

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

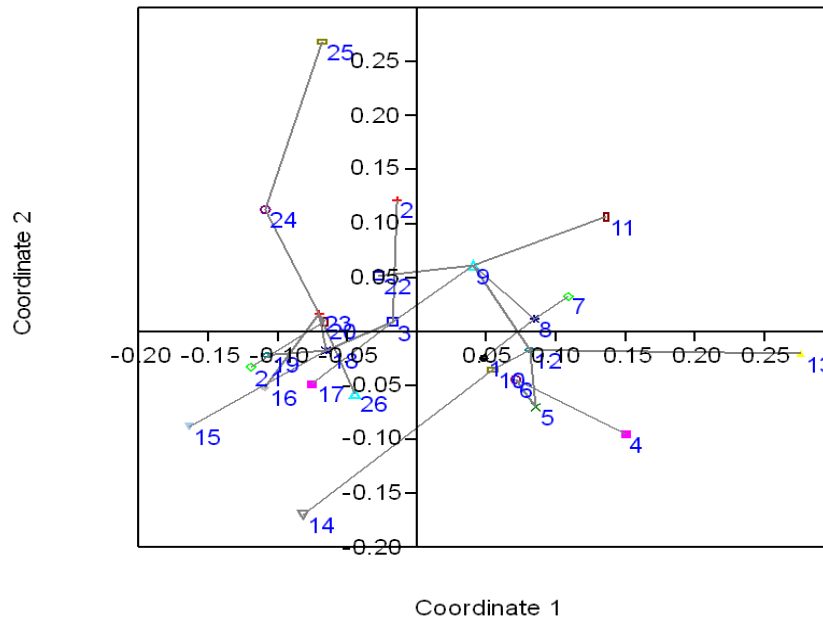


Figure 2: Principal Coordinate Axis 1 and 2 of ISSR Allelic data of twenty-six sesame accessions.
Note: Numbers in the plot correspond to accession's number as shown in Table 1

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.

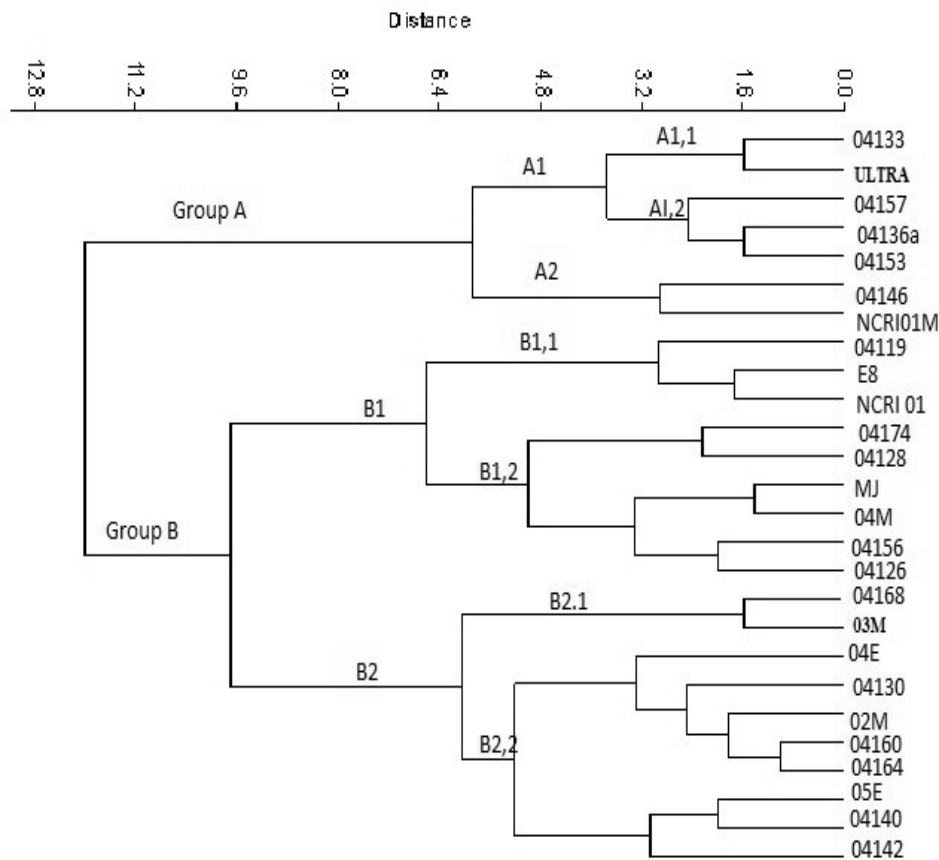


Figure 3: Dendrogram depicting genetic relationships among 26 sesame accessions based on the ISSR data using Ward's method.

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.

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