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Characterization of Nigerian Sesame (*Sesamum Indicum* L.) Using Random Polymorphic DNA (RAPD) Marker

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Abstract

The assessment of genetic diversity among 23 sesame genotypes (*Sesamum indicum* L.) obtained from different locations across 10 states in Nigeria was carried out using Random Amplified Polymorphic DNA (RAPD) technique. The field trial tests were carried out on the 23 sesame accessions for two seasons to have uniform genotypes from each accessions. A standard protocol of CTAB with slight modifications was employed for DNA extracted from the harvested seeds. The extracted DNA samples were observed under UV illumination using agarose gel electrophoresis after staining with ethidium bromide. A total of 7 primers were used for PCR amplification, 5 of which have been previously used to discriminate sesame genotypes from other countries. Only 3 of the 7 primers considered produced strong amplification with the selected 23 sesame samples. A total of 47 amplified products were produced by the 3 primers among the 23 accessions all of which are 100% polymorphic. The estimates of similarity index for the 23 accessions ranged from 0.29 to 0.92. Cluster analysis revealed 2 main clusters with some of the accessions from different geographical origin cluster together in the same group which might indicate the involvement of human factor in the spread of sesame varieties in Nigeria. The relevance of RAPD to this study was evident from the high level of polymorphism reported in this study. There is therefore existence of adequate genetic diversity among the 23 Nigerian sesame accessions for sesame breeders to develop improved varieties.

Keywords: Genetic; Diversity; RAPD; Polymorphism; Primers.

1. Introduction

Sesamum indicum L. (Pedaliaceae), commonly known as sesame is an ancient oilseed crop cultivated in almost all continent [1]. The high drought tolerance of sesame helps it to thrive well in the savanna zone of west and central Africa where other crops fail [2]. Sesame is multipurpose crop use as raw materials in the production of confectionery and bakery products; while the oil is use in the industry to produce soap, perfume, carbon paper, pharmaceuticals and edible vegetable oils [3, 4]. It provides food for human beings, folder for livestock and poultry as well as use as organic fertilizer [5]. Sesame is an under-utilized crop with a great economic potential [6].

However, the potentials available for optimum sesame production in Nigeria, the reported estimated production of 300,000 tons is still low compared to the 4.8 million metric tons produced worldwide in the year 2016 [2]. Most sesame genotypes cultivated by farmers are low yielding landraces which eventually leads to the recent decline in production [7, 8]. There is urgent need for molecular characterization of different sesame landraces for genetic diversity to improve sesame breeding programme [3]. The traditional methods for characterizing and assessing genetic variability based on morphological, physiological and agronomic traits are often not adequate, since these traits are developmentally regulated or influenced by the genotype-environment interaction and agronomic practices [9, 10]. Molecular markers provide excellent opportunity for genetic characterization and allow direct comparison of different genetic material independent of environmental influences [11].

Despite the nutritional, medicinal and cultural importance of sesame, research on genetic diversity using molecular marker has been scarce [3, 12-14]. Knowledge of the genetic diversity of germplasm as well as genetic relationships among accessions will be required to achieve optimum improvement in the crop [3, 15-17].

Against this background a study on genetic diversity of some sesame (*Sesamum indicum* L.) genotypes cultivated in Ghana Using morphological and molecular marker was carried out [3]. In Nigeria, efforts have been made to characterize sesame varieties using morphological markers [18], proximate composition [19], and protein profiling [17]. Breeding efforts in Nigeria have concentrated majorly on characterization and cataloguing of germplasm collections using morpho-agronomic traits [20-22]. It is therefore obvious that there is information dearth on the use of molecular markers such as RAPD to characterize Nigerian sesame. The aim of this study will be to use Random Amplified Polymorphic DNA (RAPD) technique to assess the level of genetic diversity among 23 sesame accessions obtained from four geopolitical zones in Nigeria.

2. Materials and Methods

2.1. Sample Collection

Twenty three accessions of sesame, comprising of eighteen traditional and five improved accessions from 10 states in North-West, North-East, North-Central and South-West geographical zones of Nigeria were collected. The traditional varieties were collected directly from farmers during harvest. The seeds were packed and sealed in paper envelops, each of which was given an accession numbers. To obtain uniform genotypes from each accession, field

trial tests were conducted on each accession for two growing seasons at the research garden of Department of Biological Sciences, Kogi State University, Anyigba. A brief description of the 23 sesame accessions studied is shown in Table 1.

Table-1. Description of the twenty three *Sesamum indicum* accessions considered for the Study

Acce No	Accession Names	Local Names	Sample Sources (States)	Geopolitical Zones	Brief Morphological Description of Samples at the Field Trial Location
1	03M	Esso	Badeggi (Niger)	North Central	Erect stem, green branched, whitish pink flower with light brown seeds.
2	E8	Esso	Badeggi (Niger)	North Central	Erect stem, green branched, whitish pink flower with light brown seeds.
3	01M	Esso	Badeggi (Niger)	North Central	Erect stem, green branched, whitish pink flower with light brown seeds.
4	02M	Esso	Badeggi (Niger)	North Central	Erect stem, green, branched, whitish pink flower with light brown seeds.
5	EXSUDAN	Esso	Badeggi (Niger)	North Central	Erect stem, green, branched, whitish pink flower with light brown seeds.
6	IBA I	Eku	Ibadan (Oyo)	South West	Erect stem, green, branched, whitish pink flower with dark brown seeds.
7	IBA II	Eku	Ibadan (Oyo)	South West	Erect stem, green, branched, whitish pink flower with light brown seeds.
8	OKE	Igorigo	Okene (Kogi)	North Central	Erect stem, green, branched, whitish pink flower with light brown seeds.
9	YOL I	FariRidi	Yola (Adamawa)	North East	Erect stem, green, branched, whitish pink flower with light brown seeds.
10	MAI I	FariRidi	Maiduguri (Borno)	North East	Erect stem, green, branched, whitish pink flower with dark brown seeds.
11	KAN III	FariRidi	Kano (Kano)	North West	Erect stem, green, branched, whitish pink flower with white seeds.
12	KAN II	FariRidi	Kano (Kano)	North West	Erect stem, green, branched, whitish pink flower with light brown seeds.
13	KAN I	FariRidi	Kano (Kano)	North West	Erect stem, green, branched, whitish pink flower with light brown seeds.
14	MAK I	Ishwa	Makurdi (Benue)	North Central	Erect stem, green, branched, whitish pink flower with light brown seeds.
15	OUT	Ishwa	Otukpo (Benue)	North Central	Erect stem, green, branched, whitish pink flower with light brown seeds
16	ZAR I	BekiRidi	Zaria (Kaduna)	North Central	Erect stem, green, branched, whitish pink with dark brown seeds
17	ANY I	Igorigo	Anyigba (Kogi)	North Central	Erect stem, green, branched whitish pink flower with light brown seeds
18	ANY II	Igorigo	Anyigba (Kogi)	North Central	Erect stem, green, branched, whitish pink flower with dark brown seeds
19	OKE I	Igorigo	Okene (Kogi)	North Central	Erect stem, green, branched, whitish pink flower with dark brown seeds
20	ILO I	Eku	Ilorin (Kwara)	North Central	Erect stem, purple, branched, purple flower with black seeds.
21	ILO II	Eku	Ilorin (Kwara)	North Central	Erect stem, purple, profusely branched, pink flower, black seeds
22	OFU	Eku	Offa (Kwara)	North Central	Erect stem, green, branched, pink flower, black seeds
23	JAL I	FariRidi	Jalingo (Taraba)	North East	Erect stem, green, moderately branched, whitish pink flower with light brown seeds

3. Seed Genomic DNA Extraction and Electrophoresis

Genomic DNA was extracted from seeds of the 23 sesame genotypes using CTAB method of Porebski, *et al.* [23] with slight modifications. The modification made was intended to improve the quantity and the quality of the DNA. In this method the fine powdered plant materials were immediately transferred into 13ml Falcon tubes containing 6ml of pre-warmed lysis solution. Tubes containing the samples were then incubated in a water bath at 65^0C with gentle shaking for 30min and left to cool at room temperature for 5mins. Isoamyl alcohol-chloroform mixture (1:24) was added to each tube and the phases were mixed gently for 5min at room temperature to make a homogenous mixture. The cell debris was removed by centrifugation at 5,000rpm for 15mins and the resulting

highly viscous solution (containing DNA) were transferred to new sterile tubes. The steps from the addition of isoamyl alcohol was repeated twice.

The nucleic acids in the highly viscous solution were precipitated by adding equal volume of cooled isopropanol. The contents were mixed gently and centrifuged at 4000rpm for 10min. The formed DNA pellet was washed twice with 70% ethanol, and the ethanol was discarded after spinning with flash centrifugation. The remaining ethanol was removed by leaving the pellet to dry at room temperature. The pellet was dissolved in TE buffer (10mM Tris, 1mM EDTA, pH 8) and stored at -20^{0}C for further use. The extracted DNA samples were observed under UV illumination after staining with ethidium bromide and agarose gel electrophoresis. Impurities suspected to be secondary metabolites and polysaccharides that precipitated along with the DNA were removed using the methods described by Alege [15]. The good quality DNA samples were now observed and snapped under UV illumination after staining with ethidium bromide using Agarose gel electrophoresis.

4. RAPD Analysis and Primer Selection

A total of 7 primers were considered for PCR amplification, 5 of which have previously been used to discriminate sesame genotypes from other countries. Only three of the primers produced strong amplified polymorphic bands with the samples for RAPD-PCR analysis. The PCR reaction was conducted in 50ml reaction volume 2 containing 1xPCR buffer, 1.5mM MgCl, 0.2ml mM of each dNTPs, 1mM of forward and reverse primers, 1 U Taq DNA (promega) polymerase and 10ng genomic DNA. Hot start and touchdown PCR temperature profile was used as follows: an initial denaturizing step at 94^{0}C for 5min, followed by 10 cycles of touchdown annealing temperature 60 to 50^{0}C for 60seconds in which the annealing temperature was decreased by 1^{0}C every cycle. Another 35 cycles started and then a final extension step at 72^{0}C for 7min was performed.

The PCR product were mixed with 2.5µl of 10 X loading dye (0.25% bromophenol blue, 0.25% xylene cyanol and 405 sucrose, w/v) and spun briefly in a microfuge before loading. The PCR products and 1000kp DNA ladder were electrophoresed using 2% agarose gel at 100 volts followed by staining with ethidium bromide and photographed on Polaroid 667 film under ultra-violet light. Each PCR reaction was repeated twice in order to ensure that RAPD banding patterns were consistent and reproducible.

5. Data Analysis

To avoid ambiguity in the data, only clear and consistent bands were considered for data recording. Bands clearly visible in at least one genotype were scored (1) for present, 0 for absent and entered into a data matrix. Similarity Matrix (coefficient) proposed by Nei and Lei [24] was used to calculate the degree of similarity (S_{ab}), between two cultivars a and b according to the formula:

$$S_{ab} = 2N_{ab} / (N_a + N_b)$$

Where $N_{ab=}$ number of bands common to both species a and b; $N_{a=}$ number of bands in species a; $N_{b=}$ number of bands in species b. Hierarchical clustering (dendogram) was constructed using Unweighted Pair Group Method with arithmetic Average (UPGMA) with SPSS v 21 window software.

6. Results

6.1. Molecular Markers for the 23 Sesame Genotypes

Primers OPA01, OPA09, S7 and S14 did not produce amplified bands with any of the 23 sesame accessions studied despite the fact that some of them have been used successfully for such RAPD study elsewhere (Table 2).

Table-2. List of Primers Screened for the Study and their Amplification Status

S/No	Primer Names	Primer Sequences	Previous researcher on sesame	Region where research was carried out.	Amplification status
1	OPA 01	GTGATCGCAG	[17] [14]	Iranian sesame Iranian and some exotic sesame	No amplification
2	OPA 09	AGGTGACCGT	[25] [26]	Sudanese sesame Pakistan sesame	No amplification
3	OPA 10	GGGTAACGCC	[17] [26]	Iranian sesame Pakistan sesame	Amplified
4	OPA 18	ACCCGGTCAC	[26] [14]	Pakistan sesame Iranian and some exotic sesame	Amplified
5	ODP 20	CAGGCCCTTC	[27]	Venezuelan sesame	Amplified
6	S 7	GAAACGGGTG	Nil	Nil	No amplification
7	S 14	TCTGTGCTGG	Nil	Nil	No amplification

Plates 1a-c showed the pattern of amplification among the 23 sesame accessions generated by the 3 primers. Generally the sesame accessions exhibited different banding pattern. Some of the accessions shared some of bands

with others but none of the 23 accessions showed unique bands. Each of the 3 primers considered varied greatly in their ability to determine variability among the accessions.

A total of 47 amplified products were produced by the 3 primers among the 23 accessions all of which are 100% polymorphic (Table 3, Plate 1a, b, c). The number of amplification products ranged from 14 (OPA-18) to 17 (OPA-10).

Plate-1a. Polymorphic RAPD-Agarose Gel image of the 23 sesame genotypes obtained with primer OPA 10

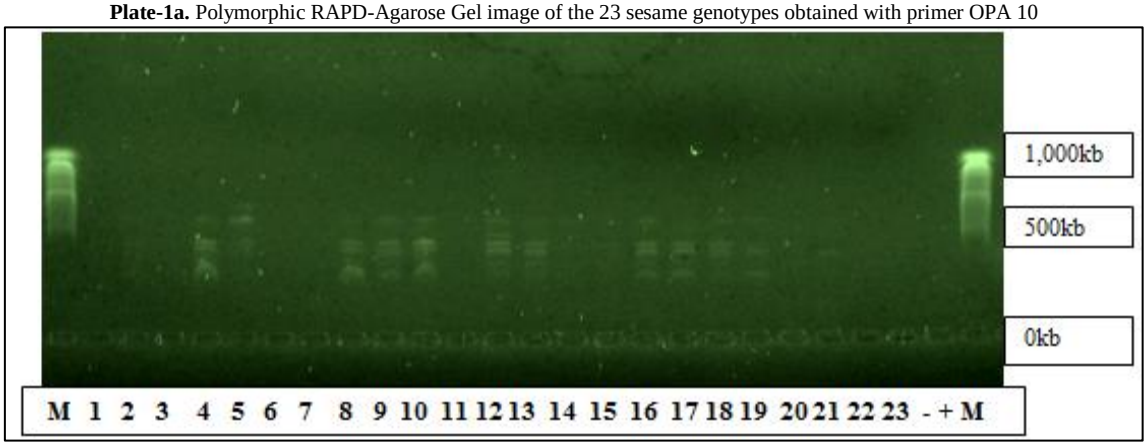


Plate-1b. Polymorphic RAPD-Agarose Gel image of the 23 sesame genotypes obtained with primer OPA 18

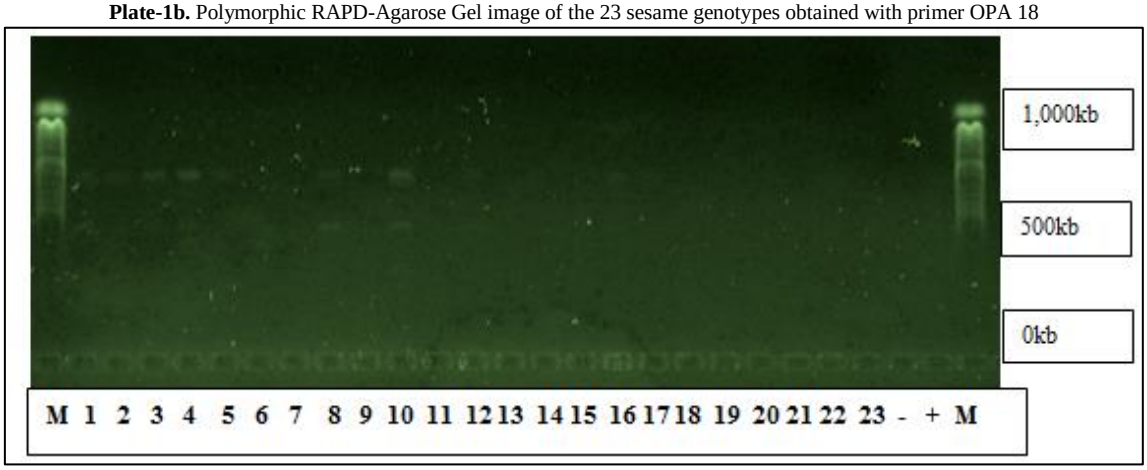
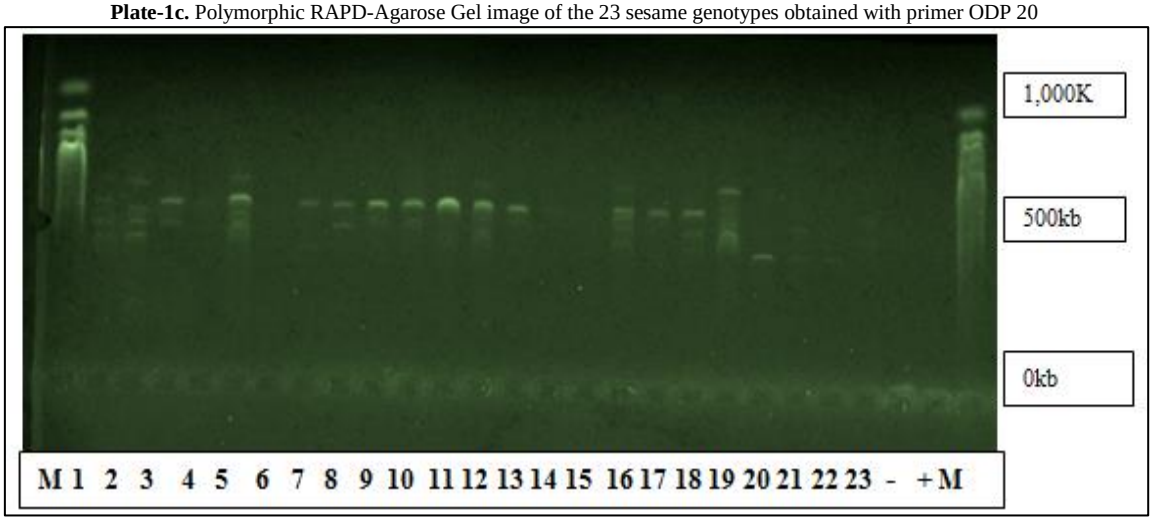


Plate-1c. Polymorphic RAPD-Agarose Gel image of the 23 sesame genotypes obtained with primer ODP 20



Key: 1-23 Accession numbers, M-Markers

The estimates of similarity for the 23 accessions (Table 4) ranged from 0.29 to 0.92. Accessions 10 (MAI) and 18 (ANY II) as well as 22 (OFF) and 23 (JAL) being the closest genotypes with 92% similarity index while accessions 1(03M) and 11 (KAN III) were the least similar accessions with similarity index of 0.29%. No accession was exactly the same with the other accessions.

Cluster analysis for the 23 accessions using RAPD (Figure 1) revealed 2 main clusters with the first group consisting of 11 accessions (i.e 03M, 01M,OFF, JAL, KAN I, ANY I, IBA II, ILO III, MAK, ZAR and OTU). The second cluster consisted of 12 accessions (i.e E8, KAN II, EXSUDAN, YOL, OKE II, 02M, IBA I, OKE I, ILO II, MAI, ANY III and KAN III). Each of the clusters was subdivided into 2 sub-groups. However, some of the accessions from the same geographical origin were grouped together in the same cluster. Some of these accessions

include 03M and 01M, MAK and OTU, E8 and EXSUDAN. Though in most cases, accessions from the same geographical origin fail to cluster together in the same sub-cluster as observed between OKE I and OKE II, KAN I, KAN II and KAN III, ANY I and ANY II in Figure 1.

Table-3. Polymorphism Detected by the use of 3 Random Primers on the 23 Sesame, Genotypes

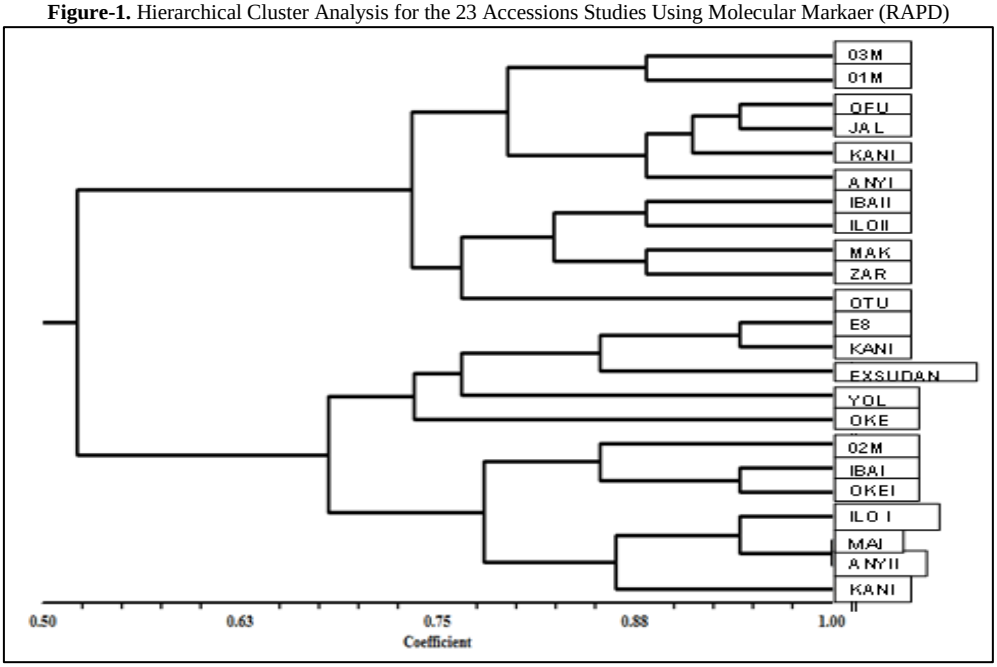
S/NO	Primer Name	Primer Sequence (5'-3')	Total Numbers of Bands	Numbers of Polymorphic Bands	Percentage of Polymorphism (%)
1	OPA 10	GGGTAACGCC	17	17	100
2	OPA 18	ACCCGGTCAC	14	14	100
3	ODP 20	CAGGCCCTTC	16	16	100
Means			47	47	100

Table-4. Similarity Matrix among the 23 sesame genotypes based on molecular markers

AN	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
1	-																						
2	0.47	-																					
3	0.88	0.41	-																				
4	0.52	0.59	0.53	-																			
5	0.52	0.82	0.53	0.53	-																		
6	0.65	0.35	0.77	0.88	0.52	-																	
7	0.82	0.41	0.82	0.47	0.47	0.82	-																
8	0.65	0.71	0.65	0.83	0.59	0.91	0.47	-															
9	0.41	0.79	0.53	0.76	0.75	0.53	0.59	0.75	-														
10	0.59	0.65	0.59	0.72	0.59	0.35	0.41	0.84	0.71	-													
11	**0.29**	0.35	0.88	0.53	0.41	0.88	0.46	0.53	0.65	0.71	-												
12	0.47	0.90	0.47	0.71	0.81	0.35	0.53	0.75	0.71	0.65	0.47	-											
13	0.77	0.65	0.59	0.82	0.59	0.59	0.65	0.82	0.62	0.77	0.71	0.77	-										
14	0.71	0.41	0.82	0.59	0.41	0.82	0.88	0.59	0.71	0.47	0.54	0.53	0.77	-									
15	0.65	0.47	0.77	0.47	0.35	0.88	0.82	0.53	0.65	0.41	0.68	0.47	0.71	0.82	-								
16	0.53	0.70	0.53	0.59	0.68	0.57	0.47	0.77	0.77	0.71	0.41	0.88	0.71	0.91	0.84	-							
17	0.47	0.65	0.59	0.82	0.59	0.59	0.65	0.82	0.57	0.75	0.71	0.77	0.74	0.77	0.71	0.71	-						
18	0.29	0.77	0.59	0.71	0.77	0.47	0.65	0.82	0.72	**0.92**	0.79	0.77	0.88	0.65	0.59	0.88	0.88	-					
19	0.53	0.82	0.53	0.53	0.85	0.53	0.59	0.62	0.74	0.59	0.53	0.79	0.71	0.59	0.52	0.77	0.71	0.76	-				
20	0.53	0.47	0.65	0.53	0.41	0.76	0.71	0.53	0.65	0.89	0.77	0.47	0.71	0.71	0.77	0.41	0.71	0.81	0.65	-			
21	0.59	0.53	0.77	0.47	0.35	0.71	0.90	0.59	0.59	0.53	0.71	0.41	0.65	0.65	0.88	0.41	0.65	0.65	0.71	0.82	-		
22	0.65	0.35	0.77	0.42	0.44	0.88	0.82	0.41	0.53	0.35	0.75	0.35	0.71	0.85	0.71	0.59	0.59	0.47	0.53	0.88	0.82	-	
23	0.77	0.47	0.77	0.42	0.53	0.77	0.81	0.41	0.65	0.35	0.68	0.47	0.78	0.82	0.77	0.53	0.59	0.59	0.53	0.65	0.59	**0.92**	-

AN-Accession Numbers

Figure-1. Hierarchical Cluster Analysis for the 23 Accessions Studies Using Molecular Markaer (RAPD)



7. Discussion

In this study a high level of polymorphism was detected among the 23 sesame genotypes from different geographical regions in Nigeria. The high level of polymorphism (100%) observed in this study is analogous to the

100% polymorphism reported by Salazar, *et al.* [27] on Venezuelan sesame but higher than the 75% and 78% polymorphisms reported by Akbar, *et al.* [26] on Pakistan sesame and Ercan, *et al.* [28] on Pakistani and Turkish sesame respectively. Likewise, the high level of genetic diversity obtained in this study on RAPD is in agreement with other reports by Bhat, *et al.* [29] on Indian sesame, [28] on Turkish sesame, [27] on Venezuelan sesame and Abdellatef, *et al.* [25] on Sudanese sesame. A number of other investigators have reported the use of limited number of RAPD primers for evaluating genetic variations. Schontz and Rether [30], reported very wide genetic diversity among 37 lines of Foxtail Millet (*Setaria italic* L.) using four RAPD primers.

The 3 primers considered in this study (i.e OPA 10, OPA 18 and OPD 13) have been perfectly used by Tabatabaei, *et al.* [14] Akbar, *et al.* [26] Salazar, *et al.* [27] Bhat, *et al.* [29] Suhasini [31] to discriminate Indian, Venezuelan, Dharwadian, Pakistanis and Sudanese sesames respectively. This is an indication that the base sequences of these 3 primers are widespread in sesame. Therefore these primers can be tagged 'universal primer' in sesame. In contrast, primers like Primers OPA01 and OPA09 which have been successfully used to study genetic diversity in sesame elsewhere failed to amplify with the DNA of Nigerian sesame accessions considered in this study. This indicates that the base sequences complementary to these 2 primers in sesame are not universal. Therefore, these 2 primers can easily be used to discriminate sesame accessions from Nigeria.

In this study, there is no basis to discriminate Nigerian sesame base on seed coat colour since sesame seeds with different coat colours clustered together. Sesame with black seed coat show heterogeneous clustering on the dendrogram which further strengthened the fact that selection on the basis of seed coat colour for hybridization may not be effective in sesame improvement program. This finding conforms to the report of Alege [15] on protein profile study of sesame where clustering of accessions were not according to seed coat colour. Dar, *et al.* [16] reported that white and brown sesame population were phylogenetically close as compared to black one. Though their report that at molecular level, the black sesame varieties lacked specific DNA bands that are found only in white and brown varieties did not conform to the findings of this study. The occurrence of three different seed colours in the 23 studied sesame indicates that this trait may be controlled by more than one gene.

The estimates of similarity index range of 0.29 to 0.92 for the 23 sesame accessions observed in this study is in consonance with the 0.39 to 0.92 range reported by [14] on Iranian sesame genotypes but a little at variance with the 0.72 to 0.95 reported by Sarita, *et al.* [6] on Indian sesame. This 0.29 to 0.92 range reported in this study revealed that sufficient genetic diversity exists among Nigerian sesame for their improvement.

Although a considerable level of genetic diversity was observed among the 23 sesame genotypes from various geographical regions in Nigeria, some accessions such as OFU and JAL, KAN I and ANY I, ZAR and OTU, E8 and KAN II, OKE I and YOL, and IBA I and OKE II from different ecological zones clustered together in the same group. These could be as a result of movement of sesame seeds from one geographical location to another by farmers. This finding is in agreement with the report of Adu-Gyamfi, *et al.* [3] and Akbar, *et al.* [26] where clustering of sesame accessions did not conform to their geographical origin. This finding is in agreement with the report of Pham, *et al.* [1] that selecting sesame from different geographical regions will not lead to meaningful improvement.

Some of the accessions from the same geographical zones were found to cluster together which suggested that they have similar genetic combinations. For instance, 03M and 01M, IBA II and ILO II, E8 and EXSUDAN are from close locations the same geographical regions in North Central geopolitical zone of Nigeria. This finding is in conformity with the report of Sarita, *et al.* [6] who reported strong resemblance among sesame genotypes from the same geographical region.

In conclusion, RAPD analysis revealed a considerable level of genetic diversity among sesame accessions collected from different geographical areas in Nigeria. This high level of genetic diversity among the sesame accessions studied revealed that RAPD technique can be used effectively to evaluate genetic variability, select parents for breeding programmes and sesame systematics. Therefore selecting sesame genotypes from the same geographical region in Nigeria can still maximize the genetic diversity needed for a breeding programme.

8. Conclusion

Random Amplified Polymorphic DNA (RAPD) technique have been used successfully to assess the level of genetic diversity among 23 sesame accessions obtained from four geopolitical zones in Nigeria. It can be concluded that even with the use of limited numbers of primers in this study, Random Amplified Polymorphic DNA (RAPD) technique revealed high level of genetic diversity among the 23 sesame (*Sesamum indicum*) which can be used for their discrimination during sesame improvement programme.

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