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MORPHOLOGICAL AND MOLECULAR CHARACTERISATION OF EGUSI MELON (*CITRULLUS LANATUS* (THUMB) MATSUM AND NAKAI*)* FOUND IN DIFFERENT ECOLOGICAL ZONES IN NIGERIA

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ABSTRACT

Ten accessions of egusi melon were investigated using seed morphology, percentage protein and fat content as well as their molecular characters. The morphological features of the seeds revealed, they range in size from medium to large brown or golden yellow with thin or thick edge. The results obtained for percentage seed fat content showed SMATK-KD (43.90) and MAD- NG (43.69) had the highest mean values while the least mean value for percentage seed fat content was obtained from KTGO-GMB (20.07). Results obtained for percentage seed protein content revealed that the accessions KTGO-GMB (23.50) and OKIJA-AN (17.59) had highest mean values for protein content while GUS-ZAM (13.96) and AGU-ENU (14.35) produced the least mean values. The results obtained for seed weight revealed that AGU-ENU (12.90) and KTGO-GMB (12.45) had the highest mean weight obtained for 100 seeds while OKIJA-AN (10.8) and MAS-NAS (10.90) had the least mean weight values. Total genomic DNA was extracted using Cetyl Trimethyl Ammonium Bromide (CTAB) protocol. Genetic relatedness and variation was determined by Random Amplified Polymorphic DNA (RAPD) PCR analysis. Three primers were used, OPA 13, OPA 16 and OPA 18. The results of dendrogram obtained from the pooled data revealed high level of dissimilarity or diversity of 67.5% among the accessions of the melon. RAPD markers are suitable in assessing genetic diversity and can aid in the identification of desirable qualities for the introduction of new genes into breeding materials.

Keywords: Egusi melon, RAPD PCR, Morphological

INTRODUCTION

Egusi-melon (*Citrullus lanatus* (Thumb.) Matsum & Nakai) is a member of the Cucurbitaceae family **(Schippers, 2000).** It is a variety of melon seed which is popularly called 'Egusi' in West Africa **(Akpambang *et al*., 2008**). Other common names include, 'Ibara', wild watermelon **(Abrefa, 2003**) and egusi–melon **(Ayodele and Salami, 2006; Idehen *et al*., 2008; Ojieh *et al*., 2008**). The crop is widely cultivated in Nigeria **(Ezeike and Offen, 1989; Jolaoso *et al*., 1996; Anuebunwa, 2000**) and other African countries for its seeds **(Ogbonna** and **Obi, 2007).** The seeds provide a well relished condiment for soup. It is rich in vegetable protein, fat and vitamins **(Rehn and Espig, 1991**; **Fayemi, 1999, Adewusi *et al*., 2000).** The seeds contain up to 50% oil (**Olaefa *et al*., 1994; Fakou *et al*., 2004; Achu *et al*., 2005)** and 35% protein **(Fakou *et al*., 2004).**
In spite of the nutritional value of egusi – melon, its benefit to farmers and the land this nutritional age - old resource is languishing **(DSC, 2006).** It is one of the most neglected vegetable crops in tropical agricultural research **(William** and **Isaq, 2002; Makinde *et al*., 2014**). **Idehen *et al*., (2007)** noted that the only breeding work that has been done on egusi-melon that is directed towards producing a high yielding variety has been limited to selection based on the examination of varietal differences. If given attention, the plant is likely not only to improve nutrition but also farmer's income **(DSC, 2006)**
Characterization of closely related plant species is nowadays greatly supported via examination of various decisive genetic criteria such as random amplified polymorphic DNA (RAPD) **(Adeyemo** and **Ojo, 1991**). It is reported as a promising tool for the authentication of medicinal plant species and especially useful in species or varieties that are morphologically and/or phytochemically indistinguishable **(Adeyemo** and **Ojo, 1991**). Molecular marker technologies offer alternatives for the identification of accessions and genetic diversity.
Idahosa *et al*., (2010) noted that the magnitude of genetic variability present in the base population of any crop species is important in crop improvement and as such must be exploited by plant breeders for yield improvement. Other factor important to the plant breeder includes the morphological as well the physico chemical parameters in terms of percentage fat and protein content **(Ndukauba *et al*, 2015).** RAPD has been used to resolve taxonomic relationships providing a quantitative measure of genetic diversity between species and genres **(Silberstein *et al*., 1999).** The variability among European melon breeds using RAPD molecular marker was relatively low compared to lentils **Garco-Rodrigez *et al*., 1996)**.
Furthermore, **Mliki *et al*., (2001)** studied genetic differences among African melon landraces using molecular markers**.** Their results revealed variation among landraces. **Erdine *et al*., (2015)** also studied the genetic diversity of Turkish landraces of melon by RAPD and ISSR primers, their result also showed variation between accessions. RAPD and ISSR markers selected to characterize the traditional landraces of curcumins melon groups in Palestine, because it is informative, easy to use, cheap, and quick and no sequence information required, **(Zhang *et al*., 2012).** The aim of this study is to do a morphological and molecular characterisation of Egusi-melon (*Citrullus Lanatus (*Thumb*)* Matsum and Nakai) found in different ecological zones in Nigeria.

MATERIALS AND METHODS

Evaluation of ten egusi-melon accessions

Ten egusi-melon accessions collected from five ecological zones of Nigeria were used for the experiment. Each accession was given a code name for easy identification. Two (2) of the accessions each were obtained from the Derived savannah ecological zone, Forest ecological zone, Northern Guinea Savanna, Southern Guinea Savanna and Sudan Savannah respectively

Extraction

Dried and de-hulled Egusi Seed samples per accession were ground by using mortar and pestle to fine powder and genomic DNA was extracted by using the Cetyl Trimethyl Ammonium Bromide (CTAB) method **(Doyle and Doyle, 1987).**

Random Amplified Polymorphic DNA (RAPD) assay

Three RAPD primers were used, RAPD-PCRs were performed using random decamer sets according to **Williams *et al*., (1993).**The reactions were performed twice for each primer by thermo cycler in 25µl reaction volumes containing the following: 30 ng genomic DNA, 0.2 mmo1/l primer, 0.5 unit taq DNA polymerase, 0.1 mmol/l of each dNTP, 1.5 mmo1/l $MgC1_2$, and reaction buffer

(1.5mmo1/l $MgC1_2$, 10 mmo1/l Tris-HC1 (pH = 9), 50 mmo1/l KC1, 0.1% volume fraction of Triton X-100, and 0.2 mg/ml bovine serum albumin (BSA)). Amplification included 40 cycles of 1 min at 94ºC, 90 s at 36ºC, 2 min at 72ºC, with 2 min initial denaturation, and 5 min final extension.
RAPD-PCR products was separated by gel electrophoresis (1.5% agarose, 1X Tris Acetate EDTA (TAE) buffer, and 0.5µg/ml ethidium bromide). PCR products were loaded in the gel as follows: 8µl of PCR product and 3µl of 6X sample loading buffer. Gel was run at voltage 120V for 1 hour in 1X TAE buffer, bands were visualized under a UV trans illuminator and photographed.

Table 1 Sequences of the random primers for RAPD-PCR Analysis

Primer	Primer Sequence (3'-5')
OPA 13	CAGCACCCAC
OPA16	AGCCAGCGAA
OPA 18	AGGTGACCGT

Protein and fat content analysis

The nitrogen content was determined by Kjeldahl method described by **Pearson (1976)** and nitrogen content was converted to protein by multiplying by a factor of 6.25. Crude fat content of samples was done by Heating the sample with concentrated HCl .This dissolves the fat and other materials. The fat is then extracted with suitable solvents (diethyl ether).

Data Scoring and Analysis

Amplified DNA fragments that were reproducible were translated into binary character matrices (1 for presence, 0 for absence) **(Podani, 2016)**. The commercial software package SPSS (version 16) was used to develop similarity matrices based on Jaccard coefficient and the data used to construct dendrogram for cluster analysis based on the Jaccard coefficient **(Podani, 2016)**
.

Table 2 Code name, ecological zone, description and source of seeds of ten egusi-melon accessions

Code name of accessions	Ecological zone of accessions	Description of seed of accessions	Source of accessions
	Forest regrowth zone		
OKIJ –AN	Okija in Anambra State in the forest regrowth zone in Nigeria	Large, brown seed with thin edge	Farmer
AMZI –AB	Amizi in Abia State in forest regrowth zone	Large, brown seed with thin edge	Farmer
	Derived savanna zone		
OFATK-KW	Offa in Kwara State in the derived savanna zone	Medium, brown seed with thin edge	Farmer
AGWU-EN	Agwu in Enugu State in the derived savanna zone	Medium, brown seed with thin edge	Farmer
	Northern guinea savanna		
SMATK –KD	Samaru, Zaria in the Northern guinea savanna zone	Large, brown seed with thin edge	Farmer
MAD – NG	Madala in Niger State in the northern guinea savanna zone	Large, brown seed with thick edge	Farmer
	Southern guinea savanna		
SULTK – NG	Suleija, Niger State in the southern guinea savanna zone	Medium, brown seed with thick edge	Farmer
MAS-NAS	Massaka, Nassarawa State in the southern guinea savanna zone	Large, brown seed with thick edge	Farmer
	Sudan savanna		
KTGO – G MB	Kaltungo in Gombe State in the sudan savanna zone	Large, golden-yellow seed with thin edge	Farmer
GUS-ZAM	Gusua in Zamfara State in the sudan savanna zone	Large, brown seed with thick edge	Farmer

RESULTS AND DISCUSSION

Results

Morphology of Egusi Melon Seed Accessions:

Morphological features of the ten egusi melon seeds revealed that they range in size from medium to large brown or golden yellow seeds with thin or thick edge as shown figure 1

Figure 1 Morphology of egusi-melon seeds accessions , 1 = KTGO-GMB, 2 = SMATK-KD, 3= GUS-ZAM, 4 = AMIZI-AB, 5 = OFATK-KW, 6 = SULTK-NG, 7 = OKIJ-AN, 8= MAD-NG, 9 = AGWU-EN, and 10 = MAS-NAS.

Variations in the mean seed weight is revealed in figure 3. AGU-ENU (12.90) and KTGO-GMB (12.45) has the highest mean weight obtained for 100 seeds with a range of 12.40-13.40 and 12.10-12.8 respectively.

Other results obtained for seed weight revealed that OKIJA-AN (10.8) MAS-NAS(10.90) has the least mean weight obtained for 100 seeds with a range of 10.2-11.40 and 10.10-11.70 respectively .
SMATK-KD (43.90) and MAD- NG (43.69) obtained the highest mean value for fat content with range values of 43.84-43.95 and 43.66-43.72 respectively as shown in figure 2. On the other hand, the least mean value for percentage seed fat content was obtained from KTGO-GMB (20.07) with a range of 20.05-20.09.
Variations in mean seed protein content is revealed in figure 4. KTGO- GMB (23.50) and OKIJA-AN (17.59) has highest mean values for protein content. On the other hand, GUS-ZAM (13.96) and AGU-ENU (14.35) produced the least mean values for protein content and were associated with a range of 13.91-14.00 and 14.26-14.44 respectively.

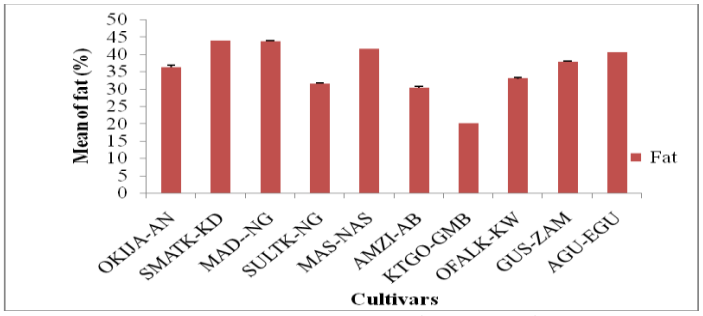

Figure 2 Variations in mean seed percentage fat content of Egusi Melon seed accessions collected from different ecological zones in Nigeria

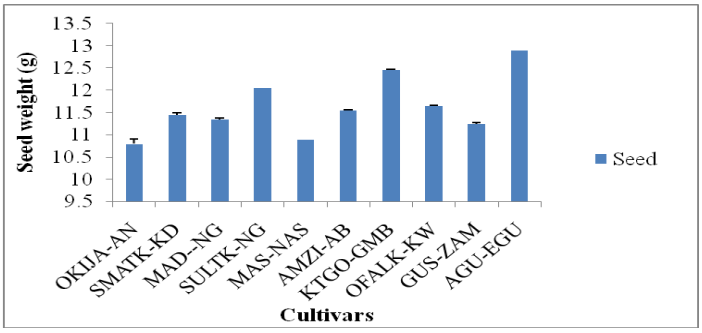

Figure 3. Variations in mean seed weight of Egusi Melon seed accessions collected from different ecological zones in Nigeria

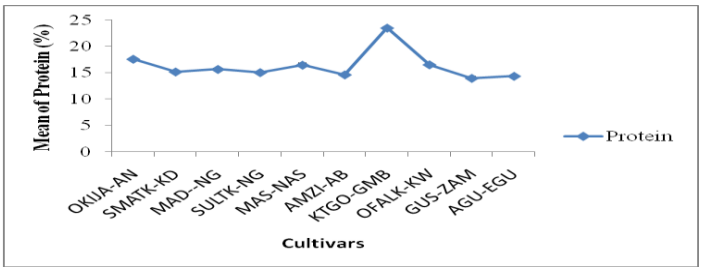

Figure 4 Variations in mean seed percentage protein content of Egusi Melon accessions collected from different ecological zones in Nigeria.

Variations in the mean seed weight, percentage fat and protein content using the Duncan multiple test is as presented in table 3 Means with the same letter and within the same column are not significantly different (P>0.05) using the Duncan's Multiple Test

Table 3 Variations in the mean seed weight, % fat and protein using the Duncan multiple test

accessions	Seed wt (g)	Mean Fat (%)	Mean Protein %
OKIJA-AN	10.8[a]	36.28[f]	17.59[b]
SMATK-KD	11.45[f]	43.9[a]	15.18[cd]
MAD—NG	11.35[g]	43.69[b]	15.66de
SULTK-NG	12.05[c]	31.49[h]	15.05[ef]
MAS-NAS	10.9	41.54[c]	16.46[c]
AMZI-AB	11.55[e]	30.39	14.62[fg]
KTGO-GMB	12.45[b]	20.07	23.5[a]
OFALK-KW	11.65[d]	32.99[g]	16.53[c]
GUS-ZAM	11.25[h]	37.77[e]	13.96
AGU-EGU	12.9[a]	40.57[d]	14.35fg

Molecular Analysis of ten egusi melon seed accessions

Three oligonucleotide primers were used of which the number of bands produced per primer varied from two for OPA 16, nine for OPA 13 and ten for OPA-18 as shown by figures 5, 6 and 7 respectively.

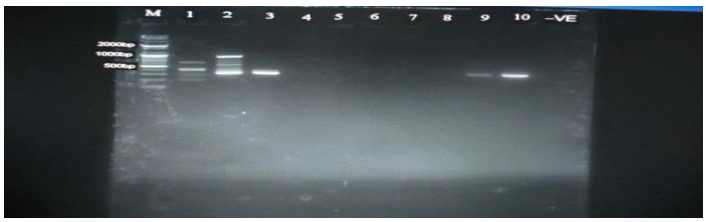

Figure 5 RAPD profiles for ten Egusi melon seed accessions amplified with primer OPA 13. Lane 1 = KTGO-GMB, lane 2 = SMATK-KD, lane 3= GUS-ZAM, lane 4 = AMIZI-AB, lane 5 = OFATK-KW, lane 6 = SULTK-NG, lane 7 = OKIJ-AN, lane 8= MAD-NG, lanes 9 = AGWU-EN, and lane 10 = MAS-NAS.

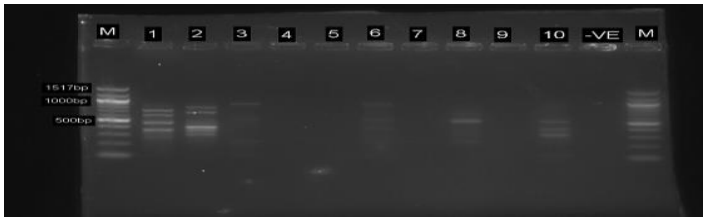

Figure 6 RAPD profiles for ten Egusi melon seed accessions amplified with primer OPA 18. Lane 1 = KTGO-GMB, lane 2 = SMATK-KD, lane 3= GUS-ZAM, lane 4 = AMIZI-AB, lane 5 = OFATK-KW, lane 6 = SULTK-NG, lane 7 = OKIJ-AN, lane 8= MAD-NG, lanes 9 = AGWU-EN, and lane 10 = MAS-NAS

Figure 7 RAPD profiles for ten Egusi melon seed accessions amplified with primer OPA 16. Lane 1 = KTGO -GMB, lane 2 = SMATK-KD, lane 3= GUS-ZAM, lane 4 = AMIZI-AB, lane 5 = OFATK-KW, lane 6 SULTK-NG, lane 7 = OKIJ-AN, lane 8= MAD-NG, lanes 9 = AGWU-EN, and lane 10 = MAS-NAS,

The Primer Specific Scoring of bands in ten (10) accessions of Egusi Melon Seed obtained from five ecological zones in Nigeria is shown in table 4.
The number of fragments, polymorphic fragments and percentage polymorphism revealed by RAPD primers is shown in table 5. While Scorable bands used for the construction of Dendrogram is shown in table 6

Table 4 Primer Specific Scoring of bands in ten (10) accessions of egusi Melon Seed

Primers	Base Pair	1	2	3	4	5	6	7	8	9	10	Band Pattern
OPA 13	1000	0	1	0	0	0	0	0	0	0	0	Unique
	900	0	1	0	0	0	0	0	0	1	0	Polymorphism
	800	0	1	0	0	0	0	0	0	0	0	unique
	700	1	1	0	0	0	0	0	0	0	0	Polymorphism
	600	0	1	0	0	0	0	0	0	0	0	Unique
	500	0	1	0	0	0	0	0	0	0	0	Unique
	400	1	1	1	0	0	0	0	0	1	1	Polymorphism
	300	1	0	0	0	0	0	0	0	0	0	Unique
	200	1	0	0	0	0	0	0	0	0	0	Unique
OPA 16	1000	0	1	0	0	0	0	0	0	0	0	Unique
	800	0	1	0	0	0	0	0	0	0	0	Unique

OPA 18	1000	0	0	1	0	0	1	0	0	0	0	Polymorphism
	900	0	1	0	0	0	0	0	0	0	0	Unique
	800	1	1	0	0	0	0	0	0	0	0	Polymorphism
	700	1	0	0	0	0	1	0	0	0	0	Polymorphism
	500	1	0	0	0	0	1	0	1	0	1	Polymorphism
	400	0	1	0	0	0	0	0	0	0	1	Polymorphism
	350	1	1	0	0	0	0	0	0	0	0	Polymorphism
	300	0	1	0	0	0	0	0	0	0	1	Polymorphism
	200	0	0	1	0	0	0	0	0	0	0	Unique
	100	0	0	1	0	0	0	0	0	0	0	Unique

RAPD profile scores of ten egusi melon seeds. 1 = KTGO -GMB, 2 = SMATK-KD, 3= GUS-ZAM, 4 = AMIZI-AB, 5 = OFATK-KW, 6 SULTK-NG, 7 = OKIJ-AN, 8= MAD-NG, 9 = AGWU-EN, and 10 = MAS-NAS

Table 5 Number of Fragments, Polymorphic Fragments and Percentage Polymorphism revealed by RAPD markers

Primers	Range of Fragment Sizes in BP	Total No of fragment	Polymorphism	% Polymorphism
OPA 13	200-1000bp	9	9	100%
OA16	800-1000bp	2	2	100%
OA18	100-1000bp	10	10	100%
Total		**21**	**21**	**100%**

Table 6 The Scorable band used for the construction of Dendrogram

KTGO-GMB	0	0	0	1	0	0	1	1	1	0	0	0	0	1	1	1	0	1	0	0	0
SMATK-KD	1	1	1	1	1	1	1	0	0	1	1	0	1	1	0	0	1	1	1	0	0
GUS-ZAM	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	1	1
AMIZI-AB	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OFATK-KW	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
SULTK-NG	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	1	0	0	0	0	0
OKIJ-AN	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
MAD-NG	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
AGWU-EN	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
MAS-NAS	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	1	0	1	0	0

The genetic distance (%) in ten egusi melon accessions based on RAPD pooled over three primers is shown in table 7. Accessions AMIZI-AB, OFATK-KW and OKIJ-AN shows high level of similarity because all the accessions mentioned clustered at the same level of coefficient which shows 100% similarity.

Dendrogram depicting ten egusi melon seed accessions based on genetic characteristics is shown in Figure 8

Table 7 Genetic distance (%) in Ten melon accessions based on RAPD pooled over the 3 primers

KTGO-GMB	SMATK-KD	GUS-ZAM	AMIZI-AB	OFATK-KW	SULTK-NG	OKIJ-AN	MAD-NG	AGWU-EN	MAS NAS
1.0000000									
0.3333333	1.0000000								
0.5238095	0.2380952	1.0000000							
0.6190476	0.3333333	0.8095238	1.0000000						
0.6190476	0.3333333	0.8095238	1.0000000	1.0000000					
0.6666667	0.1904762	0.7619048	0.8571429	0.8571429	1.0000000				
0.6190476	0.3333333	0.8095238	1.0000000	1.0000000	0.8571429	1.0000000			
0.6666667	0.2857143	0.7619048	0.9523810	0.9523810	0.9047619	0.9523810	1.0000000		
0.6190476	0.4285714	0.8095238	0.9047619	0.9047619	0.7619048	0.9047619	0.8571429	1.0000000	
0.6190476	0.4285714	0.7142857	0.8095238	0.8095238	0.7619048	0.8095238	0.8571429	0.8095238	1.0000000

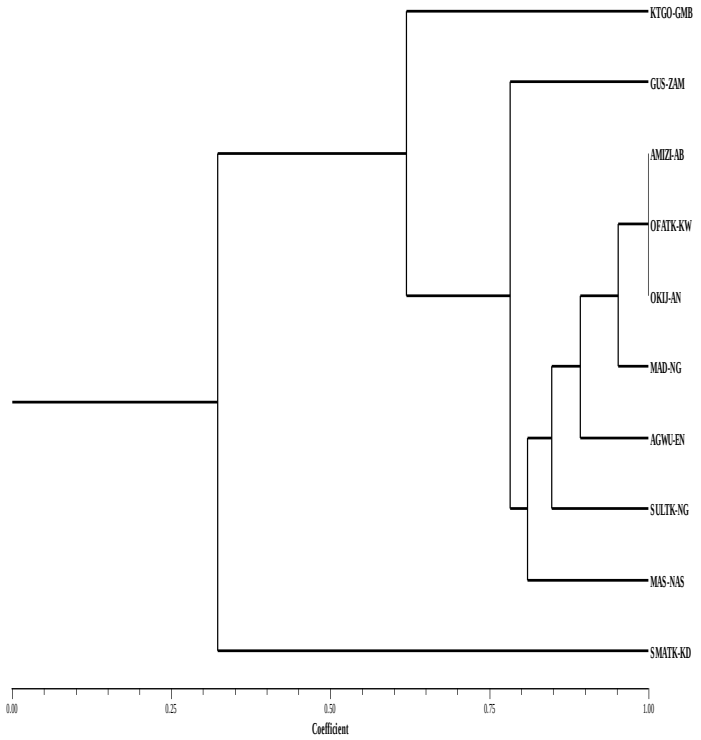

Figure 8 Dendograms depicting Ten egusi Melon seeds accessions based on their genetic characteristics.

DISCUSSION

This study was conducted to determine ten Egusi melon seed accessions morphology including mean seed weight, percentage seed fat and protein content as well as its genetic relatedness and variation. Observed among the accessions studied were the Range of seed sizes and their colors. The morphological features of the seeds revealed they range in size from medium to large brown or golden yellow seeds with thin or thick edge which supports the studies by **Mohr (1986)** that seeds of melon crops, ranged in size from very small seed size to large seed sizes.

The mean percentage protein content ranged from 13.90-14.00 and 23.36-23.63% with KTGO-GMB (23.5) having the highest value, which was significantly different ($p < 0.05$) from other samples. The results obtained for these egusi melon seeds does not agree with the findings of **Ndukauba *et al*., (2015)** and **Fakou *et al*., (2004)** who revealed a range of 28.62-30.67% in twenty eight melon seeds and a range of 24.3 - 41.6% for protein content in five melon seeds respectively. The values obtained from this study were closer to the values (23.7 - 30.68%) and (25.05-25.82) reported by **Olaofe *et al*., (1994)** and **Onyemize *et al*., (2017)** respectively for melon seeds. KTGO-GMB and OKIJA-AB could be used to supplement food products because of their excellent protein content.

Egusi seed accessions SMATK-KD (43.90) and MAD-NG (43.69) had the highest percentage mean fat value with a range of 43.84-43.95 and 43.66-43.72 respectively while KTGO-GMB (20.07) had the least value with a range of 20.05-20.09, which was significantly different ($p < 0.05$) from the other accessions. This result agrees with that of **Abiodun** and **Adeleke, (2010**) that fat content of four species of four melon seeds ranged from 40.26 - 45.21%. It also agrees with the findings of **Ndukauba *et al* (2015),** for 28 melon seeds accessions. These results were also closer to the values (48.89 -49.96) reported by **Onyemize *et al*.,(2017)** for fat content in two varieties of egusi melon All the samples except KTGO-GMB have high fat contents, this classifies Egusi melon seeds accessions as excellent sources of dietary oil **(Abiodun** and **Adeleke, 2010).**

The results obtained for seed weight revealed that AGU-ENU (12.90) and KTGO-GMB (12.45) has the highest mean weight obtained for 100 seeds with a range of 12.40-13.40 and 12.10-12.80, which were significantly different ($p < 0.05$) from other accessions. Other results obtained for seed weight revealed that OKIJA-AN (10.8) and MAS-NAS (10.90) has the least mean weight obtained for 100 seeds with a range of 10.2-11.40 and 10.10-11.70, which were significantly different ($p < 0.05$) from other accessions. These results agree with the findings of **Ndukauba *et al*., (2015),** which revealed a range 8.23-13.03 for 28 melon seed genotypes. These results were also closer to the values (14.27-15.58) obtained by **Idehen, (2017)** for mean weight of100 melon seeds.

RAPD analysis was done individually with 3 random decamer primers (OPA 13; OPA 16 and OPA 18) according to the method of **Williams *et al*., (1993).**

The RAPD profiles of ten accessions of egusi melon were separately compared to find out the differences among them by the occurrence of polymorphic bands. The percentage polymorphism was calculated using Nei .and Li formula **(Nei and Li, (1979).** 21 scorable bands were produced in ten accessions with 3 primers. The number of bands produced per primer varied from two for OPA 13, nine for OPA 16 and ten for OPA-18. The average number of bands per primer was 7 out of 21 and 10 were polymorphic (47.62%). The average number of polymorphic bands was 7 per primer only. These findings concurs with that of **Mihalte *et al*., (2011)** that RAPD fragments vary with primers as well as species. All the identifiable RAPD bands for the three primers were polymorphic. The primers OPA-13, OPA-16 and OPA-18 produced 100 per cent polymorphism. This result is close to the findings of Idehen *et al* (2007) that 50 accessions of egusi Melon show 93.60% polymorphism**.** The results of dendrogram obtained from the pooled data revealed high level of dissimilarity or diversity of 67.5% among the accessions of the melon. The results agree with the findings of **Jacob *et al*., (2016**) that 5 melon seeds obtained (51%) variation and that these variations were attributed to variation within individuals. The dendrogram constructed for pooled data show two major clusters, the KTGO-GMB and SMATK-KD. The accession KTGO-GMB was found in another cluster distinctly separated from other accessions. Within the sub-clustered GUS-ZAM accession revealed some level of dissimilarity when compared with other accessions such as AMIZI-AB, OFATK-KW, OKIJ-AN,, MAD-NG, AGWU-EN, SULTK-NG and MAS-NAS. However it was interesting to observe that within this sub-cluster, AMIZI-AB, OFATK-KW and OKIJ-AN shows high level of similarity because all the accessions mentioned clustered at the same level of coefficient which shows 100% similarity. The rest of the accessions were found in another sub-cluster showing low level of dissimilarity among each other.

The amount of genetic diversity observed in molecular studies depends on the number and the types of primers used and amount of diversity among the accessions used in the investigation (**Mliki *et al*., 2001)**

The genetic diversity observed in this study could be attributed to either the divergent lines examined or the wide geographical spread of the melon accesssions. Furthermore, the selected primers were able to recognize the genetic differences among accessions.

CONCLUSION

It is known that egusi melon is an excellent source of important nutrients such as fat and protein. From the results, SMATK-KD, MAD-NG, and AGU-ENU are excellent sources of fat while KTGO-GMB and OKIJA-NG are excellent sources of protein. These egusi melon seeds are valuable dietary supplements especially for people in the rural areas where there are lack of adequate access to dairy foods and meats.

RAPD markers are suitable in assessing genetic diversity and can aid the identification of desirable qualities for the introduction of new genes into breeding materials. This study serves as a basis to encourage further characterization of Egusi melon seed accessions.

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REFERENCES

Abiodun O.A and Adeleke R.O, (2010). Comparative Studies on Nutritional Composition of Four Melon Seeds Varieties. *Pakistan Journal of Nutrition*, 9: 905-908. http//doi:10.3923/pjn.2010.905.908.

Abrefa, D. J. (2003). In: Tropical Crops, agricultural science and resource management in the tropics and subtropics Field and vegetable crops PTS 130 Wintersemester Bonn. Germany pp.5-8.

Achu, M. B., Fokou, E., Techigang, C., Fotso, M. and Techonanguep, F. M. (2005). Nutritive value of some cuansitacea ion seed from different regions in Cameroon. *Afr.I.Biotech*, 4:1329 – 1334 http//doi.org/10.5897/AJB2005.000-3263

Adewusi, H., Ladipo, D.O., Sarumi, M.B., Adebisi, A.A. and Vodonhe, R. (2000). Egusi production, utilization and diversity in Nigeria.CompIled by Akoroda, M.O. In: Agronomy in Nigeria, Dept. of Agronomy, University of Ibadan, Ibadan, Nigeria. pp 67-75

Adeyemo, M.O, and Ojo, A.A. (1991).Genetic variability and associations of some agronomic traits and seed yield in Sesame (*Sesamum indicum* L.). *Nigeria J. Genet. VIII*: 39 – 44.

Akpambang, V. O. E., Amoo, I. A. and Izuagie, A. A. (2008). Cooperative compositional analysis on two varieties of melon (*Colocynthis citrllus* and *Cucumeropsis edulis*) and a variety of almond (*Prunus amydalus*).*Research Journal of Agriculture and Biological Science* 4(6): 639 – 642.

Anuebunwa, F.O. (2000). A Bio – economic on – farm evaluation of the use of sweet potato for complementary weed control in a yam/ maize/egusi/cassava inter crop in pigeonpea hedgerous in the rainforest belt of Nigeria. *Biological Agriculture and Horticulture* 18:95 – 102. http//doi.org/10.1080/01448765.2000.9754872

Ayodele, O. J. and Salami, A. E. (2006). Population requirements of egusi melon (*Citrullus lanatus*) In a humid environment. Proceedings of 23rd Annual Conference. pp. 286 – 291.

Development, Security and Cooperation (DSC) (2006). Lost crops of Africa. Vol II: Vegetables. 500 Fifth St. N. W. Washington D. C. 2001, pp 154 – 171. http//doi.org/10.17226/2305

Doyle, J. J. Doyle, J. L. (1987). A rapid DNA Isolation procedure of Small quatities of fresh leaf tissue. Phytochem Bull; 19, 11-15.

Erdine A., (2015) Morphological and Molecular Chararterization of Turkish landraces of cucumis melo L *J. Amer. Soc. mol Sci*. 111: 335 – 380.

Ezeike, G.O.I and Otten, I. (1989).Two–Compartment model for drying unshelled egusi (melon) seeds. *Am. Soc. Agric. Eng*. 89:1 – 20.

Fakou, E., Achu, M. B. and Techonarguep, M. F. (2004). Preliminary nutritional evaluation of five species of egusi seeds in Cameroon. *Afr. J. Food Agric*. 4 (1): 1-3 http//doi.org/10.43/ajfand.v4i1.19151

Fayemi, P. O. (1999). Nigerian vegetables. 1. Heinemann Educational Books. Nigeria. 237

Garcia, E., M. Jamilena, J.I. Alvarez, T. Arnedo, J.L. Oliver and R. Lozano, (1998). Genetic relationships among melon breeding lines revealed by RAPD markers and agronomic traits. *Theor. Applied Genet*. 96: 878-885.http/doi.org/10.1007/s001220050815

Garco-Rodrigez, E., I. Alvarez and R. Lozano, (1996). Random Amplified Polymorphic DNAs (RAPDs) as markers to determining genetic relationships among *Cucumis melo* L. genotypes. Proceedings of the 6th Eucarpia Meeting on Cucurbit Genetics and Breeding, May 28-30, 1996, Malaga, Spain, pp: 187-193.

Idahosa, D. O., Alika, J. E. and Omgregre, A. U. (2010). Genetic variability, heritability and expressed genetic advance as indices for yield and yield components selection in cowpea (*Vigna unguiculata* (L) walp. *Academia Arena* 2 (5): 22 – 26.

Idehen E.O. (2017) Evaluation of agronomic characters of Egusi Melon genotypes from various agro ecological zones Of Nigeria, *Pragon Czech republic journal of biotechnology 17-19*

Idehen, E. O., Kehinde, O.B. and Adegbite, A.E. (2008). Somatic chromosome counts and yield performance of some accessions of 'egusi' melon (*Citrullus lanatus*) *African Journal of Biotechnolgy* 5 (22): 2049 – 2052. http//doi:10.4314/njg.v2oi1.42253

Idehen, E.O., Kehinde, O.B. and Ariyo, O. J. (2007). Numerical analysis of variation among Nigerian accessions of 'egusi' melon (*Citrullus lanatus* (Thumb.) Matsum and Nakai. *Journal of American Science* 3 (2): 1 – 6.

Jacob M., Hussein L., Alfred O. (2016) Simple sequence repeat markers reveal diversity within and among landrace collections of citron and used water melon from South Africa *African Journal of Agricultural Research* 2 (8): 114-13.http//doi:10.21273/jashs03870-16

Jolaoso, M.A., Ojeifo, I.M. and Aiyelaagbe, I.O.O. (1996).Productivity of plantain (*Musa* AAB) melon mixtures in Southeastern Nigeria. .*Biol. Agric Hort.* 13: 335 – 340.http//doi:10.1080/01448765.1996.9754791

Makinde E. A., O.T.Ayuola, O. T. and Akande, M. U. (2014) Effects of Organo – mineral Fertilizer Application on the Growth and Yield of 'Egusi' melon. *Australian Journal of Basic and Applied Science* 1(1):15 – 19

Mihalte, L. Sestras, R. Feszt., Sestraz, A. F. (2011) Variability of seed traits in interspecific and inter-generic combination between different genotypes N. *Bot Horti Agrobo* (38(3): 246-252.

Mliki, A., J.E. Staub, S. Zhangyong and A. Ghorbel, (2001). Genetic diversity in melon *Cucumis melo* L. an evaluation of African germplasm. *Genet. Res. Crop.* Evol. 48: 587-597

Mohr, H.C. (1986).Watermelon breeding.In Basset M. J. Breeding Vegetable crops.Westport, C.T. AVI Publ. pg 37 – 66.

Ndukauba J., G. E. Nwofia , P. I. Okocha, E. E. Ene-Obong (2015) Variability in Egusi-Melon Genotypes (Citrullus lanatus (Thumb) Matsum and Nakai) in Derived Savannah Environment in South-Eastern Nigeria. *International Journal of Plant Research 2015, 5(1): 19-26. http//doi:10.5923/j.plant20150501.04*

Nei M., Li W.H (1979) Mathematical model of studying genetic variation in terms of restriction endonucleses Pro Natl Acad Sci. USA 10:5269.

Ogbonna, P.E. and Obi, I. U. (2007). Effects of time of planting and poultry manure application on growth and yield of egusi melon (*Colocynthis citrullus* L.) In a Derived Savannah Agro – Ecology. *Agro – Science* 6(2):33 – 38. http/doi.org/10.4314/as.v6i2.1568

Ojeifo, I.M., and F.N. Emuh (2009). Effect of fruit size, injury Infection Techniques and Post Injury handling on Rolting of Citrullus lanatus(Egusi – melon). Fruit for seed extraction. *Agricultura Tropica et subtropica* 42(4): 187 – 189

Ojieh G, oluba O, Ogunlowo K (2008) Compositional stdies of Citrullus lanatus (Egusi Melon) seed. *Journal of nutrition and wellness volume 6, number 1. http//doi.org/10.5580/e6fs*

Olaefa, O., Adeyemi, F. O. and Adeniran, G.O. (1994). Amino acid and mineral composition and functional properties of some oil seeds. *J. Agri. Food Chem*., 42:878 – 884. http//doi.org/10.1021/jf00040a007

Onyemize U.C, Ozumba I, Chukwu C (2017) comparative evaluation of manually and mechanically shelled Egusi Melon. *American journal of food sciences and nutrition research vol 4, no 2.*

Pearson, D. (1976). *Chemical analysis of foods*, 7th edition, Churchil Hill Living Stone London.

Podani, J. E. (2016) Extending Gower's general coefficient of similarity to ordinal characters and Taxon. Longmans, Green and Co. ltd. 227 pages

Rehm, S., Espig, G. (1991). The cultivated plant of the tropics and subtropics: Cultivation, economic values and utilization. Institute of Agronomy in the tropics, University of Gottingen. 321pages http//doi.org/10.1017/s026646740000612X

Schippers, R. R. (2000). African Indigenous vegetables. An Overview of the cultivated species. Chattam, Uk: Natural Resources Institute/ACP – EU Technical Centre for Agricultural and Rural Cooperation, 224pages

Silberstain A., and Wagar A., (1999) DNA finger printing of essential commercialized medicinal plants from Pakistan, School of Biological Sciences University of the Punjas Lahore Pakistan

Staub, J.E., Y. Danin-Poleg, G. Fazio, T. Horejsi, N. Reis and N. Katzir, (2000). Comparative analysis of cultivated melon groups *Cucumis melo*L. using random amplified polymorphic DNA and simple sequence repeat markers. *Euphytica*, 115: 225-241.

http//doi.org/10.21273.hortsci.35.3.397b

Williams, J.T. and Isaq, N. (2002).Global research on underutilized crops.An assessment of current activities and proposal for enhancement cooperation. Southanption, Uk: ICUC. pp 49-6

William, R. L. and Pollack, E. (1993).Theory of heterosis. .*Journal of Diary Increase* 68: 2411-2417

http//doi.org/10.3168/jds.s0022-0302(85)81117-6

Zhang, Z., Zhongfu, N., Yingyin, Y. and Qixin, S. (2012). Gibbererellins and heterosis of plant height in wheat (*Triticum aestivum* L.) BMC. Genet. 8 (40): 1471-2156. http//doi.org/10.1186/1471-1256-8-40