Genetic Diversity in Moringa Oleifera from Nigeria Using Fruit Morpho-Metric Characters & Random Amplified Polymorphic DNA (RAPD) Markers

By

Jacob O. POPOOLA,

Babafemi O. OLUYISOLA

&

Olawole O. OBEMBE*

Department of Biological Sciences,
School of Natural and Applied Sciences,
College of Science and Technology,
Covenant University, P.M.B. 1023,
Canaan Land Ota, Ogun State, Nigeria

*E-mail: olawole.obembe@covenantuniversity.edu.ng

Abstract: The present study was to evaluate the intra-specific genetic variabilities available among some accessions of Moringa oleifera collected from the six eco-geographical areas of Nigeria. The study was carried out on Covenant University farm and the Department of Biological Sciences, Covenant University, Ota, Ogun State, Nigeria between April 2012 and December 2013. The Intra-specific variabilities were evaluated using 12 fruit morpho–metric characters and five arbitrary Random Amplified Polymorphic DNA (RAPD) markers. Data obtained from reproductive characters were expressed as means with standard deviation as well as Hierarchical clustering. Data matrix from RAPD profiles were scored as present (1) or absent (0). The data obtained from scoring the RAPD bands were subjected to genetic similarity matrix using Jaccard's similarity coefficient (Jaccard, 1908). The results revealed high genetic variability among the accessions. A total number of 224 bands were obtained with 77.86% polymorphism. Cluster analysis of pod and seed characters revealed three distinct groups while dendrogram based on the RAPD data clustered the accessions into four distinct groups with one splinter subgroup. Some accessions exhibited good agronomic features such as long pods, high number of seeds per pod and high seed set percentage. Such accessions could serve as parent plants for breeding for genetic improvement, utilization and conservation.

Keywords: Intra-specific genetic variabilities; fruit morphology; polymorphism; genetic improvement.

INTRODUCTION

Moringa oleifera Lam. is an underutilized out crossing diploid (2n = 28) tree species belonging to the family Moringaceae and indigenous to the sub-Himalayan tracts (1; 2; 3). It has been successfully introduced and established in other parts of India, Pakistan, Afghanistan, Bangladesh, Sri Lanka, Southeast Asia, East and West Africa, Southern Florida, throughout the West Indies, and from Mexico to Peru, Paraguay and Brazil (4; 3).

Of all the species in the genus *Moringa*, *M. oleifera* is the most economically cultivated because all its parts are useful as medicine, food, fodder, domestic cleaning agent, green manure, rope, tanning hides and as water purifier (5; 6). However, in spite of all these economic importance, *M. oleifera* has not witnessed successful breeding for genetic improvement to further enhance its productivity. The knowledge of the levels of genetic diversity and relatedness of introduced landraces or populations to different locations in Nigeria is limited, though cultivation and utilization has greatly increased. Our recent field survey indicated possibility of transfer of similar genotypes to different locations in Nigeria, which may further narrow gene pool. The reviews of literature also indicate that knowledge of their intraspecific relationships is poorly understood and where available it is limited in scope, bearing in mind that any successful genetic improvement depends on the inter- and intra-specific variability. Also, in meeting the future demands for various uses such as food, medicine, bio-diesel and other allied products, it will be very important to select the best planting materials for higher productivity, which can only be achieved through characterization.

More scientific studies in the areas of reproductive biology, ecology and genetic characterization are therefore needed to achieve desirable success in its selection, breeding and improvement, conservation and utilization. Some earlier reports on its floral biology, pollen viability, cytology and genetic variability are available (7; 8; 9), however these reports have been limited to populations of *M. oleifera* of Indian, Kenya, Ethiopian and Brazil origin. Previous genetic variability studies (including Nigerian populations) using molecular markers did not combine them with morphological characters (10; 8; 11). Hence, this study focuses on fruit morpho-metric characters and Random Amplified Polymorphic DNA (RAPD) markers analyses of the taxon diversity in Nigeria with a view to identifying candidate accessions for

initiating a breeding and genetic improvement programme.

MATERIALS AND METHODS

Sources of materials

Seeds of *Moringa oleifera* used for this study were collected from the six eco-geographical locations covering nine states of Nigeria and were assigned specific accession numbers at the point of collection. The States are Oyo, Osun, Ondo, Ogun, Edo, Kwara, Plateau, Kano and Yobe State (Fig. 1). The accessions were raised in the nursery for 8 weeks, after which they were transplanted in the *Moringa* experimental field in Covenant University Farm. The specific locations of the collection areas within the respective states as well as the codes for each accession are given in Table 1.

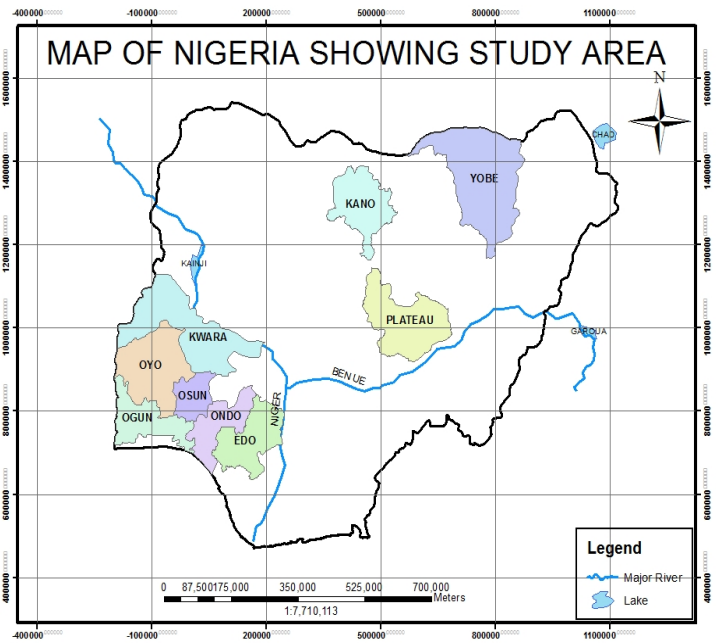

Figure 1: Map showing areas of collection of the accessions used for the study.

Table 1: *Moringa oleifera* accessions used and location / origin

S/N	Accession	Area of Collection	State	Origin	Latitude	Longitude	Height
1.	Oy01	Saki	Oyo	Philippines	8.66834	3.40054	469m
2.	Oy02	Ogbomoso	Oyo	Jos	8.13367	4.24892	234m
3.	Os03	Ife	Osun	India	7.50715	4.52791	255m
4.	Os04	Iwo	Osun	Sokoto	7.63293	4.18416	205m
5.	Og05	Abeokuta	Ogun	Kano	7.16051	3.34756	245m
6.	Os06	Ejigbo	Osun	Unknown	7.91439	4.30853	198m
7.	Kw07	Sobi Barrack	Kwara	Kaduna	8.57457	4.55744	420m
8.	Yo08	Burkati	Yobe	Yobe	12.82285	11.0066	455m
9.	Pl09	Jos	Plateau	Unknown	9.95093	8.88948	98m
10.	Ka10	Kafanchan	Kaduna	Unknown	9.58365	8.51021	321m
11.	On11	Okitipupa	Ondo	Ghana	6.51232	4.78234	199m
12.	Ed12	Ehanlen-Ewu	Edo	Unknown	6.73394	6.17659	326m
13.	Kn13	Army Barracks	Kano	Unknown	12.03227	8.5102	98m

Morphological characterization

Thirteen (13) representative accessions were selected based on their observed agronomic features from the *Moringa* experimental field in Covenant University. The quantitative characters were measured, counted and weighed using metric rulers, weighing balance and venial calipers.

Pod and seed characters

All lots were dried under similar temperature and humidity conditions to reach constant weight. Ten measurements were taken for each of the quantitative characters measured or weighed, with means and standard error calculated. Characters evaluated include; pod length (cm), pod width (cm), number of seeds per pod, number of locules per pod, seed set percentage and 100 seed weight (g). Seed set percentage was estimated as follows:

$$\text{Seed set percentage} = \frac{number\ of\ seeds\ per\ pod}{number\ of\ locules\ per\ pod} \times 100$$

Germination percentage (GP) was also evaluated as the portion of number of germinated seeds to that of plated seeds in germinating

plastics and expressed in percentage.

RAPD-PCR amplification

Leaves for molecular studies were harvested from six week-old plants and DNA extracted using ZR plant/seed DNA extraction kit protocol. The concentration and purity of the extracted DNA was estimated using a Nanodrop spectrophotometer and the DNA was stored at -20 °C until used for RAPD-PCR. 2 g (1%) agarose was employed for gel electrophoresis, and viewed under a UV light Transilluminator to check the quality of the extracted DNA.

Ten (10) decamer primers were screened out of which only 5 produced clear and bright fragments after electrophoresis. The five primers utilized for this study are shown in Table 3.

The RAPD-PCR amplification was carried out using five arbitrary RAPD primers OPR-02, OPC-05, OPC-04, OPI-05 and OPC-04 from Operon Technology (Almeda, CA, USA).

The PCR was performed in 25 µl of a reaction mixture containing DNA (10-200 ng), 200 µM of each deoxynucleoside triphosphates (dNTP) (Promega), 2.5 mM $MgCl_2$, 1X PCR Buffer, 20 pMol primer, 2.5 unit of *Taq DNA* polymerase (Promega) and sterile distilled water. Thermal cycling was conducted in an Eppendorf Nexus Thermal Cycler for an initial denaturation of 94 °C for 5 min followed by 40 amplification cycles of 1 min at 94 °C; 1 min at 28 °C and 1 min at 72 °C. This was followed by a final extension step of 10 min at 72 °C. The amplification products were separated on 1% agarose gel electrophoresis using 0.5 X TBE buffer (44.5 Mm Tris/Borate, 0.5 Mm EDTA, pH 8.0) or 12% polyacrylamide gels and visualized by ethidium bromide staining under UV light. 1 kb DNA ladder procured from Promega was used as DNA molecular weight standard.

Data Analysis

Data obtained from reproductive characters were expressed as means with standard deviation as well as Hierarchical clustering. Data matrix from RAPD profiles were scored as present (1) or absent (0). The data obtained from scoring the RAPD bands were subjected to genetic similarity matrix using Jaccard's similarity coefficient (12). Phylogenetic relatedness of the accessions was determined by cluster analysis using UGPMA (unweighted pair-group method with arithmetic averages) with the NTSYS-pc software version (13). The cluster generated from the mean values of reproductive characters was compared with that of RAPD bands.

RESULTS

Morphological Characterization

Pod and Seed Characters

Table 2 presents means of 12 characters obtained for each of the 13 accessions used for this study. The earliest seedling emergence (7 days) was observed with Accession Os06 while the least days to peduncle initiation and flowering were recorded for Oy01. Accession On11 had the highest days to flowering. Accession Os03 and Os06 had the least and the highest number of pods per peduncle, respectively. Pod length and number of seeds per pod were highest in Os06, while pod length and number of seeds per pod were least in Kn13. Seed set percentages were estimated to be very high in all the accessions with Os06 having the highest (98.74%) and Oy02 having the least (84.76%). Ten pods weight per plant varied from 58.99 g in Oy01 to 98.74 g in Os06. Germination percentage was generally high in all the accessions and ranged from 60% in Og05, Ka10 and Ed12 to 100% in Os06.

Table 2 shows the correlation coefficient of pair of 12 characters used to characterize the 13 representative accessions. The correlation matrix showed that days to peduncle initiation (DPI) was strongly correlated with 50% days to seedling emergence (DSE) and days to 50% flowering (DF). Pod length and pod width were also significantly associated with each other. Similarly, the number of pods per peduncle and number of locule per pod also correlated. Additionally, the number of seeds per pod (NSP) correlated with pod per peduncle (PPP), pod length, pod width and number of locule per pod. Seed set % was not found to be correlated with other agronomic features. However, it is interesting to note that 100 seed weight was significantly correlated with pod length, pod weight, number of seed per locule and number of seed per pod.

Table 2. Quantitative data on Pod and Seed characters of *Moringa oleifera* accessions studied

Acc No	DSE	DPI	DF	PPP	PODL (cm)	PODW (cm)	NLP	NSP	SS	PW (g)	W g)	GP
Oy01	8	185^{L}	206^{L}	3	46.7	2.54^{H}	16.4^{H}	15.2	92.52	58.99^{L}	33.17	80
Oy02	9	197	233	2	41.8	2.36	15.8	13.4	84.76^{L}	61.19	33.77	90
Os03	9	199	221	1^{L}	32.6	1.88^{L}	10.8	10	92.8	59.26	32.46	90
Os04	10	205	235	2	44	2.26	15.6	13.6	86.9	77.81	34.35	70
Og05	11	235	213	3	38.1	2.3	14.2	14	98.46	71.02	31.17	60^{L}
Os06	7^{L}	188	210	4^{H}	50^{H}	2.92	16.2	16^{H}	98.74^{H}	86.92^{H}	35.9^{H}	100^{H}
Kw07	8	205	238	2	34.5	1.98	9.2	8.6	93.56	62.76	29.21	90
Yo08	9	210	240	2	29.6	1.92	8.4^{L}	8	95.78	80.74	29.63	80
Pl09	9	220	245	2	38.6	2.02	13.6	11.8	87.8	80.01	32.52	70
Ka10	12^{H}	244^{H}	259	2	36.7	2	13.6	13.2	96.78	80.61	34.92	60^{L}
On11	12	238	260^{H}	3	37.9	2.17	12.2	11.52	94.53	78.21	32.44	70
Ed12	12	207	239	2	33.4	2.08	13.6	13.2	95.92	84.68	29.97	60^{L}
Kn13	10	198	245	2	28.7^{L}	1.98	7.6	7.2^{L}	96.46	59.08	27.31^{L}	80
G. Mean	9.69	210.1	234.2	2.31	38	2.19	12.9	11.98	93.46	72.41	32.06	77
±SE	0.46	5.23	4.798	0.21	1.8	0.08	0.84	0.77	1.234	2.963	0.691	3.6
Variance	9	355.6	299.3	0.56	41	0.09	9.08	7.709	19.8	114.2	6.216	173
P value	**	**	**	**	**	**	**	**	**	**	**	**

Significant at P <0.05; L= Least value, H=Highest value

DSE: days to seedling emergence; DPI- days to peduncle initiation; DF- days to 50% flowering; PPP- pod per peduncle; PODL- pod length; PODW- pod width; NLP- number of locules per pod; NSP- number of seeds per pod; SS- seed set percentage; PW- 10 pod weight; SW- 100 seed weight; GP- Germination Percentage

Table 3. Correlation coefficient of pair of characters of *Moringa oleifera*

	DSE	*DPI*	*DF*	*PPP*	*PODL*	*PODW*	*NLP*	*NSP*	*SS*	*PW*	*SW*	*GP*
DSE	1											
DPI	0.76**	1										
DF	0.61**	0.61**	1									
PPP	-0.19	-0.03	-0.38	1								
PODL	-0.39	-0.27	-0.51*	0.67**	1							
PODW	-0.42	-0.39	-0.63	0.84**	0.89**	1						
NLP	-0.07	-0.09	-0.43	0.48*	0.89**	0.74**	1					
NSP	-0.02	-0.04	-0.47	0.59**	0.87**	0.79**	0.97**	1				
SS	0.20	0.23	-0.09	0.41	-0.23	0.07	-0.30	-0.07	1			
PW	0.29	0.39	0.27	0.34	0.17	0.17	0.24	0.32	0.27	1		
SW	-0.14	0.03	-0.22	0.35	0.81**	0.60**	0.81**	0.79**	-0.25	0.33	1	
GP	-0.85**	-0.73**	-0.42	0.10	0.23	0.34	-0.10	-0.13	-0.13	-0.37	0.10	1

Significance **= *P<0.05*

RAPD DNA Band

Five Operon primers were used to amplify the DNA fragments of each accession. The sequences of each primer, the total number of bands per primer, polymorphic bands and percentage polymorphisms were recorded in Table 4. The DNA profile of gel electrophoresis is shown in Figure 2. Number of amplified fragments per primer ranged from 28 (OPR-02) to 56 (OPI-04). Primer OP1-05 produced the highest number of polymorphic bands (55) while OPC-10 produced the least (24) (Table 4 and Fig. 2).

Table 4: Primers, sequences, number of bands and percentage polymorphic

S/N	**Primers**	**Sequences**	**Number of bands**	**Polymorphic bands**	**%**
1.	OPI 05	TGTTCCACGG	55	55	100
2.	OPR 02	CACAGCTGCC	28	20	71.4
3.	OPC 05	GATGACCGCC	51	48	94.1
4.	OPC 04	CCGCATCTAC	56	46	82.1
5.	OPC 10	TGTCTGGGTG	34	24	41.7
	Total		224	193	77.86%

Cluster Analysis

A dendrogram cluster was generated from pod and seed characters using SPSS 15.0 for windows, which grouped the 13 accessions into 3 groups, cutting across the geographical locations (Fig. 3). Group I has seven accessions, which include Ka10, On11, Ed12, Yo08, Og05, Os04 and Pl09; Group II is a single cluster and distinctly isolated from others while Group III has five accessions comprising Os03, Kw07, Kn13, Oy01 and Oy02.

The cluster generated from RAPD profiling grouped them into four distinct groups, which are quite different from that of pod and seed characters (Fig. 4). Group I is made up of two members, S_9 and S_{10} (Pl09 and Ka10), Group II comprises of three accessions, S_5 S_6 and S_7 (Og05, Os06 and Kw07); Group III has two accessions, S_4 and S_8 (Os04 and Yo08) while Group IV is made up of six members, which are further divided into two subgroups. S_2 and S_3 (Oy02 and Os03) are grouped in a unique cluster while S_1, S_{11}, S_{12} and S_{13} (Oy01, On11, Ed12 and Kn13) are also clustered together. This Cluster showed high level of genetic variation (77.86%) among the accessions, which ranged from 0.5 to 1.0 with S_2 and S_3 (Oy02 and Os03) accessions found to have highest genetic similarity (1.0), while S_7 (Kw07) possessed least similarity coefficient compare to others (Fig. 4).

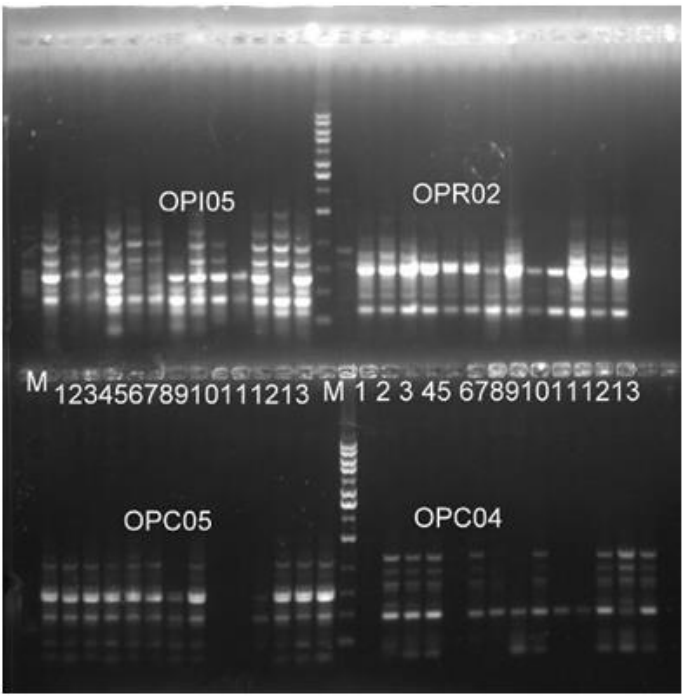

Fig. 2. RAPD DNA banding profile 1% gel electrophoresis of OPI05, OPR02, OPC05 and OPC04 (M = Marker; 1 – 13 = Accession numbers Oy01, Oy02, Os03 to Kn13 in Table 1)

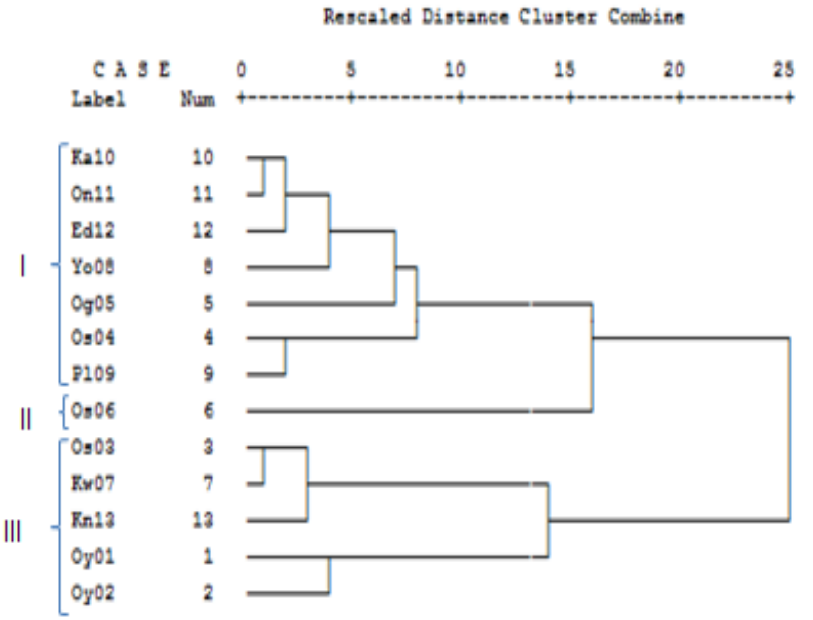

Fig. 3. Hierarchical cluster dendrogram based on Morphological characters of the 13 accessions of *M. oleifera* studied

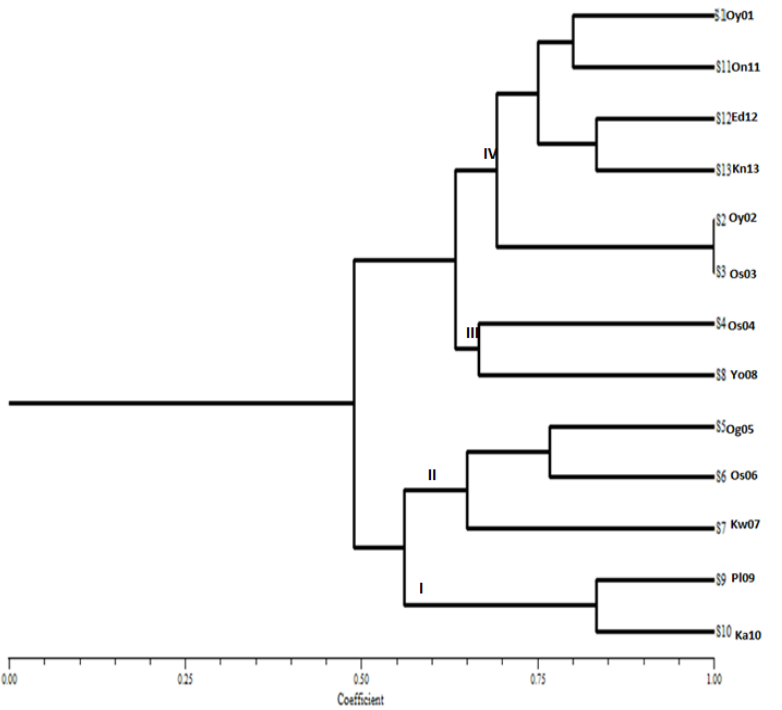

Fig. 4. Hierarchical cluster dendrogram based on RAPD profile of the 13 accessions of *M. oleifera* studied

DISCUSSION

We have combined both morphological characters and RAPDs to study intra-specific variability of 13 representative accessions of *Moringa oleifera* collected from different areas in Nigeria. Significant levels of intra-specific variations were found among the 13 accessions with both means of characterization. The pods from Southern part of Nigeria had higher number of seeds while those from the Northern part were shorter but wider and with bigger seed. Pod length, ten pods weight and 100 seed weight were traits that contributed significantly to the variations observed among the 13 accessions of *M. oleifera* evaluated. Pod length, number of locules per pod and number of seeds per pod were also observed to contribute significantly to the high seed set percentages in all the accessions. On the other hand, days to seedling emergence, days to peduncle initiation and seed weight did not influence the seed set percentage. Thus, it was not surprising to observe positive significant correlation between number of

seeds per pod and number of pods per peduncle (r=0.59), pod length (r=0.87) and number of locules per pod (r=0.97). However, it was unexpected to observe 100 seed weight to have significant correlation with pod length (r=0.81), number of locules per pod (r=0.81) and number of seeds per pod (r=0.79). Therefore, selection based on these characters will positively enhance genetic improvement and enlargement of the reported narrow gene pool of *Moringa oleifera*.

Ten (10) decamer primers were screened out of which only 5 produced clear and bright fragments after electrophoresis. (14) screened 16 primers and only 6 were selected for genetic diversity studies of *Citrus jambhiri*. 15 also screened nine (9) random primers and only four (4) were selected to characterize 70 accessions of Cowpea from Republic of Benin. Though the primers have not been utilized for *Moringa oleifera,* they have been found useful in tree species and medicinal plants (16; 17; 18). This study revealed that the five primers have the ability to detect more polymorphisms compared to others used for *Moringa oleifera* by other Researchers.

The RAPD analysis gave 193 polymorphic bands out of a total 224 bands (78%). Primer OP1-05 produced the highest number of polymorphic bands (100%) while OPC-10 generated the lowest (41.67%) (Table 4). The level of polymorphisms is relatively high and quite comparable to other studies that had earlier utilized RAPD for genetic diversity of *Moringa* (10; 11; 19), though they did not evaluate morphological characters in their studies.

Though, RAPD markers are relatively fast, cheap, easy to carry out in comparison with other methods such as AFLP, ISSR and SNP in detecting polymorphisms, they are nevertheless not easy to reproduce (20; 21). It will continue to be relevant particularly in genetic diversity studies of underutilized medicinal species as long as other DNA-based methods remain unavailable in terms of cost, time and labour (20; 18; 11). Additionally, in combination with fruit morpho-metric characters, it has enabled us to establish a proof-of-concept for the existence of genetic diversity among a sub-set of the accessions of *Moringa oleifera* in Nigeria. As such, it has provided baseline information, which can be leveraged on for future comprehensive analyses of intra-specific genetic diversity among the Nigeria populations.

With respect to cluster analysis, morphological dendrogram grouped the 13 accessions into 3 distinct groups and provides overall pattern of variation and genetic relatedness. It was interesting to note that the clustering did not express the

influence of geographical distribution or environment. This indicates that they are all genetically distinct from one another, although some exhibited overlapping fruit characters. The cluster analysis indicates that cluster groups consist of accessions from different geographical locations suggesting that geographical diversity may not necessarily link to genetic diversity (22; 23). Group I is made up of seven accessions, which includes Ka10, On11, Ed12, Yo08, Og05, Os04 and Pl09; Group II is a single cluster and is distinctly isolated from others while Group III is made up of five members comprising Os03, Kw07, Kn13, Oy01 and Oy02.

However, the result of cluster 2 based on RAPD bands generated a different grouping though with some similarities to that of morphological cluster. The RAPD cluster generated from Jaccard's Genetic Similarity Coefficient revealed 77.86% polymorphism level of genetic variation among the accessions which ranged from 0.5 to 1.0 and produced four distinct groups. Group I comprised two members S_{10} and S_9 (Ka10 and Pl09), Group II comprised three accessions S_5 S_6 and S_7 (Og05, Os06 and Kw07); Group III contained two accessions S_4 and S_8 (Os04 and Yo08) while Group IV contained six accessions, which were further divided into two distinct subgroups comprising S_2 and S_3 (Oy02 and Os03) that stood out as a unique cluster and S_1, S_{11}, S_{12} and S_{13} (Oy01, On11, Ed12 and Kn13) that distinctly clustered together.

Accessions S_2 and S_3 (Oy02 and Os03) were found to have the highest genetic similarity (1.0) and were clustered together in Group III of morphological cluster. Accession Os06 was genetically isolated and distant from other accessions in the morphological cluster. It, however, showed a different grouping in the RAPD cluster, which clustered it in group II comprising Og05, Os06 and Kw07, all of which were widely separated in the morphological cluster. However, accessions Oy02 and Os03 showed close relatedness with highest genetic similarity index, which accounted for their cluster in Group III of morphological cluster. The study revealed that accessions from the same geographic regions were found in different clusters, which is an indication of their adaptability to similarity in nutrient requirements, heterogeneity, population genetic architecture, selection history and approach under domestic cultivation and development traits (24; 23).

It is interesting to note that both phenotypic and molecular characterizations revealed high genetic variability among the selected accessions in Nigeria. These variabilities as expressed in pod and seed characters as well as DNA profile banding could be

utilized in genetic improvement of the species since the higher the genetic variation existing among related species or close variants, the better for adoption for breeding purposes (11). This study is in tandem with other genetic diversity studies of *M. oleifera* such as the work of (8), which utilized AFLP, and the high polymorphisms ranging from 60% to 85% in the reports of (10), (11) and (25). The observation made in this study can, therefore, be utilized in genetic improvement, conservation and transformation of the species. It was observed that *M. oleifera* is a tall plant that needs to be genetically improved to develop short plants with wider canopies that will attract farmers. The increasing awareness on the economic importance of *M. oleifera* calls for more concerted research efforts to breed for shorter and high yielding lines of the species that will be adapted to Nigerian ecological areas. On the whole, Os06 and Kw07 exhibited good and promising agronomic characters, which make them good candidates for breeding and genetic improvement programme.

CONCLUSION

The study revealed that fruit morpho-metric characters and DNA polymorphisms detected by RAPD analyses can be applied as veritable tools in evaluating intra-specific genetic variabilities in crop species especially underutilized crop like *Moringa oleifera.* The genetic variabilities expressed by the 13 representatives of *M. oleifera* could be exploited for breeding and genetic improvement. Further characterizations involving larger number of accessions, using a more robust, highly efficient molecular maker tool, simple sequence repeat (SSR), will certainly enhance the use of its genetic resources.

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COMPETING INTERESTS: The authors declare that there are no competing interests.

AUTHOR'S CONTRIBUTIONS

Jacob O. Popoola carried out the study design, field work, data collection, statistical analysis and interpretation of results, literature search and manuscript preparation. Babafemi O. Oluyisola collected data, provided assistance in literature search while Olawole O. Obembe supervised the work, assisted in data interpretation, reviewed, corrected and approved the final manuscript.

REFERENCES

Fahey, J.W. 2005. *Moringa oleifera*: A review of the medical evidence for its nutritional, therapeutic & prophylactic properties. Part 1. Trees Life J. Vol. 1.

Jyothi, P.V., Atluri, J.B., and Subba Reddi, C. 1990. Pollination ecology of *Moringa oleifera* (Moringaceae). Proc. Ind. Acad. Sci. (Plant Sci.). 100: 33-42.

Parrotta, J.A. 2009. *Moringa oleifera* Lam. Edited by Dr. Bernd Stimm. Wiley-VCH. Verlag GmbH and Co. KGaA, Weinheim.

Ramachandran, C., Peter, K.V. and Gopalakrishnan, P.K. 1980. Drumstick (*Moringa oleifera*): A multipurpose Indian Vegetable. Economy Botany. 34: 276-283.

Adebayo, A.G., Akintoye, H.A., Olufolaji, A.O., Aina, O.O., Olatunji, M.T., and Shokalu, A.O. 2011. Vegetative development and nutrient uptake of *M. oleifera* lam in the nursery. Asian Journal of Plant Sciences. 10: 74-79.

Popoola, J.O. and Obembe, O.O. 2013. Local knowledge, use pattern and geographical distribution of *Moringa oleifera* Lam. (Moringaceae) in Nigeria. Journal of Ethnopharmacology. 150:682-691.

Bhattacharya, A. and Mandal, S. 2004. Pollination, pollen germination and stigma receptivity in *Moringa oleifera* Lam. Grana. 43:48-56.

Muluvi, G.M., Sprent, J.I., Soranzo, N., Provan, J., Odee, D., Folkland, G., McNicol, J.W., and Powell, W. 1990. Amplified fragment length polymorphism (AFLP) analysis of genetic variation in *Moringa oleifera* Lam. Molecular Ecology. 8:463-470.

Silva, N., Mendes-Bonato, A.B., Sales, J.G.C. and Pagliarini, M.S. 2011. Meiotic behaviour and pollen viability in *Moringa oleifera* (Moringaceae) cultivated in Southern Brazil. Genetics and Molecular Research. 10(3): 1728-1732.

Abubakar, B.Y., Wusirika R., MuA'zu S., Khan A.U., and Adamu, A.K. 2011. Detection of Genetic variability using RAPD markers in some accessions of *Moringa oleifera* lam. in Northern Nigeria. International Journal of Botany. 3: 237-242.

Ojuederie, O.B., Igwe, D.O., Okuofu, S.I. and Faloye, B. 2013. Assessment of Genetic diversity in some *Moringa oleifera* Lam. Landraces from Western Nigeria using RAPD Markers. The African Journal of Plant Science Biotechnology. 7(1): 15-20.

Jaccard, P. 1908. Nouvelles recherché sur la distribution florale. Bulletin de la Societe Vaudoise des Sciences Naturelles. 44: 223 – 270.

Rohlf, F.J. 1998. Numerical Taxonomy and Multivariate Analysis System (NTSYS-pc) version: 2.202i. Users Guide. Exeter Software, New York, USA. 31pp.

Akhter, S., Ferdous, M.J., Hossain, M.R., and Rabbanni, G. 2009. Molecular characterization of Jamir (*Citrus jambhiri*) accessions of Bangladesh through PCR based RAPD markers. Journal of Agrofor. Environ. 3(1): 21 – 24

Zannou, A., Kossou, D.K., Ahanchede, A., Zoundjihekpon, J., Agbicodo. E., Struik, P.C. and Sanni, A. 2008. Genetic variability of cultivated cowpea in Benin assessed by random amplified polymorphic DNA. African Journal of Biotechnology. 7(24): 4407 – 4414.

Arif, I.A., Bakir, M.A., Khan. H.A., Al Farhan, A.H., Al Homaidan, A.A., Al Sadoon, M., and Shobrak M. 2010. Application of RAPD for Molecular Characterization of Plant species of medicinal value from arid environment. Genet Mol Res. doi:10.4238/vol9-4gmr848

Nanda, R.M., Nayak, S. and Rout, G.R. 2004. Studies on genetic relatedness of *Acacia* tree species using RAPD markers. Biologia, Bratislava. 59(1):115 – 120.

Li, J., Wan, D.R., and Chen, K.L. 2007. RAPD analysis of Eight (8) Medicinal species of *Selaginella*. Zhong Yao Cai. 30(4):403 – 406.

Rufai, S., Hanafi, M.M., Rafii, M.Y., Ahmed, S., Arolu, I.W. and Ferdous, J. 2013. Genetic Dissection of New Geenotypes of Drumstick Tree (*Moringa oleifera* Lam.) using Random Amplified Polymorphic DNA marker. BioMed Research International. http://dx.doi.org/10.1155/2013/604598.

Ferzi, B. 2001. Random Amplified Polymorphic DNA (RAPD) markers. Turkey Journal of Biology. 25(185 - 196).

Singh, D.R., Srivastara, A.K., Srivastara, A., and Srivastara, R.C. 2011. Genetic diversity among three *Morinda* species using RAPD and ISSR markers. Indian Journal of Biotechnology. 10: (285-293).

Virangama, A.V. and Goyal, S.N. 1994. Genetic divergence in pigeon pea. Gujarath Agriculture University Resource Journal. 19: 65-71.

Olawuyi, O.J. and Fawole, I. 2005. Studies on genetic variability of some quantitative and qualitative characters in Pigeon pea – *Cajanus cajan* (L.)

Millsp. (Fabaceae). *Journal of Life and Physical Sciences* acta SATECH. 2(1): 30 – 36.

Murthy, C.R. and Arunachalam, V. 1986. The nature of divergence in relation to breeding system system in some crops plants. Indian Journal Genetics. 28: 151-155.

Cruz da Silva, A.V., Ferrara dos Santos, A.R., Ledo, A., Feitosa, R.B., Almelda, C.S., Mello da Silva, G., and Range, M.S.A. 2012. *Moringa* genetic diversity from germplasm bank using RAPD marker, Tropical and Subtropical Agroecosystems. 15: 31-39.