

RESEARCH ARTICLE

ASSESSMENT OF GENETIC DIVERSITY AMONG NIGERIAN SESAME (*SESAMUM INDICUM* L.) ACCESSIONS USING INTER SIMPLE SEQUENCE REPEATS MARKERSFelicia Adejoke Durodola^{a*}, Amos Oladimeji Adubi^{a,b}, Musibau Adewuyi Azeez^a^aDepartment of Pure and Applied Biology, Faculty of Pure and Applied Sciences, Ladoko Akintola University of Technology, Ogbomoso, Nigeria.^bSchool of Science, Department of Biology, Oyo State College of Education, Lanlate, Nigeria.*Corresponding Author Email: fadurodola79@lautech.edu.ng

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ARTICLE DETAILS

Article History:

Received 23 September 2023
Revised 26 October 2023
Accepted 25 November 2023
Available online 30 November 2023

ABSTRACT

Genetic diversity among 26 sesame accessions in Nigeria was evaluated using inter simple sequence repeat (ISSR) markers. The samples' genomic DNA was extracted from the leaf, and eleven ISSR markers were used in polymerase chain reaction (PCR) to determine allelic polymorphism, marker effectiveness and genetic relationship of the accessions. Fifty-two loci comprising 885 alleles were amplified by the 11 markers with 82.69% polymorphism. The average number of amplified loci per primer was 4.73 with a polymorphism range of 50-100%. The Principal Coordinate Analysis revealed 86.74% variability with the first three coordinates; PCoA1, PCoA2 and PCoA3 responsible for 20.38%, 13.82%, and 13.11% of the total variation, respectively. The dendrogram separated the accessions into two groups with 9 sub-clusters at a genetic distance of 10 (90% similarity). Jaccard similarity index range was between 0.50 and 0.94. In conclusion, the ISSR markers employed for this study were informative and effective in detecting variations among the accessions. Therefore, there is substantial genetic diversity existing in the twenty-six accessions of sesame which could be used in the selection of suitable parental genotypes for initiation of breeding programme for seed yield and oil quality improvement of sesame plant in the country.

KEYWORDS

DNA, Inter Simple Sequence Repeat (ISSR), polymerase chain reaction (PCR), Dendrogram,

1. INTRODUCTION

Africa continent accounted for 51.2 % of global sesame production, followed by Asia (45%) (FAOSTAT, 2021) and Burma (Myanmar) as the largest producers. Nigeria is ranked 6th among the major sesame producers in the world between the year 2009-and 2019 (FAOSTAT, 2021) and is one of the major suppliers of sesame seed to Japan, the world's largest importer. Though the crop is gaining recognition in Nigeria agricultural system as the number of production area has increased in the last ten years, but the seed yield production is not increasing. According to the FAOSTAT (2021), there was a gradual decrease in yield quantities of sesame in Nigeria from 2012 till date.

Farmers in major sesame-growing nations like Nigeria rely extensively on sesame as a good source of edible oil and protein-rich food. Sesame seeds produce high-quality, odourless oil that is edible and serves as an excellent source of protein and fat for both humans and animals (Adebisi *et al.*, 2005). It is used as a cooking medium mainly in the Indian subcontinent and African countries. Sesame is also used in making different cuisines in different parts of the world. In Nigeria, young fresh leaves are used in making soups while dried stems are used locally as a source of fuel, with the ash being used in producing traditional local soap. The seeds which may be roasted or used raw are not only widely used in European and North American bakery Industries in garnishing bread and other flour products but also gaining importance in Nigerians bakery industries.

The oil, apart from being used as a cooking medium, also has industrial applications in the production of cosmetics, soaps, insecticides, paints and vanishes, and pharmaceutical products (Ashri,1998). The meal left after oil extraction is used in making feed for poultry and livestock. Sesame oil

is highly desirable commercially owing to the presence of high unsaturated fatty acid (oleic acid) and low levels of saturated fatty acids. Sesame lignans have antioxidant and health promoting activities (Harikumar *et al.*, 2010). The beneficial effects of sesame oil in reducing plasma cholesterol level and consequently lowering blood pressure in humans has been reported (Sankar *et al.*, 2005; Frank, 2005). Also, the antifungal property of chloro-sesamone from the roots of sesame was reported by (Begum *et al.*, 2000).

The use of molecular markers in the assessment of genetic diversity among crop plants has been shown to be more effective compared to conventional breeding methods (morphological, agronomic, and biochemical markers). This has been attributed to the fact that environmental factors have no effect on the markers, as very low variation could be detected and large number of plants/samples could be screened at a time. Advancement of modern plant breeding has been accelerated by the research on molecular markers, which has also enhanced genetic gain with potential to shorten the breeding cycle in many crop species (Lennarts *et al.*, 2019). The usefulness of a particular molecular marker depends on its capacity to identify nucleotide polymorphisms that allow segregation between different molecular marker alleles (Hassan *et al.*, 2021).

Different authors have reported the use of molecular markers in diversity study of different sesame genotypes and examples include SRAP, SSR, RAPD and ISSR (Zhang *et al.*, 2012; Nweke *et al.*, 2015; Uncu *et al.*, 2015; Adu-Gyamfi *et al.*, 2019; Pham *et al.*, 2011; Olorunshola, 2019; Parsaeian *et al.*, 2011). There were few reported studies on the use of molecular breeding on Nigerian sesame. For instance, in a recently conducted investigation, Olorunshola (2019) employed RAPD markers to assess

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DOI:

[10.26480/asm.02.2023.66.74](https://doi.org/10.26480/asm.02.2023.66.74)

genetic diversity among 23 sesame genotypes. Nweke (2015) studied genetic diversity in sesame with respect to phytochemical composition using simple sequence repeats (SSR) markers and high genetic variation was observed among the 30 accessions used. In another study, SSR markers were employed by (Oduoye *et al.*, 2020), to assess genetic diversity among 22 sesame accessions from germplasm collections. However, there have been no reports on the use of ISSR markers in the determination of genetic diversity among Nigerian sesame. Therefore, the aim of this study was to evaluate the extent of genetic variability among some Nigerian sesame accessions using ISSR markers in order to identify genetically diverse accessions to be used for purposeful planning of breeding programme towards sesame yield and quality improvement in the country.

2. MATERIALS AND METHODS

2.1 Plant Materials

The materials used for the study comprised Twenty-six accessions of sesame (Table 1) which were collected from two research Institutes in Nigeria (NACGRAB: National Centre for Genetic Resources and Biotechnology and NCRI: National Cereal Research Institute, Badegi). The seeds of the twenty-six accessions were planted in planting pots that were replicated three times and arranged using Complete Randomized Design (CRD).

Table 1: List of the Sesame accessions used for ISSR Analysis	
Accession Number	Accessions Name Code
1	04119
2	04128
3	04130
4	04133
5	04136a
6	ULTRA
7	E8
8	NCRI01
9	MJ
10	04157
11	04156
12	04153
13	04146
14	04142
15	04140
16	04E
17	05E
18	04160
19	04164
20	04168
21	03M
22	04M
23	02M
24	04126
25	04174
26	NCRI01M

2.1.1 DNA extraction

Genomic DNA was extracted from the young leaf of each accession of sesame at four weeks old using Plant DNA Extraction Kit (Zymo Research, USA) according to the manufacturer's instructions. Quality of the gDNA was determined by visualization of the band intensity produced from 1% agarose gel electrophoresis under UV light. The extracted DNA from the samples were then stored at -20°C.

2.1.2 Polymerase chain reaction analysis

A few of the accessions were initially selected and tested for amplification with selected primers. The markers were designed and developed from

data mined from the NCBI platform (<http://www.ncbi.nlm.nih.gov/nuccore?term=sesamumn>) and the sequences of the primers were similar to the base sequences of primers reported by Spandana *et al.* (2012). The primers that produced clear-cut DNA fragments were selected for amplification of the DNA of each accession. The amplification reaction was carried out according to other study with adjustments in annealing temperature and reaction volume (Uzun and Cagirgan, 2009; Animasaun *et al.*, 2020).

Amplification of the genomic DNA through Polymerase chain reaction (PCR) by the ISSR primers (Eurofins Germany) was performed on 96-well thermal cycler. The Genomic DNA extracted was used as the template for the PCR reactions of a final 25 µL volume PCR reaction, in a thin-walled 0.2 ml PCR tube containing the following:

MM (Master mix.)	= 12.5 µl
FP (Forward primer)	= 1 µl
RP (Reverse primer)	= 1 µl
ddH ₂ O (Double distilled water)	= 8.5 µl
gDNA	= 2.0 µl
Total Reaction Volume	= 25.0µl

The amplification of ISSR primers was performed in a programmed thermo-cycler (Prime Cycler, Germany) with initial denaturation at 94 °C for 5 min, followed by 35cycles of denaturation at 94 °C for 30 sec. Primer annealing temperature was 55.4 °C for 45 sec and 72 °C for 1 min of elongation. The final extension was at 72 °C for 10 min and the setup was on hold at 4 °C for 10 min (Animasaun *et al.*, 2018). The PCR products (the Amplicons) were resolved on 2% agarose gel in 1X TBE buffer which was stained with ethidium bromide (0.5 µl /µg) and electrophoresed for 90 minutes at 110 Volt. A 1kb DNA ladder (Thermo Fishers, USA) was used as a gene ruler, the DNA fragments on the gel was visualized and detected using a UV-trans-illuminator before the image was captured. In order for the primer to bind to the template, a suitable working temperature was ensured. The primer annealing temperature was specific for each primer based on the primer sequence. These temperatures were calculated according to Borah (2011) using the expression $[4(G + C) + 2(A + T)] - 2^{\circ}C$.

2.1.3 ISSR Data Analysis

Data were generated by scoring for the presence and absence of DNA bands on the gel. A score of "1" was used to indicate the presence of a distinct and reproducible band on the electropherograms as predicted by the DNA ruler while "0" was used to indicate its absence. For diversity analysis, the data were entered into a binary matrix format. The electropherograms were used to determine the allelic frequency of each primer, and the percentage polymorphism was employed to determine the marker efficiency. The Polymorphic information content (PIC) for each primer was calculated according to Deriek *et al.* (2001), where 'PIC' for dominant markers is equal to $2f(1-f)$ with 'f' representing the frequency of allele present, and a matrix was generated. The PAST software version 4.03 was used to carry out a principal coordinate (PCoA) analysis. Cluster analysis was performed by an agglomerative technique using the unweighted pair group method of arithmetic average (UPGMA) algorithm method in NTSYS (Numerical taxonomy and multivariate analysis system) software package version 2.1. A dendrogram was used to visually depict the genetic diversity and relationships within and between the accessions.

3. RESULTS

3.1 ISSR primer effectiveness

The effectiveness of the ISSR primers used as markers in assessing the genetic diversity and the relationship among the sesame accessions is shown in Table 2. Eleven ISSR primers generated 52 fragments /loci in total and 885 alleles were amplified by the markers. The markers had an average of 4 loci per marker, with loci frequency ranging from 3 to 7. ISSR marker Si-4 produced the maximum number of loci (7) and next to this were Si-3 and Si-10 which amplified 6 fragments each, while Si-2 and Si-7 amplified the minimum number of 3 loci. Allelic frequency per marker ranged from 42 to 161 with a mean allelic frequency of 80 per marker (Figure 1). The maximum number of alleles (161) was produced by ISSR Si-4, followed by Si-8 and Si-9 with values of 114 and 104, respectively, while other markers produced alleles less than 100. The minimum allelic frequency (42) was produced by ISSR Si-1. All the primers had polymorphic profiles with varied polymorphic information content. Out of the 52 loci amplified by the primers, forty-three loci were polymorphic, resulting in 82.69% polymorphism while only nine (9) of the loci were

monomorphic in nature. Ten out of the eleven markers employed in this study produced polymorphism greater than 50% (66.7-100) while only one primer ISSR 9 produced 50% polymorphism, although with high allelic frequency. Three of the markers (ISSR Si-2, ISSR Si-3 and ISSR Si-6)

had 100% polymorphism. Polymorphic information content (PIC) of the ISSR markers ranged between 0.054 (Si-9) and 0.350 (Si-3) while the average PIC for all the markers was 0.2. Gel pictures of ISSR Si-3 and Si-2 are shown on plates 1 and 2.

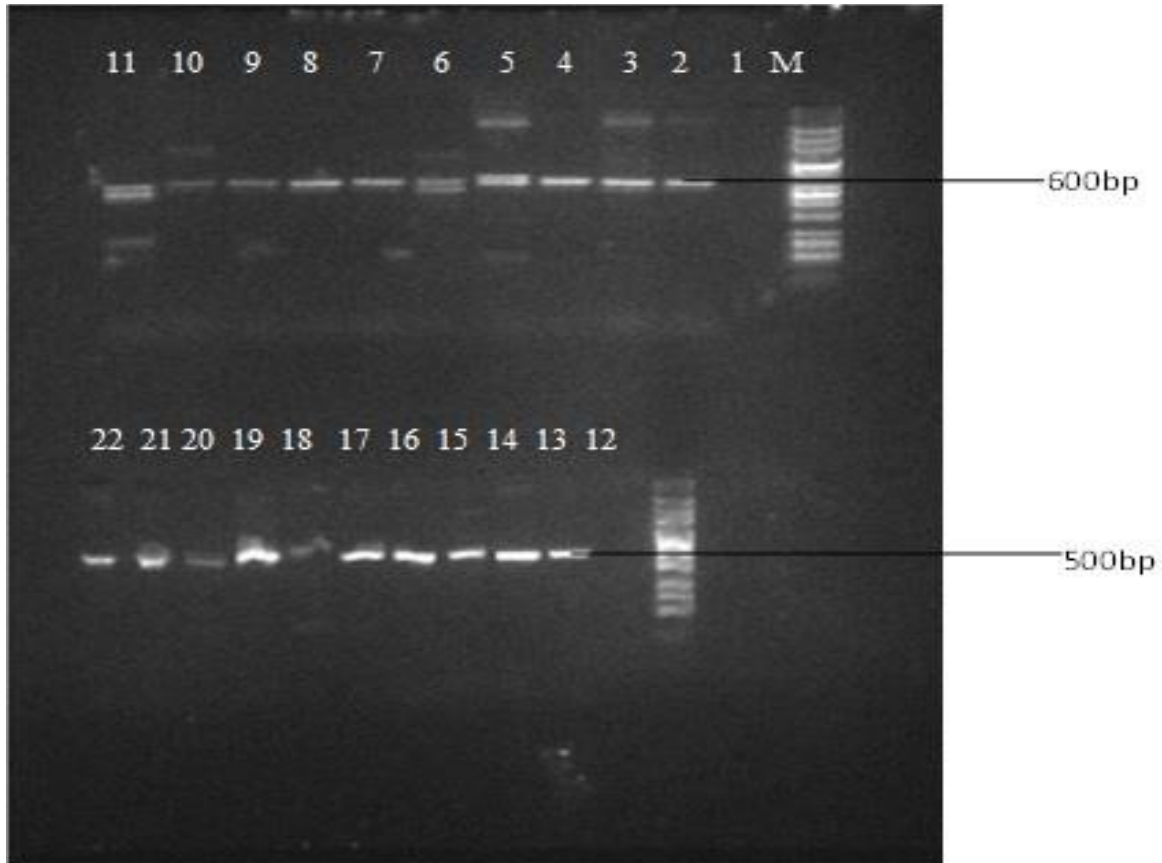


Plate 1: Amplification of ISSR primer Si-3 used for the molecular diversity in 22 sesame accessions as resolved on 1.5% Agarose gel

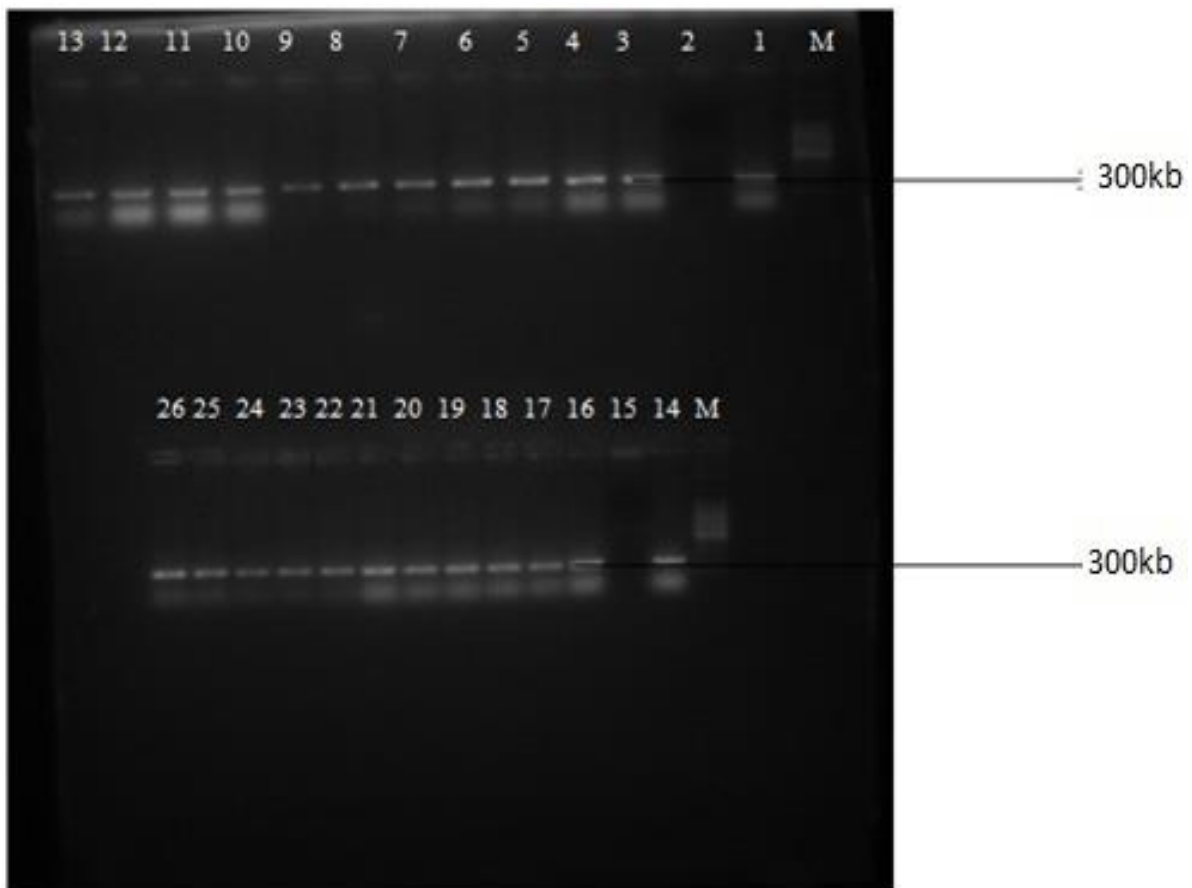


Plate 2: Amplification of ISSR primer (Si-2) used for molecular diversity in 26 sesame accessions as resolved on 1.5% Agarose gel.

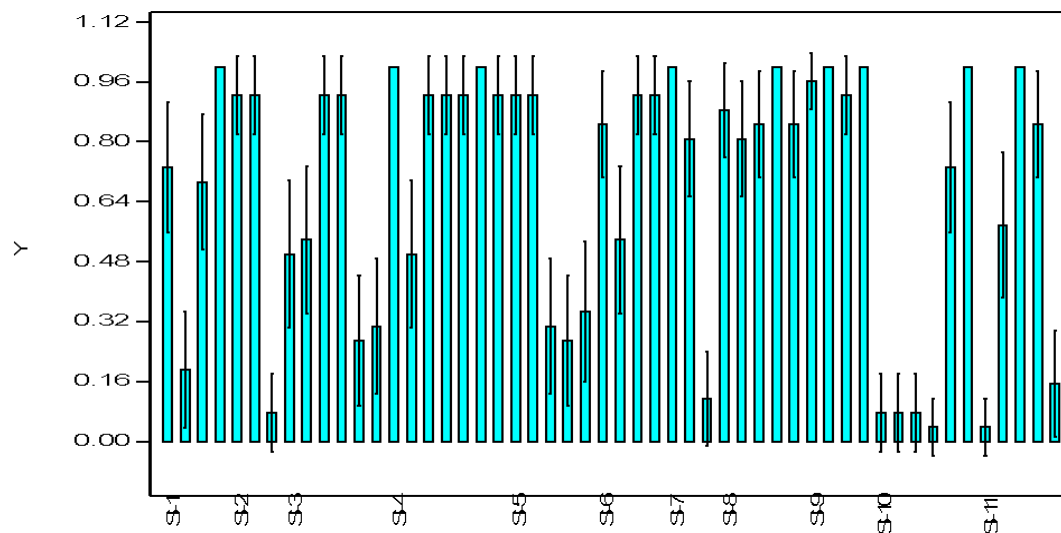


Figure 1: ISSR Primer Allelic Amplification frequency on twenty-six accessions of sesame.

Y - axis: Allelic frequency, X-axis: ISSR primers employed for the stud

Table 2: Loci and allelic polymorphism generated by inter simple sequence repeat markers used for genetic diversity of sesame accessions

S/N	Code	Forward primer (5'-3')	NAL	NML	NPL	%P	AF	PIC
1	Si-1	CTCAACAGCATCTCCACCA	4	1	3	75	42	0.283
2	Si-2	CACGATACACACATTACGAGACA	3	0	3	100	50	0.142
3	Si-3	GTCGCAGACCCCATCACTT	6	0	6	100	91	0.350
4	Si-4	CGTTTCCATCACACACCTTG	7	2	5	71.43	161	0.153
5	Si-5	TGCGTGAGTACTGCTGTAAG	5	0	5	100	72	0.311
6	Si-6	GCATCAATTTGCAGACCAGA	4	0	4	100	84	0.260
7	Si-7	CCGTGACTCGCTCTCTCTCT	3	1	2	66.67	50	0.172
8	Si-8	GGCTTTTCAGGGGAAAAAGA	5	1	4	80	114	0.207
9	Si-9	GCAGAGATTGCCGGTAAGAA	4	2	2	50	101	0.054
10	Si-10	GGCTTTTCAGGGGAAAAAGA	6	1	5	83.33	52	0.149
11	Si-11	AAGGCCAAAACACAATGGAG	5	1	4	80	68	0.217
	Total		52	9	43	82.69	885	
	Average		4.73	0.82	3.91	78.8	80	0.209
	Range		3-7	0-2	2-6	50-100	42-161	0.14-0.35

NAL= Number of Amplified Loci, NML= Number of Monomorphic loci, NPL=Number of Polymorphic loci,

%P= Percentage Polymorphism, AF= Total number of allele / Allelic frequency per primer and

PIC = Polymorphic information content.

3.2 Principal Coordinate Analysis (PCoA)

The amplified bands and allelic fragments showed the placement of the accessions into different quadrants. The first three principal coordinates such as PCo1, PCo2 and PCo3 were responsible for 20.38, 13.82, and 13.11 percent of the variation respectively (Table 3). These axes significantly contributed to the variations observed in the microsatellite regions among

the sesame genotypes (Figure 2). The first six axis with eigenvalue of 5 and above cumulatively were responsible for 70.51 % of the total variation. The plots of Coordinates 1 against Coordinates 2 revealed quadrant I containing the minimum number of accessions (4), while majority of the accessions congregated in quadrant III with a total number of ten (10) accessions. Quadrants II and IV were occupied by five (5) and six (6) accessions, respectively.

Table 3: Principal coordinates of loci and allele fragments of sesame accessions based on ISSR marker

Axis	Percentage variation	
	Individual %	Cumulative %
1	20.377	20.377
2	13.816	34.193
3	13.11	47.303
4	8.3833	55.686
5	8.146	63.832
6	6.6789	70.511
7	5.331	75.842
8	3.5113	79.354
9	2.9964	82.350
10	2.4571	84.807
11	1.9285	86.736

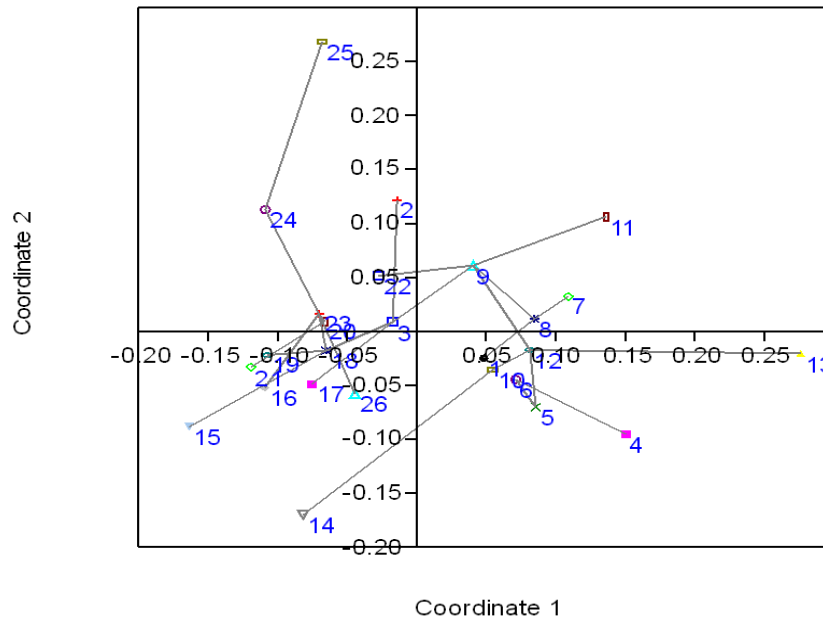


Figure 2: Principal Coordinate Axis 1 and 2 of ISSR Allelic data of twenty-six sesame accessions.
Note: Numbers in the plot correspond to accession's number as shown in Table 1

3.2.1 Clustering analysis

The accessions were delineated into two primary groups (A and B) by the dendrogram generated according to ISSR marker region amplifications. Group A separated into two major clusters (A1 and A2), where A1 was further separated into two clusters (A1,1 and A1,2) and A2 contained two closely related accessions (04146 and NCRI 01M) at a genetic distance of 3 (Figure 3). Cluster A1,1 comprised of two accessions such as 04133 and

ULTRA whereas A1,2 contained three accessions. Group B was separated into two clusters (B1, B2) at a genetic distance of 10. Cluster B1 split into B1,1 and B1,2 at a genetic distance of 7, where B1,1 had three accessions (04119, E8 and NCRI01) and B1,2 with six accessions. B2 was also divided into two subclusters B2,1 and B2,2 at a genetic distance of approximately 6.4 with B2,1 containing two accessions (04168 and 03M) while B2,2 had eight accessions that were further subdivided into two at a genetic distance of approximately 5.2.

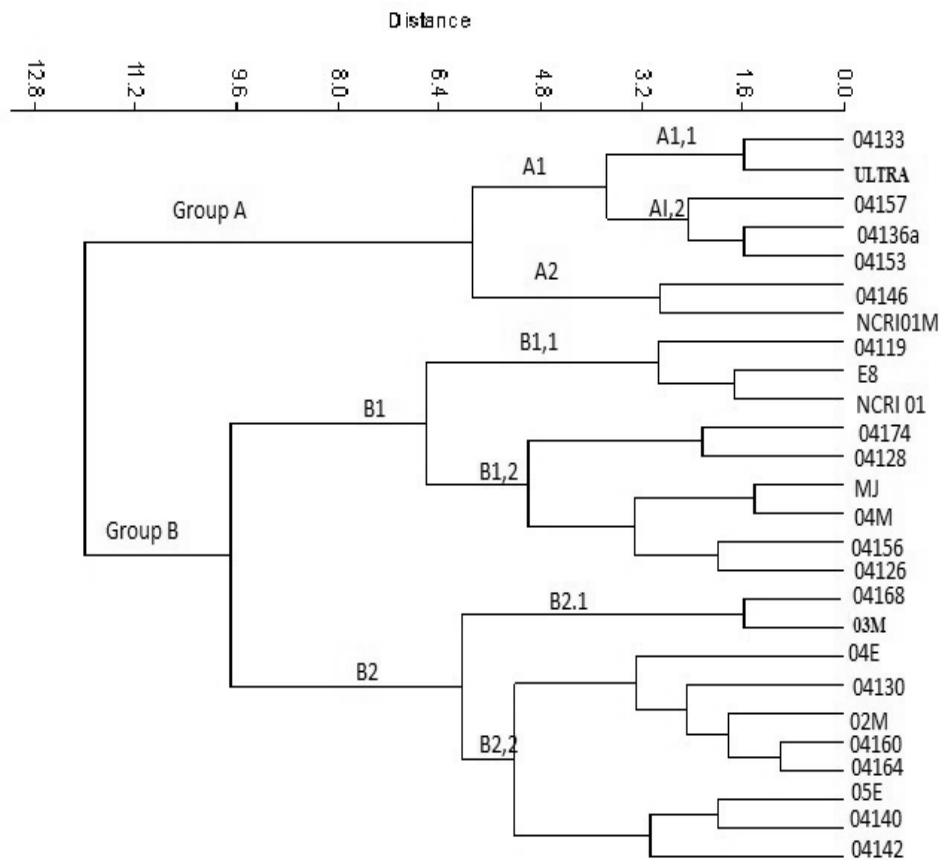


Figure 3: Dendrogram depicting genetic relationships among 26 sesame accessions based on the ISSR data using Ward's method.

3.2.2 Genetic Similarity Matrix among the accessions based on ISSR markers

The similarity index estimates from this study range from 0.50 to 0.94

among the 26 sesame accessions (Table 4). The lowest similarity index was between accessions 04146 and 04140 while the highest value was between 04160 and 04164. Accession 04160 and 04164 had 94% similarities while 04146 was 50% genetically similar to accession 04140.

Table 4: Genetic Similarity matrix among 26 sesame accessions based on ISSR markers

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	
1																										
2	0.67																									
3	0.74	0.74																								
4	0.70	0.65	0.76																							
5	0.66	0.65	0.84	0.84																						
6	0.68	0.63	0.79	0.88	0.86																					
7	0.68	0.63	0.79	0.71	0.74	0.69																				
8	0.78	0.72	0.79	0.79	0.74	0.78	0.83																			
9	0.74	0.69	0.85	0.76	0.80	0.79	0.79	0.84																		
10	0.66	0.61	0.76	0.76	0.84	0.79	0.71	0.75	0.85																	
11	0.66	0.61	0.76	0.76	0.80	0.79	0.75	0.75	0.85	0.80																
12	0.71	0.70	0.81	0.81	0.88	0.83	0.71	0.80	0.85	0.85	0.81															
13	0.58	0.53	0.60	0.71	0.67	0.66	0.66	0.66	0.67	0.67	0.71	0.72														
14	0.65	0.56	0.67	0.67	0.71	0.66	0.58	0.62	0.67	0.76	0.64	0.72	0.56													
15	0.61	0.68	0.76	0.67	0.71	0.74	0.62	0.66	0.71	0.67	0.60	0.68	0.50	0.75												
16	0.72	0.63	0.83	0.66	0.70	0.68	0.64	0.68	0.74	0.70	0.66	0.74	0.55	0.73	0.78											
17	0.72	0.67	0.84	0.79	0.70	0.77	0.68	0.78	0.79	0.74	0.66	0.71	0.58	0.74	0.78	0.76										
18	0.74	0.73	0.89	0.71	0.83	0.78	0.74	0.79	0.80	0.80	0.71	0.85	0.60	0.75	0.79	0.82	0.83									
19	0.68	0.68	0.84	0.67	0.79	0.73	0.69	0.74	0.79	0.79	0.67	0.80	0.55	0.74	0.79	0.77	0.83	0.94								
20	0.66	0.65	0.85	0.68	0.80	0.74	0.79	0.79	0.80	0.72	0.72	0.77	0.57	0.67	0.76	0.78	0.74	0.85	0.79							
21	0.62	0.61	0.73	0.68	0.68	0.75	0.67	0.76	0.73	0.68	0.64	0.69	0.53	0.72	0.76	0.70	0.80	0.76	0.76	0.86						
22	0.69	0.64	0.80	0.71	0.75	0.74	0.74	0.74	0.89	0.76	0.76	0.76	0.63	0.67	0.75	0.73	0.78	0.79	0.79	0.85	0.76					
23	0.72	0.71	0.87	0.70	0.77	0.76	0.73	0.77	0.83	0.83	0.74	0.83	0.62	0.73	0.78	0.85	0.81	0.92	0.86	0.87	0.79	0.82				
24	0.60	0.59	0.79	0.59	0.70	0.69	0.65	0.65	0.79	0.75	0.79	0.71	0.55	0.66	0.70	0.73	0.73	0.79	0.78	0.79	0.76	0.79	0.86			
25	0.57	0.70	0.64	0.56	0.57	0.63	0.58	0.62	0.73	0.64	0.73	0.65	0.52	0.55	0.59	0.62	0.66	0.68	0.67	0.64	0.64	0.72	0.70	0.76		
26	0.57	0.52	0.64	0.60	0.60	0.62	0.62	0.66	0.71	0.71	0.60	0.68	0.63	0.67	0.67	0.65	0.69	0.71	0.70	0.67	0.68	0.71	0.73	0.66	0.59	

Numbers 1-26 represent the accessions as shown in Table 1

4. DISCUSSION

4.1 Molecular Diversity Study Using Inter-Simple Sequence Repeat

The determination of the magnitude and degree of genetic variability/diversity of crop plants has been achieved in the past using morphological and biochemical markers (Azeez et al., 2013; Animasaun et al., 2017; Azeez et al., 2017; Nahak et al. 2018; Bharat et al., 2020). However, utilizing morphological (phenotypic and agronomic) variables to characterize and quantify diversity is frequently constrained and influenced by environmental factors (Chen et al., 2014). Biochemical markers also have limitations despite the fact that the environment has less influence on the markers, very low variation may not be detected if a small genome

section is screened (Rao, 2004). In order to conserve genetic resources and forecast the potential of breeding materials to combine, it is essential to assess the inter- and intra-specific genetic diversity of crop germplasm using molecular approaches.

There was a substantial genetic variation at the DNA level among the sesame accession studied based on the inter-simple sequence repeat analysis result. High polymorphism was detected with the use of eleven ISSR markers /Primers. The high polymorphism percentage (82.69%) is an indication of high genetic variation among the test materials and thus demonstrated the usefulness/efficiency of the markers employed for the genetic diversity study. Microsatellite markers' effectiveness in genetic variability and characterization has been reported in crop plants (Rana et al., 2014; Animasaun et al., 2015; Olatunji and Afolayan 2019). The

polymorphism level obtained in this study was higher than the earlier reports on the use of ISSR markers in diversity study in sesame by Parsean et al. (2011) who recorded 76.47%, Nyongesa (2013) reported 70.6%, Kumar et al., (2014) documented 73.09%, and recently by EL Harfi et al. (2021) who recorded 80.7%. However, the percent polymorphism was lower than the value obtained by Abate et al. (2015) who reported a mean polymorphism of 92.2% among Ethiopian Sesame genotypes. High allelic numbers and high polymorphism are necessary to estimate the genetic diversity of germplasm correctly. Microsatellite profiles in diversity studies are commonly interpreted using allele phenotypes (Ess Link et al., 2004). Moreover, the extent of diversity and the effectiveness of the markers are determined by the magnitude of polymorphism (Pfeiffer et al., 2011).

The following factors such as genotypes employed, the composition of the ISSR Primers and the annealing temperatures of the primers could be the contributing factors to the variations in polymorphism levels in all these studies. An increase in non-specific amplification causing artefact bands has been attributed to low annealing temperatures (Sanchez et al., 1996). The adjustment of the annealing temperature has significant effects on the richness and readability of fingerprints (Bornet and Bran chard, 2001). The eleven primers produced 885 alleles in total and allelic frequency among the primers ranged between 42 and 161 with a mean value of 80. The discriminate ability of primers or Polymorphism Information Content (PIC) value is employed as a comparative indicator of the degree of polymorphism. In essence, PIC is used to assess a genetic marker's usefulness for linkage investigations. The PIC values for monomorphic and polymorphic ISSR markers that are present in 50% of plants and absent in the remaining 50% of plants, respectively, frequently fall within the range of zero to 0.5 in plants (Roldán-Ruiz et al., 2000).

The PIC (Polymorphic information content) which represents a relative indicator of polymorphism, varied from 0.05 (SI- 2) to 0.35 (SI-3) indicating that the primers are informative and appropriate in discriminating the accessions. This is in line with the report in which PIC values ranged between 0.002 and 0.35 among Moroccan sesame populations but at variance to the PIC value ranges of 0.5-0.91, 0.26-0.76, and an average PIC of 0.675 by (El Harfi et al., 2021; Kumar et al., 2014; Abate et al., 2015; Singh et al. 2015). The differences in PIC values from these studies could be attributed to populations of sesame used and the nature and number of markers employed. Though all the primers employed were polymorphic in nature, the PIC value was low for most of them with the exception of primers 1, 3, 5, and 6 having PIC values greater than 0.25. From this study, primers with lower polymorphism were associated with low PIC value except for ISSR Primer 2 which manifested 100% polymorphism but a low PIC value of 0.14.

Allelic frequency is typically used to determine polymorphic information that is connected to the anticipated heterozygosity (Animasaun et al., 2015). Therefore, ISSR Si-3 was the most informative as well as the appropriate primer for the accessions' diversity study and this was followed by ISSR Si-6 and Si-8 for producing high number of polymorphic loci with high number of alleles and therefore able to distinguish between genotypes.

The accessions were placed in spatial coordinates based on Principal Coordinates Analysis (PCoA) of the amplified microsatellite loci and the alleles. The PCoA analysis revealed two important axes for the observed variation. The first two axes (CoA1 and CoA2 explained 20.34% and 13.82% of the variation, respectively, and cumulatively were responsible for 34.2% of the total variation. Accessions on the same quadrant are relatives, while the ones overlapping within a spatial quadrant are more similar and genetically related. Based on this, from quadrant IV, accession 04128 is more similar and genetically related to 04M than accession 02M, 04126, and 04174 despite being within the same quadrant. The Plots showed that all the accessions are relatives and all have a common ancestor (the accessions were of the same species) but there still existed variation among them, hence the grouping into different quadrants.

The Dendrogram constructed showed the accessions being separated into two groups with two major clusters each, at a genetic distance of 12 (88% similarity) and each of these clusters further separated into subclusters of accessions with high genetic affinities. Consequently, the twenty- six accessions were grouped into nine (9) sub-clusters. The degree of relationship (relatedness) is dependent on the magnitude of the genetic distance between the accessions. The lower the genetic distance, the more closely related the accessions. The most closely related accessions are 04160 and 04164 due to the fact that the genetic distance between these two accessions was less than 1.6 (92% similarity).

Genetic similarity index according to Jaccard similarity varied from 0.50 (50%) to 0.94 (94%). This shows that there is low genetic variation present among the tested materials (sesame accessions). Accessions 02M and 04164 had the highest similarity index. Thus, suggesting that these two accessions are closely related while the lowest occurred between accessions 04146 and 04140. This result was at variance with the one reported among sesame genotypes (0.09-0.55) from Iran by (Parsean et al., 2011). But in consonance with the range (0.29-0.92) reported among Nigerian sesame, and 0.72-0.95 among Indian sesame genotypes (Olorunshola, 2019; Sarita et al., 2019). Although, the two later studies employed RAPD markers, the differences observed may also be attributed to the nature of the genetic background of the plant materials used. In related studies, El Harfi et al. (2021) reported a similarity index ranging from 0.509 to 1.00, with an average of 0.870 among Moroccan sesame populations (El Harfi et al., 2021). Abate et al. (2015) reported a dissimilarity range of 0.01-0.88 in sesame populations from Ethiopia (Abate et al., 2015).

The most closely related accessions from this study were 04164 and 04160. The least similar accessions (the most dissimilar) were 04146 and 04140, which is suggestive of the maximum genetic distance between the pair. Not a single pair of the accessions were genetically the same as there was no pair with a similarity index of 1.0. The dissimilar accessions could be exploited to broaden the genetic variability of the sesame accession through hybridization.

CONCLUSION

This study has shown the successful utilization of the ISSR technique in assessing genetic diversity in some accessions of Nigerian sesame. Percent Polymorphism and the markers' PIC revealed that the ISSR markers employed for the study were informative and effective in detecting variation among the accessions. Therefore, based on the result of this study, there is substantial genetic diversity existing in the twenty-six germplasm of sesame which could be used in the selection of suitable parental genotypes for initiation of breeding programme for yield and quality improvement of sesame plant in the country.

ACKNOWLEDGEMENT

The authors acknowledge the managements of the National Centre for Genetic Resources and Biotechnology, Ibadan and the National Cereal Research Institute, Badegi, Niger State for releasing some of the seeds used for this study. We also appreciate the expertise of Dr. D. A. Animasaun of Plant Biology Department, University of Ilorin.

REFERENCES

- Abate, M., Ayana, A., Mekbib, F., Nigussie, M., 2015. Assessment of Genetic Diversity in Ethiopian Sesame (*Sesamum indicum* L.) Germplasm Using ISSR Markers. *British Biotech. J.* 8(4), 1-13. DOI: 10.9734/BBJ/2015/18481
- Adebisi, M.A., Ajala, M.O., Ojo, D. K., Salau, A.W., 2005. Influence of population density and season on seed yield and its component in Nigerian sesame genotypes. *J. Trop. Agric.* 43 (1-2), 13-18. ISSN 0971-636X; eISSN 0973-5399
- Animasaun, D.A., Morakinyo, J.A., Mustapha, O.T., Krishnamurthy, R., 2015. Assessment of genetic diversity in accessions of pearl millet (*Pennisetum glaucum* and napier grass (*Pennisetum purpureum*) using microsatellite (ISSR) markers. *Iranian J. of Genet. And Plt. Breed.* 4(1), 25-35.
- Animasaun, D.A., Shuaibu, B., Oyedeji, S., Azeez, M.A., 2017. Fractionalization of seed storage protein in some selected Pea and Bean varieties use as food in Nigeria by SDS PAGE. *Annals. Food Sci. Tech.* 18 (2), 255-262. Available on-line at www.afst.valahia.ro
- Animasaun, D.A., Awujoola, K.F., Oyedeji, S., Morakinyo, J.A., Krishnamurthy, R., 2018. Diversity Level of Genomic Microsatellite Among Cultivated Genotypes of Digitaria Species in Nigeria. *Afri. Crop Sci. J.* 26 (2), 305-313. 10.4314/acsj.v26i2.11
- Animasaun, D.A., Afeez, A., Adedibu, P.A., Akande, F.P., Oyedeji, S., Olorunmaiye, K.S., 2020. Morphometric variation, genetic diversity and allelic polymorphism of an underutilized species *Thaumatococcus daniellii* population in Southwestern Nigeria. *J. Plt. Biotechnol.* 47, 298-308.
- Adu-Gyamfi, R., Prempeh, R., Zakaria, I., 2019. Diversity Assessment of

- some Sesame (*Sesamum indicum* L.) Genotypes Cultivated in Northern Ghana Using Morphological and Simple Sequence Repeat (SSR) Markers. *Adv. Agric.* <https://doi.org/10.1155/2019/6067891>
- Ashri, A., 1998. Sesame breeding. In: *Plant Breeding Reviews*, 16, 179-228.
- Azeez, M.A., Aremu, C.O., Olaniyan, O.O., 2013. Assessment of genetic variation in accessions of sesame (*Sesamum indicum* L.) and its crosses by seed protein electrophoresis. *J. Agroalimentary Process and Tech.* 19, 383-391.
- Azeez, M. A., Olowookere, M.B., Animasaun, D.A., Bello, B.O., 2017. Utility of some floral characters in the assessment of genetic diversity in sesame (*Sesamum indicum* L.). *Acta Agric. Slov.* 109 (1), 61-70. <https://doi.org/10.14720/aas.2017.109.1.06>
- Begum, S., Furumoto, T., Fukui, H., 2000. A new chlorinated red naphthoquinone from roots of *Sesamum indicum*. *Biosci. Biotech. Biochem.* 64, 873-874. <https://doi.org/10.1271/bbb.64.873>
- Bharat, P., Dileep, T., Siddharth, P., Manohar, P., Yogendra, S., Gita, K., 2020. Genetic variability, heritability and genetic advance studies in sesame (*Sesamum indicum* L.). *J. Pharm. and Phytochem.* 9 (3), 1679-1683.
- Borah, P., 2011. Primer designing for PCR. *Sci. Vis.* 11 (3), 134 -136.
- Bornet, B., Branchard, M., 2001. Non-anchored Inter Simple Sequence Repeat (ISSR) markers: reproducible and specific tools for genome fingerprinting. *Plt. Mol. Biol. Rep.* 19, 209-215.
- Chen, S., Pang, X., Song, J., Shi, L., Yao, H., Han, J., Leon, C., 2014. A renaissance in herbal medicine identification: from morphology to DNA. *Biotechnol. Adv.* 32 (7), 1237-1244. <https://doi.org/10.1016/j.biotechadv.2014.07.004>
- Deriek, J., Calsya, E., Everaert, I., Van Bockstaele, E., DeLoose M., 2001. AFLP-based alternatives for the assessment of distinctness, uniformity, and stability of Sugar beet varieties. *Theor. Appl. Genet.* 103, 1254-1265. <https://doi.org/10.1007/s001220100710>
- El Harfi, M., Charafi, J., Houmanat, K., Hanine, H., Nabloussi, A., 2021. Assessment of genetic diversity in Moroccan sesame (*Sesamum indicum*) using ISSR molecular markers. *Oilseeds & Fat Crops and Lipids* 28 (3), 1-8. <https://doi.org/10.1051/ocl/2020072>
- Esslink, G.D., Nybom, H., Vosman, B., 2004. Assignment of allelic configuration in polyploids using the MAC-PR (Microsatellite DNA allele counting-peak ratios) method. *Theor. Appl. Genet.* 109, 402-408. <https://doi.org/10.1007/s00122-004-1645-5>
- FAOSTAT, 2021. Food and Agriculture Organization of the United Nations. Statistical Database. Accessed on August 12, 2021. Retrieved from <http://www.fao.org/faostat/en/>
- Frank, J., 2005. Beyond vitamin E supplementation: An alternative strategy to improve vitamin E status. *J. Plt. Physiol.* 162, 834 - 843. <https://doi.org/10.1016/j.jplph.2005.04.017>
- Harikumar, K.B., Sung, B., Tharakan, S. T., Pandey, M.K., Joy, B., Guha, S., Krishnan, S., Aggarwal, B.B., 2010. Sesamin manifest chemopreventive effects through suppression of NF-KB regulated cell survival, proliferation, invasion and angiogenic gene products. *Molecular Cancer Res.* 8, 751-761.
- Hasan, N., Choudhary, S., Naaz, N., Sharma, N., Laskar, R.A., 2021. Recent advancements in molecular marker-assisted selection and applications in plant breeding programmes. *J. Genet. Eng. and Biotech.* 19(1), 1-26. <https://doi.org/10.1186/s43141-021-00231-1>
- Kumar, H., Kaur, G., Banga, S., 2014. Molecular Characterization and Assessment of Genetic Diversity in Sesame (*Sesamum indicum* L.) Germplasm Collection using ISSR Markers. *J. Crop Improv.* 26, 540-557. <https://doi.org/10.1080/15427528.2012.660563>
- Lenaerts, B., Collard, B.C.Y., Demont, M., 2019. Improving global food security through accelerated plant breeding. *Plant Sci.* 287, 110207. <https://doi.org/10.1016/j.plantsci.2019.110207>
- Nahak, S.C., Nandi, A., Sahu, G.S., Tripathy, P., Dash, S.K., Patnaik, A., Pradhan, S.R., 2018. Studies on variability, heritability and genetic advance for yield and yield contributing characters in chilli (*Capsicum annum* L.). *J. Pharma. Phytochem.* 7(1), 2506-2510. E-ISSN: 2278-4136
- Nweke, F.N., 2015. Genetic diversity of Nigerian Sesame cultivars (*Sesamum indicum* L.) based on simple sequence repeat (SSR) markers and its relationship with phytochemicals. *Int. J. Curr. Microbiol. App. Sci.* 4(1), 898-908. <http://www.ijcmas.com/>
- Nyongesa, B.O., Were, B.A., Gudu, S., Dangasuk, O.G., Onkware, A.O., 2013. Genetic Diversity in Cultivated Sesame (*Sesamum indicum* L.) and Related Wild Species in East Africa. *J. Crop. Sci. Biotech.* 16 (1), 9-15.
- Oduoye, O.T, Oluwasanya, O.A., Arikawe, O.O., Sunday, A., Ayekun, O.A., Oyenpemi, O.S., 2020. Genetic Variation via SSR Polymorphic Information Content and Ecological Distribution of Nigerian Sesame. *Afric. J. of Biotech.* 19(4), 165-170. <https://doi.org/10.5897/AJB2019.16980>
- Olatunji, T.L., Afolayan, A.J., 2019. Evaluation of genetic relationship among varieties of *Capsicum annum* L and *Capsicum frutescens* L. in West Africa using ISSR markers. *Heliyon.* 5, e01700. <https://doi.org/10.1016/j.heliyon.2019.e01700>
- Olorunshola, A.G., 2019. Characterization of Nigerian Sesame (*Sesamum Indicum* L.) Using Random Polymorphic DNA (RAPD) Marker. *J. Biotech. Res.* 5 (9), 77-84. <https://doi.org/10.32861/jbr.59.77.84>
- Parsaeian, M., Mirlohi, A., Saeidi, G., 2011. Study of genetic variation in Sesame (*Sesamum indicum* L.) using Agro-Morphological traits and ISSR Markers. *Russian J. Genet.* 47(3), 314-321. <https://doi.org/10.1134/S1022795411030136>
- Pfeiffer, T., Roschanski, A.M., Pannell Korbecka, G., Schnitter, M., 2011. Characterization of microsatellite loci and reliable genotyping in a polyploidy plant, *Mercurialis perennis* (Euphorbiaceae). *The J. of Here.* 102, 479-488. doi:10.1093/jhered/esr024
- Pham, T.D., Geleta, M., Bui, T., Bui, T.C., Merker, A., Carlsson, A.S., 2011. Comparative analysis of genetic diversity of sesame (*Sesamum indicum* L.) from Vietnam and Cambodia using agro-morphological and molecular markers. *Hereditas* 148, 28-35. <https://doi.org/10.1111/j.1601-5223.2010.02196.x>
- Rana, M., Sharma, R., Sharma, P., 2014. Estimation of genetic diversity in *Capsicum annum* L. germplasm using PCR-based molecular markers. *Natl. Acad. Sci. Lett.* 37 (3), 295-301
- Rao, N.K., 2004. Plant genetic resources: advancing conservation and use through biotechnology. *Afr. J. Biotechnol.* 3, 136-145. DOI:10.5897/AJB2004.000-2025
- Roldan-Ruiz, I., Van Eeuwijk, F.A., Gilliland, T.J., Dubreuil, P., Dillman, C., Lalleman, J., Baril, C.P., 2001. A Comparative study of molecular and morphological methods of describing relationships between perennial ryegrass (*Lolium perenne* L.) varieties. *Theor. And Appl. Genet.* 103(8), 1138-1150.
- Sankar, D., Sambandam, G., Ramakrishna, R.M., Pugalendi, K.V., 2005. Modulation of blood pressure, lipid profiles and redox status in hypertensive patients taking different edible oils. *Clin. Chem. Acta.* 355, 97- 104. <https://doi.org/10.1016/j.cccn.2004.12.009>
- Sarita, T., Nishant, B., Vijay, H., Sandhya, M., 2019. 'RAPD approach for varietal identification among sesame genotype.' *Inter. J. Curr. Eng. & Sci. Res.* 6, 37-41.
- Sanchez de la Hoz, M.P., Davila, J.A., Loarce, Y., Ferrer, E., 1996. Simple sequence repeats primer used in polymerase chain reaction amplifications to study genetic diversity in barley. *Genome* 39, 112-117. <https://doi.org/10.1139/g96-015>
- Singh, K.M., Kumar, D.B., Kumar, D.S., Manorama, D.S., 2015. Assessment of genetic diversity among Indian Sesame (*Sesamum indicum* L.) accessions using RAPD, ISSR and SSR markers. *Res. J. Biotech.* 10, 35-47.
- Spandana, B., Prathap, V., Prasanna, G.J., Anuradha, G., Sivaramakrishnan, S., 2012. Development and Characterization of Microsatellite Markers (SSR) in Sesame (*Sesamum indicum* L.) Species. *Appl. Biochem. Biotech.* 168, 1594-1607. <https://doi.org/10.1007/s12010-012-9881-7>

Uncu, A.O., Gultekin, V., Allmer, J., Frary, A., Doganlar, S., 2015. Genomic Simple Sequence Repeat Markers Reveal Patterns of Genetic Relatedness and Diversity in Sesame. *The Plant Genom.* 8, 1-12. <https://doi.org/10.3835/plantgenome2014.11.0087>

Uzun, B., Cagirgan, M.I., 2009. Identification of molecular markers linked

to determinate growth habit in sesame. *Euphytical* 166, 379-384.

Zhang, Y.X., Zhang, X.R., Che, Z., Wang, L.H., Wei, W.L., Li, D.H., 2012. Genetic diversity assessment of sesame core collection in China by phenotype and molecular markers and extraction of a mini-core collection. *BMC Genet.* 13, 102. <https://doi.org/10.1186/1471-2156-13-102>

