers, 2000). They are known by many local names in different part of the world. The crop is famous for its wide range of edible parts (Osawaru and Diana-Ogbe, 2010). The world Okra production was estimated at 4.8 million tons (as of 2007) with India leading the production followed by Nigeria, Pakistan, Ghana, Egypt and Iraq (Gulsen *et al.*, 2007, UN/FAO, 2012).

The Common Okra can be cultivated in areas with limited rainfall and in the drier part of the continent, sometimes under irrigation whereas the West African Okra is found throughout the high rainfall zones, humid coastal zones and more sparingly in its savanna belt Guinea. However, there is an overlap of cultivated species in their natural distribution. Siemonsma (1982) opine that whereas *A. esculentus* is almost distributed throughout the region while *A. caillei* is becoming more frequent towards the east and the drier regions and rare towards the humid equatorial area. However both species are wide spread between 8⁰ N and 12⁰ N (Siemonsma and Hamon, 2002).

African Okra grows naturally in Nigeria (Adeniji *et al.*, 2007) and is bigger in size than the Common Okra. They also vary in their number of chromosomes (Ariyo, 1993). Okra is a nutritious vegetable containing 86.1 % of water, 2.2 % of protein, 0.2 % fat, 9.7 % carbohydrate, 1.0 % fiber and 0.8 % ash (Saifullah and Rabbani, 2007).

The value of germplasm collection depends not only on the number of accessions it contains, but also upon the diversity present in those accessions (Ren *et al.*, 1995). Characterization and quantification of the genetic diversity and information on the genetic diversity within and among closely related crop varieties is essential for a rational use of plant genetic resources. Diversity based on phenotypic and morphological characters usually varies with environments and evaluation of traits requires growing the plants to full maturity prior to identification. Omonhemin and Osawaru (2005) reported that high degree of wide morphological variation exist among accessions of Okra especially in West African type. Notwithstanding the great value of the crop, information on characterization is either not accessible or simply unavailable. Characterization and evaluation of crop is done to provide information on diversity among crops. This permits the identification of unique entries (accession) necessary for curators of gene banks and plant breeders.

This study aims to characterize Okra accessions using agro-morphological characteristics as well as using biochemical characterization techniques. SDS-PAGE will be used to characterize soluble protein extracted from the Okra accessions. This will contribute to elucidating the taxonomy of the genus and aid in the distinction of *A. esculentus* and *A. caillei*. Findings will assist germplasm managers in the conservation of the genetic resources of *Abelmoschus*. Traditional agriculture will stand to benefit as an improve understanding of the crop leading to increased cultivation. More so, the result will contribute to the database on Okra genetic resources.

MATERIALS AND METHODS

Study Area

The morphological characterization of the *Abelmoschus* samples was conducted in the experimental and teaching garden of Plant Biology and Biotechnology Department, University of Benin, Nigeria (6.20 N; 5.73 E) located within tropical rainforest zone. The climate includes high rainfall up to 2000 mm – 3000 mm of bimodal pattern with peaks at July and September respectively, high temperature ranging between 20 – 40 °C and high atmospheric humidity (Omuta, 1980). Radiation is fairly high and varies according to different period of the year; above 1,600 hours per year have been reported (Onwueme and Singh 1991).

Plant Materials

Three accessions of *Abelmoschus* were obtained from active collection at National Center for Genetic Resources and Biotechnology (NACGRAB), Ibadan. These were labeled NHGB-09-008A, NGAE-96-0011B and NGAE-96-0064C.

Table 1: Identity of Okra accessions collected from NACGRAB

Accession	Status	Genus	Source Location
NHGB-09-008A	Landraces	<i>Abelmoschus</i>	NACGRAB Ibadan
NGAE-96-0011B	Landraces	<i>Abelmoschus</i>	NACGRAB Ibadan
NGAE-96-0064C	Landraces	<i>Abelmoschus</i>	NACGRAB Ibadan

Plant Husbandry

Five stands per accession were planted in a single line in the experimental ground of Plant Biology and Biotechnology Department in University of Benin, Benin City. Three seeds were planted into 3 holes of 3 cm deep and lightly covered with soil without tillage, at spacing of 75-100 cm with fertilizer. Crops were rain fed throughout the period of cultivation. Thinning was done two weeks after planting to retain two plants per stand. Weeding and pest control was done using methods outlined by Osawaru and Dania-Ogbe (2010).

Data Collection

Morphological data were collected from five randomly selected plants for each accession based on International Board for Plant Genetic Resources (IBPGR) recommended descriptor procedures for Okra (Charrier, 1984) and on germination as outlined by Osawaru (2008). Data were collected as specified including the seedling characteristics: Days to 50% germination, others are:

Vegetative Characters**Main stem:** 1-erect, 2-medium, 3-procumber**Stem colour:** 1-green, 2-green with red, 3-red, 4-purple**Leaf colour:**

a) Adaxial surface: 1-green, 2-green with red, 3-red, 4-purple

b) Abaxial surface: 1-green, 2-green with red, 3-red, 4-purple

Petiole colour: 1-green, 2-green with red, 3-red, 4-purple**Branching:** 1-unique orthotropic, 2-dense branching at base followed by an orthotropic axis, 3-base without branches at apex, 4-densely branched, branches all over the plant.**Plant height and maturity (cm)****Stem base diameter (cm)****Stem pubescence:** 1-glabrous, 2-slight, 3-conspicuous**Leaf pubescence:** 1-glabrous, 2-slight, 3-conspicuous**Leaf shape:** (7th leaf)**Leaf length (cm):** 7th leaf**Leaf width (cm):** 7th leaf**Petiole length (cm):** 7th leaf**Fruit Characters****Fruit colour (At maturity) (FCMa)**

(i) Brown (ii) Dark brown

Fruit colour (fresh) (FCMb)

(i) Yellowish green (ii) Green (iii) Green with red patches (iv) Red

Fruit shape at maturity (FSH) 1-15**Nature of fruit tip (NEF)**

(i) Fruit acuminate (ii) Acute -1-3mm (iii) Obtuse -4-6mm

Nature of fruit base (NFS)

(i) Ringed or protrude (ii) Ringless or flat (iii) sunken

Numbers of ridges on fruit (NRF)

(i) Smooth (ii) 5-7 few (iii) 8-10 many (iv) 10 very many

Nature of ridges (NRR)(i) Smooth (ii) Runs full length of fruit (iii) $\frac{2}{3}$ length of fruit**Number of epicalyx segment (NES)**

(i) 5-7 (few) (ii) 8-10 (many) (iii) 10 (very many)

Shape of epicalyx (SHE)

(i) Linear (ii) Lanceolate (iii) Triangular

Persistence of epicalyx (PEE)

(i) Non-persistent (ii) Partially persistent (iii) Persistent

Fruit length at maturity (FLM) (cm)

(i) <7cm short (ii) 8-15cm medium (iii) >15cm long

Fruit width at maturity (FWM) (cm)

(i) <2cm small (ii) 3-4cm medium (iii) >5cm large

Pedicle length at fruit maturity (PEL) (cm)

(i) 1-3cm short (ii) 4-6cm medium (iii) >6cm long

Number of fruit

(i) 1-3; few (ii) 3-6; medium (iii) above 6; high

Biochemical Analysis

This study was carried out in the Biochemistry Division, Nigerian Institute of Medical Research (NIMR), Lagos using the SDS-PAGE technique.

Protein Extraction and Gel Preparation: Harvested Okra fruits were transferred in sealed paper envelopes four weeks after harvest to the laboratory and used in the protein extraction. The fruit capsules were open and seeds collected. The seeds were tested for viability using floatation technique. Viable seeds were collected and crushed to powder. Protein extraction was performed as outlined by Saraswati *et al.*, (1993). Sample buffer was added to 0.3 g of seed flour (powder) as extraction liquid and mixed thoroughly in Eppendorf tube and vortex at 50 rpm (4°C). The extraction buffer contained the following final concentration: 0.5 M Tris-HCl (pH 6.8), 10 % SDS, urea, cold acetone and 5 % 2-merkaptoethanol. Before centrifugation at 1000 rpm for 5 minutes at 4°C in a micro centrifuge, the samples buffer was boiled for 5 min. The supernatant were collected into a micro centrifuge tube according to the label and kept at 40°C in the refrigerator. The

resultant supernatant was subjected to Gel Electrophoresis. The Gel used consists of two portions; Resolving gel and Stacking gel as well as the gel running buffer. After preparation of the gels 0.012 ml of the sample and 0.06 ml of mercaptol ethanol was added at 100 °C and then allowed to cool.

SDS-PAGE: SDS-PAGE was performed in Protean II electrophoresis cell (Bio-Rad) at 20 mA by a standard method on the vertical slab gel. Bromophenol blue was added to the supernatant as tracking dye to watch the movement of protein in the gel. Seed proteins were analyzed using 10 % polyacrylamide gel (Laemmli, 1970). The cell was allowed to run until the bromophenol dye front had reached the bottom of the gel. After electrophoresis, the protein bands were visualized by staining with Coomassie Brilliant Blue G-250 for 30 min at 67°C and destained for 3-4hours at same temperature. Marker proteins (Fermentas) of size range 14.4–116.0 kD were used to estimate molecular mass of protein. Molecular weights of the protein bands were estimated by their relative mobilities. The photograph of the gel was taken using camera. Protein profiles distance was calculated graphically, RF, and similarity indices were calculated according to Bhat and Kudesia (2011). The molecular mass of the protein was estimated graphically against the marker.

Data analysis: The polymorphic bands were scored visually as present (1) or absent (0) of protein bands. The matrix obtained was analyzed using PAleontological Statistics software (PAST) for Genetic similarities among genotypes on Li and Nei homology and Cluster analysis utilizing the Unweighted Pair Group Method using Arithmetic Averages (UPGMA) for phenogram grouping.

RESULTS

The results are presented in Tables 2 - 5 and also in Figures 1&2.

Table 2 show days to 50 % germination. The results show that all the accessions had over 50 % germination from the fourth day.

Table 2: Days to 50% percent germination

Accession	Days/Percentage of Germination						
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
NHGB-09-008A	-	-	47	100	100	100	100
NGAE-96-0011B	-	-	27	67	80	87	100
NGAE 96-0064C	-	-	33	60	100	100	100

Germination was noticed in the three accessions from the 3rd day of planting. On the 4th day, over 50 % germination was recorded for all the accessions. As from the 5th day, germination was 80 % in accession NGAE-96-0011B while accessions NHGB-09-008A and NGAE-96-0064C had 100 % germination. Accession NGAE-96-0011B had 100 % germination on the seventh day.

Table 3: Qualitative morphological characteristics of the collected Okra accessions

Accession	Main stem	Stem			Leaf					Petiole colour
		Colour	Branching*	Nature of Branches**	Pubescence***	Shape +	Colour (a)	Pubescence (b)	+++	
NHGB-09-008A	Erect	Green	1	1	1	10	1	1	1	Green
NGAE-96-011B	Erect	Green	2	2	2	7	2	2	2	Purple
NGAE-96-0064C	Procumbent	Purple	1	1	1	10	4	4	1	Purple

Key:

* 1- (unique orthotropic), 2-(dense branching at base followed by an orthotropic axis), 3- (base without branches but densely branched at apex), 4-(densely branched, branches All over the plant)

**1-(no branches), 2-(rarely more than 10cm long), 3-(frequently more than 10cm long)

***1- (glaborous), 2-(slight), 3-(conspicuous)

+ - See leaf shape

+++ adaxial surface – 1- (green), 2-(green with red), 3-(red), 4-(purple)

b++ abaxial surface – 1- (green), 2-(green with red), 3- (red), 4- (purple)

+++ 1-(glaborous), 2-(slight), 3-(conspicuous)

High level of variability was observed in the quantitative characters from the trials as shown in Table 4. The range, mean and standard deviation (an index of the disparity between the characters) showed a high degree of variability between the accessions.

Table 4: Range, mean and standard deviation for some quantitative characters the okra accession

Accession	Plant Height at Maturity (cm)			Stem Base Diameter at Maturity (cm)			Leaf Length (cm)			Leaf Width (7th Leaf)			Petiole length at 7 th leaf (cm)		
	Range	Mean	SD	Range	Mean	SD	Range	Mean	SD	Range	Mean	SD	Range	Mean	SD
A	45.2-53.1	48.2	1.4	1.2-20.3	1.5	0.1	3.5-5.1	3.8	0.3	2.3-3.5	2.7	0.7	1.3-2.5	1.7	0.2
B	85.3-190.3	105.3	7.2	3.9-5.2	4.4	0.6	6.3-14.9	9.3	1.0	5.7-7.3	6.5	0.8	3.3-5.1	4.1	1.3
C	70.3-110.7	94.4	15.1	4.3-5.1	4.6	0.4	7.2-11.5	9.1	0.9	6.1-7.0	6.8	0.7	3.9-5.0	4.3	1.2

A= NHGB-09-008A B= NGAE-96-011B C= NGAE-96-0064C

Plants were still growing when these results were collected.

Fruit characters both quantitative and qualitative according to IBPGR (1984) are presented in Table 5. Fruits from the five replicates were all similar in qualitative characters. NGAE-96-011 was yet to flower thus fruits characters were not recorded as when the readings were taken.

Table 5: Fruit characters of Okra (Abelmoschus) accessions studied

Accession	FCM	FSH	NFT	NFB	NRF	NRR	NES	SHE	PEE	FLM (cm)	FWM (cm)	PEL (cm)	NOF	DOS
NHGB-09-008A	Brown	1	Flat acuminate	Ringless or flat	8-10 (many)	Run full length of fruit	NA	NA	NA	8-5 (medium)	3-4 (medium)	4-6 (medium)	3 ±0.2	45 ±0.11
*NGAE-96-011B	-	-	-	-	-	-	-	-	-	-	-	-	-	-
NGAE-09-0064C	Brown	1	Flat acuminate	Ringless or flat	8-10 (many)	Run full length of fruit	NA	NA	NA	8-5 (medium)	3-4 (medium)	4-6 (medium)	2 ±0.2	65 ±0.11

FCM - Fruit colour (at maturity) FSH - Fruit shape at maturity NFB - Nature of fruit base

NRR - Nature of ridge

SHE - Shape of epicalyx

NOF - Number of fruit

FLM - Fruit length at maturity

PEL - Pedicel length at fruit maturity

NFT - Nature of fruit tip

NRF - Numbers of ridges on fruit NES - Number of epicalyx segment DOS - Day from sowing to first fruit

PEE - Persistence of epicalyx

FWM - Fruit width at maturity

NA - Not available at the time of collection

* Plants are yet to fruit

The result biochemical characterization using SDS-PAGE presented in Figure 1, suggests distinction for all the accessions analyzed in the study. Although two accessions were more similar.

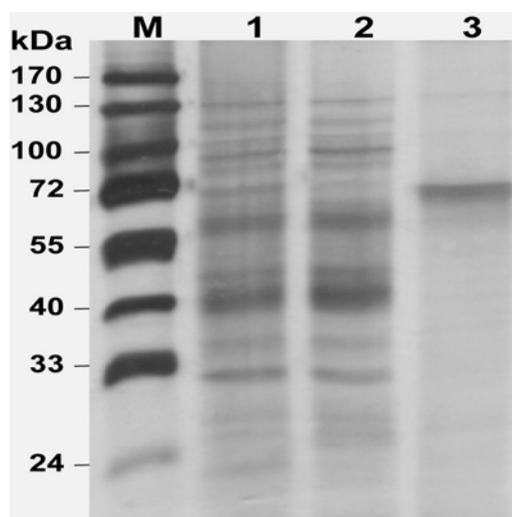


Fig. 1: SDS-PAGE profile for the three accessions of Okra (*Abelmoschus*)

KEY

1= Accession NGAE-96-0064C

KDa= KiloDalton

2= Accession NGAE-96-0011B

M= Marker

3= Accession NHGB-09-008A

The profile suggest accessions NHGB-09-008A is more distinct biochemical while NGAE-96-0011B produced more similar bands to accession NGAE-96-0064C based on the soluble protein extracted from the samples for the analysis. The band matrix obtained was used in the construction of UPGMA neighbor joining clustering diagram (Fig. 2)

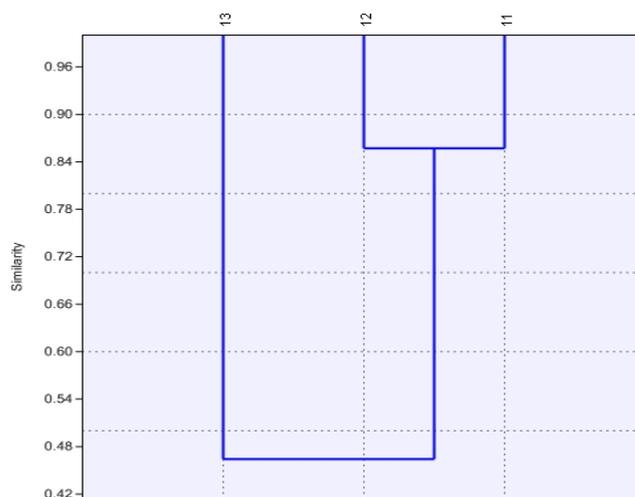


Fig. 2: Clustering analysis of the *Abelmoschus* accessions

KEY:

11= Accession NHGB-09-008A

12= Accession NGAE-96-0011B

13= Accession NGAE-96-0064C

DISCUSSION

The analysis from morphological and protein profiling of three accessions of two cultivated Okra- *Abelmoschus esculents* and *A. caillei* were studied. The morphologic characters based on some qualitative and quantitative traits of fruits and vegetative organs. The soluble protein profile was done using Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE).

The three accessions showed 50 % and above germination on the 4th day after sowing. IBPGR/Charrier (1984) descriptor for okra states that 50 % germination of Okra seeds sown is expressed in number of days from sowing to germination. They suggested two days as been required to attain 50 % germination. From this study, it was observed that 50 % germination was recorded on the 4th day. Osawaru and Dania-Ogbe (2007) reported 3 days from date of sowing to germination for Okra.

A high level of variability and overlapping was observed among the three accessions based on morphological traits. The characters assessed also showed relationships between them, which is in agreement with the earlier suggestions of Stevel (1988, 1990); Siemosma (1982) that the cultivated Okra is of two distinct species. The study suggest that two of the accessions are *esculentus* (NGAE-96-0064C and NGAE-96-0011B) while NHGB-09-008A is *caillei*.

Furthermore, the results from the following morphological characteristics such as stem type; procumbent or erect, petiole colour; green, red and purple, leaf shape, branching and internodes length are important characteristic parameters for plant vigour and architecture. It was observed that the accessions studied possessed the important vegetative traits.

According to the report of Ominhimwin and Osawaru (2005), the strong profuse branches observed are potential high production sites, therefore the higher the number of branches, the higher the number of fruit produced. Due to the fact that straight strong stems support the Okra plants in carrying and spreading vegetative parts such as leaves, fruits and flowers in order to avoid shading hereby enhancing photosynthesis, pollination and harvesting.

Pigmentation of the organs is remarkably diverse in this study. The *esculentus* species showed a green-red coloration, while Red/purple pigmentation was dominant among the *caillei* studied.

The three accessions of cultivated species of okra studied belonged to *A. esculentus* and *A. caillei*. Their biochemistry was also investigated using SDS-PAGE technique (using soluble protein profiling), the results showed that the proteins used as markers were effective in characterization of Okra in systematic studies. Visible and highly reproducible bands were produced. The indications of bands in the protein profiling showed more similarities among the *esculentus* species and distinguished them from the *caillei* species in the band compositions. The *esculentus* species had clear bands at 30, 55 and 100 KDa while the *caillei* had clear band 72 KDa. The clear band produced at 72 KDa was close to those produced by the *esculentus* species at 55 KDa. More so, the bands produced by the *esculentus* species suggest high degree of polymorphism at some points.

From this study, it is obvious that the accessions are of valuable collection due to their high variability. Thus, it can be suggested that the value of a germplasm collection

depends not only on the number of accessions it possesses but also upon the diversity present between both accessions. Similarly, the various diversity reported may play significant role in breeding programmed for biotic and abiotic stress in Okra. The leaves which are hardly consumed perhaps owing to the hairy (pubescent) nature could be another source for fats and crude protein. Notwithstanding the great value of the crop, information on characterization is either not accessible or simply unavailable. Characterization and evaluation of crops is done to provide information on diversity within or among crops. This permits the identification of unique entries (accessions) necessary for curators of gene banks and plant breeders.

The traditional approach to characterization and evaluation is based on morphological features. Though such phenotypic evaluations are important, the data is not understood at gene level. This is because most economic characters are polygenically inherited and exhibit considerable interactions between genotype and environment. It is, therefore, essential that genetic diversity within collections be assayed in the context of total available genetic diversity for each species. Molecular markers may extend and complement characterization based on morphological and agronomic descriptions, providing more accurate and detailed information than classical phenotypic data (Karp *et al.*, 1997). Other molecular markers recognized as important for use in characterization includes isoenzyme, total protein, seed storage protein, RAPDs, AFLPs and Microsatellites (Burr *et al.*, 1983). Isoenzymes exhibit co-dominance at a locus making it possible to differentiate between heterozygotes.

Seed storage protein analysis represents a valid alternative and or improved approach to varietal identification, which currently is based morphological traits recorded in the field (AOSA 1991; ISTA, 1993). Seed storage markers are highly polymorphic and environmental influence on their electrophoretic pattern is limited (Gepts *et al.*, 1986). Seed storage protein markers have been used to study crops including *Solanum* spp. (Menella *et al.*, 1999).

It is generally known that the methods of Okra identification based on morphological traits of plants and fruits are not precise because they are highly influenced by environmental conditions. For these purposes, it is therefore reasonable to search for stable traits of Okra. Availability of genetic variation is important for genetic improvement of the crop. Local and exotic germplasm can be used as sources of genetic variation. Protein markers can effectively to study the genetic variation of germplasm for its utilization in crop breeding programmes.

The study showed that diversity exists for protein profiles and seed proteins have potential for aiding species classification and for serving as markers for interspecific hybridization studies. Low interspecific diversity has been reported for the species by Lanham *et al.* (1994). Generally, all the Okra accessions recorded wide variation among the Okra species studied. Using DNA marker techniques to determine phenotypically similar cultivars would be of great interest in Okra breeding programmes (Duzyaman, 2005).

CONCLUSION

This present investigation has assessed the morphological and biochemical distinctiveness and relationships among three Okra accessions and suggests similarities

within species and distinction between species. Variability observed in this study for traits may be indicative of differences in the genetic make-up of the cultivars considered. More so, the effectiveness of seed components as a selection criterion showed high significant among accessions for seed quality characters. This suggests therefore, that selection for seed quality traits is possible due to large variability present. Hence, it may be concluded that no amount of cultural operations and careful husbandry will compensate, if seed sown is of poor quality which produce weak plants that are not capable to exploit the environment in which they are cultivated.

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