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Assessment of Genetic Diversity in Improved and Local Accessions of Okra (*Abelmoschus esculentus* and *Abelmoschus callei*) Using RAPD Markers

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Authors' contributions

This work was carried out in collaboration between all authors. Authors OSO and ID designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors MAG and OAO managed the analyses of the study. Author OSO managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The work was conducted to ascertain the level of genetic diversity in some local and improved accessions of okra in Ibadan. This is to contribute to the conservation of plant genetic resources efforts by breeders.
Place and Duration of Study: Improved accessions of okra were collected from National Centre for Genetic Resources and Biotechnology (NACGRAB), Ibadan; National Institute of Horticulture, (NIHORT), Ibadan; and Agronomy Department, University of Ibadan, Ibadan; while local accessions were collected from local farmers in Ibadan between January and June, 2012.
Methodology: Nine accessions of okra (*Abelmoschus esculentus* and *Abelmoschus callei*), which cut across improved and local accessions, were assessed for genetic diversity. Genomic DNA was extracted from the 9 okra accessions, and thirteen (13) RAPD markers in the OPB, OPT and OPH series; which exhibited a high level of DNA polymorphism, were used to PCR amplify it (DNA). The

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PCR products were checked on 1% agarose gel and the banding pattern visualised under UV-light and then photographed. Presence and absence of bands on the gel were scored '1' and '0' respectively to generate a binary data matrix. Using NTSYS-pc, a pair wise distance matrices was calculated and a dendogram generated by UPGMA cluster analysis.

Results: All the 13 primers used showed polymorphism across the 9 accessions. The genetic similarity of the studied accessions ranged from 76% to 88%. At 88% similarity level 55.6% of the accessions were distinct while 44.4% displayed minimum genetic variation. Maximum genetic variation was observed between *A. callei* UI accession (an improved accession) and Igala (a local accession).

Conclusion: The accessions displayed genetic diversity. The maximum genetic diversity observed between an improved and local accession, and the minimum genetic diversity observed between two local accessions and two improved accessions shows RAPD markers are still very much useful for genetic diversity study.

Keywords: Genetic diversity; RAPD markers; Abelmoschus esculentus; Abelmoschus callei.

ABBREVIATIONS

UPGMA: Unweighted Pair-Group Method of Arithmetic Average
PIC: Polymorphic Information Content
PCR: Polymerase Chain Reaction
UI: University of Ibadan
TE: Tris EDTA Buffer
EDTA: Ehtylene Diamine Tetra Acetic Acid
PVP: Poly Vinyl Pyrroliodione
CTAB: Hexadecyl Trimethyl Ammonium Bromide
PCI: Phenol:Chloroform: Isoamylalcohol

1. INTRODUCTION

The word okra is of West African origin and is cognate with ọkwurụ in the Igbo language spoken in Nigeria [1]. Okra, also known as "lady's fingers" is one of the vegetable crops grown in Southwestern Nigeria. In 2016, the world production of okra was estimated at 8.9 million tons, Nigeria producing 1.98 million tons as the largest African producer and second largest producer in the world, next to India which produced 5.51 million tons in 2016 [2].

Okra, with numerous accessions which include *Abelmoschus esculentus* and *Abelmoschus callei*, has a lot of economic importance. It is known for its high antioxidants content [3], series of culinary uses and industrial applications in the food and pharmaceutical industries [4]. Besides these, Okra has even been reported to be a good source of biofuel [5]. It is therefore an economic crop which is yet to be fully tapped though greatly cultivated in Nigeria.

Different accessions of this crop are cultivated in various locality in Nigeria, and are identified with the name of the locality where they are predominant with respect to their cultivation. While some of these local accessions can be morphologically distinguished easily, there are some which morphological differences are not distinct. It is essential to know whether these are duplication of the same accession or unique accessions, and genetic diversity study is the best tool for this. Genetic diversity studies help in distinct identification of genotypes thereby eliminating, to a reasonable extent, the confusion associated with morphological characterisation which include multiple cultivar registration [6].

Besides, considering the economic potential of this crop; which is yet to be fully tapped, there is a need for conservation of the plant besides its genetic improvement to suit specific purpose and various humans' needs. For this to happen genetic diversity studies are inevitable. This study aims at identifying the dissimilarities and similarities between some accessions of local and improved accessions of okra in Ibadan. This will help plant breeders in the genetic improvement of the crop.

Over the years RAPD markers have proven very useful in genetic diversity studies and has been reported as a great tool for sequence polymorphism [7]. There are many recent studies which have successfully used RAPD markers for genetic diversity studies [6,8,9,10].

This study was conducted using five (5) local accessions of okra and four (4) improved accessions of okra gotten from research institutes in Ibadan, Nigeria.

2. MATERIALS AND METHODS

2.1 Plant Material

Dried viable seeds of improved accessions of okra were collected from the department of Agronomy, University of Ibadan, Ibadan; National Institute of Horticulture, Ibadan and National Centre for Genetic Resources and Biotechnology, Ibadan. For the local accessions, dried pods were collected from some farmers in Ibadan. Three seeds of each of the okra accessions were then planted in a well labelled perforated pots, filled with top soil, and watered once every forty-eight hours. The seeds germinated between five to seven days after planting. Three weeks after germination, fresh apical portion of the plants were harvested. The harvested leaf samples were put in a well labelled polythene bags inserted into an ice pack and quickly taken to the laboratory for DNA extraction. The accessions used for this study and their sources are as stated in Table 1 while some of their pictures are presented in Fig. 1.

2.2 DNA Extraction and PCR Amplification

0.5g of the harvested leaf samples was grinded in liquid nitrogen and DNA was extracted using CTAB method [11] with little modifications. To the ground leaf was added 600µl of 2xCTAB buffer (50mM Tris HCl pH 8.0, 500mM NaCl, 10mM EDTA, 2% hexadecyltrimethylammonium bromide, 2% w/v PVP and 0.1% 2-mercaptoethanol). This was incubated for 20 minutes at 65^0C with occasional swirling. The samples were then cooled to room temperature and 600ml solution of Phenol:Chloroform:Isoamylalcohol (25:24:1) was added to the sample and mixed gently for 5 minutes. The sample was then centrifuged for 8 minutes at 9,000rpm. The supernatant was then transferred to a new labelled Eppendorf tube. PCI was added again and centrifuged, after which an equal volume of isopropanol was added and the solution incubated at -20ºC overnight. DNA was then pelleted at 4ºC by centrifuging at maximum speed for 15minutes. The supernatant was decanted and the DNA pellet washed with 70% ice-cold ethanol and then centrifuged for 10 minutes at maximum speed. The DNA pellet was then air dried and dissolved in 50ml TE.

Following the Williams et al. [12] protocol with slight modifications, the DNA was pcr amplified in a 25µl reaction volume that contains: 2.5µl PCR 10x buffer; 1.2µl $MgCl_2$ (50mM); 2.0µl dNTPs (2.5mM); 1.0µl DMSO; 1.0µl Primer (10 µM); 2.0µl template DNA; 0.2µl Taq polymerase and 15.1µl ultra-pure water. The pcr was conducted using MyGene^{TM} Series Thermal Cycler with the programme set at 94ºC for 3 minutes initialisation, 45 cycles of 94ºC for 20 seconds denaturation, 38ºC for 40 seconds annealing, and 72ºC for 1 minute extension; and concluded by 72ºC for 7 minutes final extention then 4ºC forever. Using 1% agarose gel in 1 X TBE buffer, gel electrophoresis was conducted with 3µl loading dye and 8µl amplified product. The gel was then visualised under UV light and photographed. The gel is shown in Fig. 2 while the primers used are stated in Table 2.

2.3 Data Analysis

The bands seen on the gel were transformed into a binary data matrix by scoring positions with bands “1” and positions without bands “0”. Using the NTSYS-pc, the transformed character matrix data were first transferred into the software data collection module from which a pair-wise distance matrices was calculated [13]. Genetic similarity was calculated using the Jaccard

Table 1. The accessions of okra used and their sources

S/No	Ascension	Source
1	Oyo	Local Farm, Ibadan
2	Iroko	Local Farm, Ibadan
3	Iwo	Local Farm, Ibadan
4	Igala	Local Farm, Ibadan
5	*Abelmuscos callei* NGAE – 96 – 0064	NACGRAB, Ibadan.
6	*Abelmuscos esculentus* 47 – 4	NIHORT, Ibadan.
7	*Abelmuscos callei* NGAE – 96 – 011	NACGRAB, Ibadan.
8	*Abelmuscos esculentus* 8 -11	Agronomy Dept, UI
9	*Abelmoscus callei* UI accession	Agronomy Dept, UI

Oyo (a local accession) | **Iroko (a local accession)** | **Iwo (a local accession)** | ***A. esculentus* 8 – 11 (an improved accession)**

Fig. 1. Dried pods of some of the accessions of okra used for the work

coefficient of similarity [14] given as J = A/(N – D), where A = number of positive matches; D = number of negative matches; and N = the sample size. Using the output data and the graphical model of the software, a dendogram was generated by the Unweighted Pair-Group Method of Arithmetic Average cluster analysis (UPGMA) [15]. The dendogram generated is displayed in Fig. 3.

3. RESULTS AND DISCUSSION

As shown in Table 2, thirteen out of the twenty five primers used showed polymorphisms. None of these thirteen showed low polymorphisms rather five of them representing 38.46% showed average polymorphisms while eight of them representing 61.54% showed high polymorphisms across the nine accessions. Variations in the banding patterns for each of the RAPD primers used indicates polymorphisms in the accessions being studied and in fact each primer was unique in the binding pattern revealed. This is consequent of the fact that the base sequence in the primers is different and this agrees with the report of Ogunbayo et al. [16].

From the dendogram as presented in Fig. 3, at 76% similarity the accessions clustered expectedly into two clusters of improved accessions (A) and local accessions (B).

Table 2. RAPD markers used with the PIC and alleles

S/No	Primer	Sequence	Total no of Alleles	Percentage Alleles (%)	PIC	Percentage PIC (%)
1	OPB-01	GTTTCGCTCG	14	2.85	0.57	6.5
2	OPT-05	GGGTTTGGCA	18	3.66	0.67	7.6
3	OPH-05	AGTCGTCCCC	10	2.03	0.32	3.6
4	OPH-06	ACGCATCGCA	10	2.03	0.56	6.4
5	OPH-03	AGACGTCCAC	20	4.07	0.50	5.7
6	OPH-04	GGAAGTCGCC	22	4.47	0.71	8.1
7	OPT-04	CACAGAGGGA	8	1.63	0.38	4.3
8	OPT-14	AATGCCGCAG	16	3.25	0.66	7.5
9	OPT-06	CAAGGGCAGA	110	22.36	0.91	10.4
10	OPT-10	CCTTCGGAAG	66	13.41	0.93	10.6
11	OPB-05	TGCGCCCTTC	74	15.04	0.89	10.1
12	OPH-07	CTGCATCGTG	78	15.85	0.87	9.8
13	OPB-04	GGACTGGAGT	46	9.35	0.83	9.4
		Total	**492**	**100**	**8.79**	**100.0**
		Mean	**37.85**	**7.69**	**0.68**	**7.7**

Cluster A contains all the improved accessions except one (*A. callei* NGAE -96-0064), which clustered with the local accessions in cluster B. This suggests that the local accessions are all closely linked, same with the improved accessions.

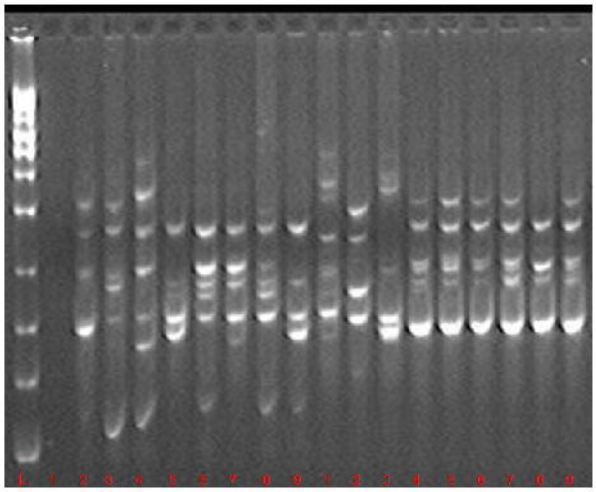
In cluster A, two sub–clusters, one for improved *A. esculentus* and the other for improved *A. callei,* would have been expected; since one would expect that the improved *A. esculentus* accessions would be more genetically related with each other than with the A. callei accessions; likewise the improved *A. callei* accessions. This was observed until unexpectedly at 81% genetic similarty, *Abelmoschus esculentus* 47 – 4 singled itself out (A2); leaving *Abelmoschus esculentus* 8 – 11 which clustered with the two *A. callei* accessions in A1. Rather than the *Abelmoschus esculentus* 8 – 11 singling out itself first from sub-cluster A1, it was *A. callei* UI accession (A1a) that singled itself out first, while *A. esculentus* 8 – 11 and *A. callei* NGAE 96-011 (A1b) showed minimum diversity unexpectedly. This implies that *A. callei* NGAE-96-011 is more related to *A. esculentus* 8-11 than the former is to *A. callei* (UI accession).

At the level of University of Ibadan, it can be acceptably said that at 83% similarity, University of Ibadan *A. esculentus* accessions diverged from University of Ibadan *A. callei* accessions. But when compared with accessions from other institutions, it is safe to say that there might be a miss up in University of Ibadan accessions. The *A. esculentus* (UI accession) might actually be *A. callei* and vice versa. This reinforces the need for DNA barcoding for classification of closely related species [17] which will eliminate miss up in genetic material storage and conservation.

Cluster B divided into two sub-clusters B1 and B2 at 79% genetic similarity. B2 sub-cluster contains only igala (a local accession) while B1 was further divided into B1a and B1b. B1b contains iroko and oyo which exhibited minimum genetic diversity while *A. callei* NGAE 96-0064 which widely diverged from other improved accessions clustered together with iwo (a local accession) till 86% genetic similarity (B1a). It is likely *A. callei* NGAE 96-0064 is from iwo accession hence their clustering together till 86% genetic similarity.

The high level of genetic similarity between *A. callei* NGAE 96-0064 and the local accessions might mean that there is still room for much breeding work to be done on *A. callei* NGAE 96-0064 to explore its full potential. It is also possible that this accession is being kept/preserved specially as a source of some good characteristics peculiar to local accessions for future breeding work.

Maximum genetic diversity was observed between *A. callei* – UI accession (improved accession) and Igala (a local accession) while minimum genetic diversity was observed between Iroko and Oyo (local accessions) and between *A. callei* NGAE-096-011 (improved accession from NACGRAB) and *A. esculentus 8 – 11* (improved accession from Agronomy department, University of Ibadan).



OPB_05 OPH 7

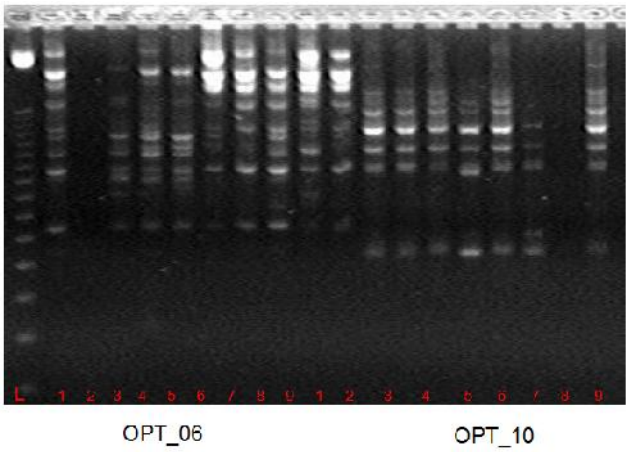


Fig. 2. RAPD Profile of 9 okra accessions UP: For primers OPB 05 and OPH 07. Down: For primers OPT 05 and OPT 10. Lanes L are for the DNA ladder while lane 1 – 9 are for various okra accessions in the order presented in Table 1

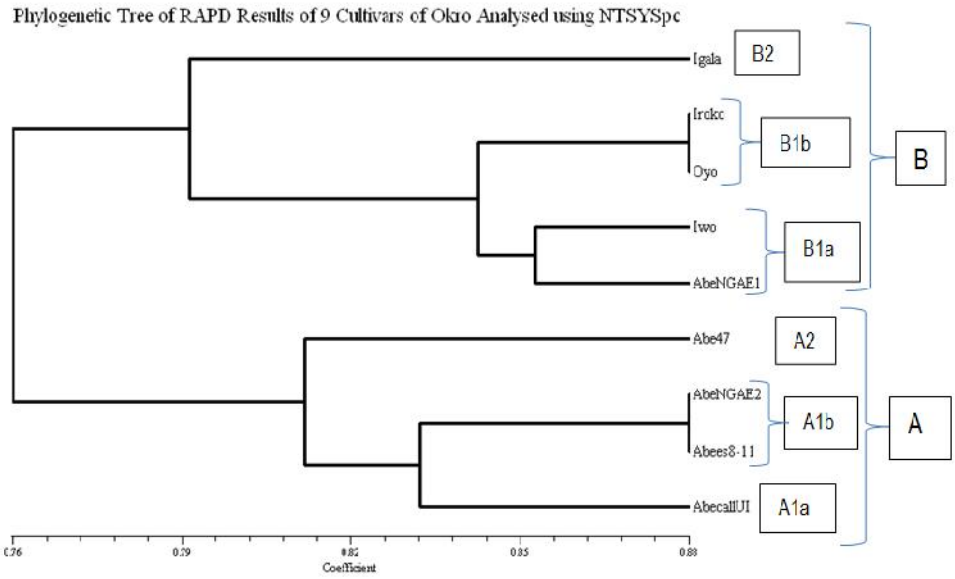


Fig. 3. Dendogram showing genetic diversity in the nine (9) accessions of okra used for the study. The accessions clustered into two main clusters A – improved accessions; and B – local accessions with one improved accession

AbeNGAE1: Abelmoschus callei NGAE 96-0064
AbeNGAE2: Abelmoschus callei NGAE 96-011
Abe47: Abelmoschus esculentus 47 - 4
Abees8-11: Abelmoschus esculentus UI accession
AbecallUI: Abelmoschus callei UI accession

4. CONCLUSION

According to Torkpo et al. [18], information on genetic relatedness among genetic resources of crop plants is useful not only for breeding purpose but also for the conservation of germplasm. The ability of 80% of the improved accessions to cluster together and 100% of the local accessions clustered together proves RAPD markers useful in genetic diversity studies

[6]. They are therefore very useful for breeding programmes. RAPD markers can also contribute to solving morphological characterisation problems not limited to multiple cultivar registration [6]. The high similarity (76% - 88%) between the studied accessions reveal there are very similar one to the other hence crosses between them might not give a good offspring.

NOTE

Orcid id is 0000-0001-6438-3379.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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APPENDICES

APPENDIX I

INFORMATION ON PHYLOGENETIC TREE GENERATION:

Software used: NTSYSpc 2.02, (C) 1986-1999, Applied Biostatistics Inc.
Clustering method: UPGMA
Matrix type = 3
Size = 9 by 9,
Missing value code ="none" (similarity)

APPENDIX II

FIT CRITERION using DARwin

Edge length sum: 1.437
Mean error: 0.002
Mean absolute error: 0.0089
Maximum absolute error: 0.0192
Mean square error: 0.0001
Cophenetic ratio: 0.9965

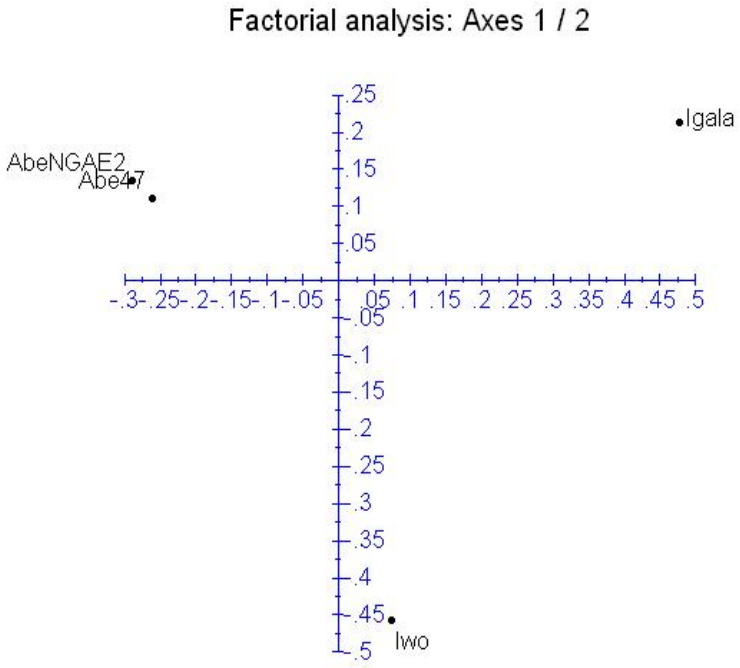

Fig. 4. Factorial analysis of the okra accessions

File Comments:
Factorial coordinates calculated from dissimilarity: Okro.dis

Axis	Eigen value	Inertia%
1	0.09634	46.83
2	0.07128	34.65
3	0.03809	18.52
4	0	0

Coordinate are calculated for the 3 finrst axes (with positive eigenvalue)
Dissimilarity calculated from data file: Okro.var (type:'single')
User selection Units: 10/10 and Variables: 146/146
Dissimilarity index: Presence / Absence - Jaccard
Missing data options:
Integer code for missing data = 9
Complete unit deletion - Remove all units with missing data

LEAST-SQUARES EDGE LENGTH RE-ESTIMATION

Fit criterion for initial tree:Okro.arb and dissimilarity: Okro.dis
Edge length sum: 1.437
Mean error: 0.002
Mean absolute error: 0.0089
Maximum absolute error: 0.0192
Mean square error: 0.0001
Cophenetic r: 0.9965
Fit criterion for adjusted tree:
Edge length sum: 1.4387
Mean error: -0.0005
Mean absolute error: 0.001
Maximum absolute error: 0.0029
Mean square error: 0
Cophenetic r: 1
The degree of confidence in association of the studied accessions is very good as revealed by the cophenetic correlation of 0.9965.

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The peer review history for this paper can be accessed here:
http://prh.sdiarticle3.com/review-history/27495