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Assessment of Genetic Diversity among Sesame (Sesamum Indicum L.) Accessions in Nigeria Using Ribulose-Bisphosphate Carboxylase (RBCL) Gene

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Abstract: In spite the medicinal, nutritional and industrial importance of sesame in Nigeria, its average annual production is still below expectation. Inadequate information on genetic diversity of the crop has been reported as the major cause of the poor production. To bridge this gap, the ribulose-1, 5-bisphosphate carboxylase (rbcL) DNA sequence was used to assess the genetic relatedness amongsesameaccessions from the six geo-political zones in Nigeria. Genomic DNA extracted from the leaves of 24 landraces and 5 improved varieties using the CTAB method was amplified and sequenced. Single Nucleotide Sequence (SNP) typing and phylogenetic analyses were performed using neighbor joining method. BLASTN nucleotide to nucleotide analysis of rbcL sequences generated for the 29 samples matched Sesamumindicum with percentage similarity ranging from 98.73% to 100%. The multiple alignments of the sequences revealed six polymorphic sites with both substitutions (transversion) and indels (insertion-deletion) observed. Codon usage bias showed that 43.75% of the codons did not show codon bias, 25% were highly preferred codons, and 18.17% exhibited low codon bias usage while 12.50% of the codons were moderately used.The 29 accessions did not cluster according to geographical origin whichsuggests exchange of seeds by farmers across the country.

Keywords: Sesamumindicum, Genetic variability, Barcoding, rbcL, Codon

1. Introduction

Sesame (Sesamumindicum L.) is an important oilseed crop cultivated for its nutritious seeds and excellent oil production (Kumaraswamyet al., 2023). The crop holds considerable economic and cultural significance in Nigeria. As a major producer and consumer of sesame, Nigeria plays vital role in its global trade (Zhang et al., 2021). According to HL-Agro (2022) Myanmar, India, China, Tanzania, Sudan, Ethiopia, Nigeria, Burkina Faso, Uganda, and Niger are the top ten producers of sesame which accounted for over 80% of world production. In Nigeria, sesame is

majorly cultivated in states like Benue, Nassarawa, Jigawa, Kano, Kebbi, Yobe, Borno, Gombe, Plateau and Taraba (Yunusaet al., 2019). Apart from the mentioned states, sesame is also grown in other parts of Nigeria on a smaller scale.

Sesame is used for several culinary, medicinal and industrial purposes (Wei et al., 2022; Chen et al., 2024). The seeds according to Abbas et al. (2022) is said to be a rich source of oil which accounted for why the crop is included in recipes for the production of food items like sesame oil, paste, candies, and several baked foods. Medicinally, sesame oil is a very good remedy for high blood pressure, cough, diabetes, high cholesterol, heart disease, hemorrhoid, constipations and other health conditions (Singletary et al., 2022). According to Orielet al. (2024) sesame oil is also used for several dermatological treatments owning to its natural anti-bacterial and anti-fungi potentials. The oil has recently been included into a number of cosmetics products because of its antiviral and anti-inflammatory properties. The shafts from processed sesame seeds have been reported by Yeasminet al. (2021) to be rich source of balance diet for farm animals.

Surprisingly, in spite the numerous medicinal and nutritional importance of sesame, its average annual production has been below expected average annual production (Shehu, 2023). In an attempt to increase the annual production of sesame in Nigeria, area of land committed to its cultivation has been significantly increasedwhich still did not get the crop to its annual yield potential (Ajibadeet al., 2023). To buttress this, Shehu (2023)reported that the average yield of sesame is roughly 300 kg/ha which is four times lower than that of other oilseed crops, such as soybeans and groundnuts.Animasaunet al. (2022) precisely implicated insufficient knowledge of genetic diversity as the major reason behind the poor production of this crop in Nigeria. Therefore, very little is known about the genetic makeup of the native sesame germplasm in Nigeria.

So far studies carried out on the genetic variations in sesame collections from Nigeria have concentrated more on morphological markers (Alake et al., 2010; Alegeet al., 2011; Ukaan and Ogbonna, 2012; Jakuskoet al., 2013; Zhigilaet al., 2015; Falusiet al., 2015; Nwekeet al., 2019; Madina, 2020; Animasaunet al., 2022). Rani et al. (2023) reported that molecular markers are more valuable tools for the assessment of genetic diversities because unlike morphological markers they are not influenced by environmental conditions, making them more reliable and reproducible. To overcome the challenges associated with morphological markers, some molecular markers have been employed for the evaluation of genetic diversity as well as species identification in sesame collections from Nigeria. The molecular markers employed so far for discriminating Nigeria sesame include SSRs (Simple Sequence Repeats) (Nwekeet al., 2011; Durodolaet al., 2023; Odesolaet al., 2020; Oduoyeet al., 2020), RAPD (Random Amplified Polymorphic DNA) (Alege 2019; Nwekeet al., 2020) and microsatellite polymorphisms (Nwekeet al., 2012). There is

information dearth on the use of DNA barcodesfor genetic characterization and species identification of Nigerian sesame.

The two major barcodes used for such studies includes nuclear barcodes like ITS2, and Cytoplasmic DNA, such as mitochondrial DNA (mtDNA) and chloroplast DNA (cpDNA). The advantages of cytoplasmic DNAs over nuclear DNA include its haploidy nature (Laczkóet al., 2022), maternally inheritance mode in most eukaryotes (Merhebet al., 2019; Chac and Thinh, 2023) and its less prone to genetic recombination (Camus et al., 2022).

Among the cytoplasmic barcoding techniques, Jianet al. (2022) stated that mitochondrial DNA sequences are more suitable for the identification of animal species, while DNA sequences in chloroplast genes are mostly used for the identification of plant species. Li et al. (2021) reported that chloroplast barcodes such as matK, rbcL, ndhF, and ycf1 have been shown to enable rapid and accurate identification of plant species. Among these four barcodes, Pere et al. (2023) singled out the rbcL (ribulose-bisphosphate carboxylase) marker as the most suitable chloroplast DNA barcode for plant species identification due to its widespread presence, conserved nature, high PCR amplification and sequence recovery rate in plants. To the best of our knowledge no known studies of the chloroplast (cp) DNA sequence of sesame is available from Nigeria. Therefore, rbcL gene sequences of sesame collected from different geopolitical zones in Nigeria will be generated and used to assess the level of genetic diversity among the samples.

2.0. Materials and Methods

Sample Collection

Seeds of sesame accessions were collected from farmers at four different locations within each geopolitical zone to give a total of 24 sesame samples as shown in table 1. Selection of locations for sample collection was done using Simple Random Sampling (SRS) techniques within each geopolitical zone. Two improved varieties were obtained from Abeokuta (Ogun State) while three improved varieties were obtained from National Cereals Research Institute (NCRI) Badeggi to make a total of 29 samples. Seeds of each sample were planted and young leaves from the plants were obtained from healthy plants for DNA extraction and amplification.

Genomic DNA extraction

The CTAB method was used to extract genomic DNA from the samples.

The PCR sequencing and Amplification

The forward and reverse primers were designed using the reference gene, ribulose 1,5 bisphosphate carboxylase/oxygenase subunit (rbcL). PCR was carried out in a

GeneAmp 9700 PCR System Thermalcycler (Applied Biosystem Inc., USA) with a PCR condition as given below:

Gene of interest	Primer name	Primer sequence 5'-3'	PCR profile
rbcL	H1f F	CCACAAACAGAGACTAAAGC	initial denaturation at 94°C for 5 min, followed by 35cycles of each cycle comprised of 30secs denaturation at 94°C, 30secs annealing of primer at 50°C, 40 sec extension at 72°C and a final extension for 7min at 72°C. Polymerase Chain Reaction
	Fofana R	GTAAAATCAAGTCCACCGCG	

Data analysis

BLASTn was used to compare the obtained sequence with DNA sequences from GenBank. The codon usage frequency table of the 29 samples was generated using the sequence manipulation suite software. Codon usage indices were calculated using CodonW as implemented on a public Galaxy server (galaxy.pasteur.fr). MEGA 11 software was used to construction the phylogenetic tree with Neighbor Joint (NJ) method (Tamura et al., 2021).

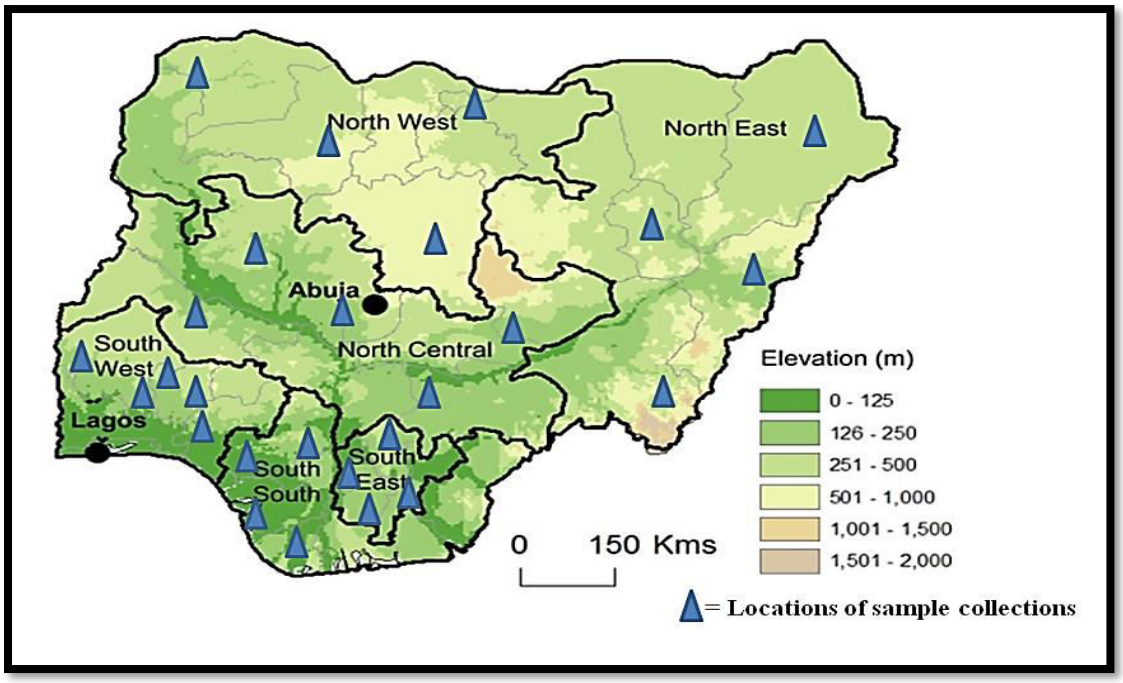

Figure 1: Samples collection locations across the six geo-political zones in Nigeria.

3.0. Result and Discussion

Agarose gel showed positive amplification between the extracted DNA from the 29 accessions and the rbcL gene sequence at approximately 550 bp (Plate 1). This revealed universality of rbcL gene in the studied accessions. According to Antilet al. (2023) the universality and ease of amplification of rbcL gene accounted for its preferable use as a DNA barcode in plant. The observed band size (around 550 bp) is consistent with the report of Xu and Tabita (1996) that the rbcL genes commonly have its bands around 500 bp.

BLASTN nucleotide to nucleotide analysis of rbcL sequences generated for the twenty-nine samples revealed strong matching with only Sesamumindicumin the database with percentage similarity ranging from 98.73% to 100% (Table 2). This indicates that all the 29 samples are Sesamumindicum.

The narrow range in the total nucleotide length (546 bp to 551 bp) and GC content (42.86% to 43.22%) of the sesame accessions studied (Table 3) suggests high degree of conservation (with very little sequence variation) in the sequence of the rbcL gene across the samples. This finding is consistent with the report of Wang et al. (2024) that the rbcL gene is well conserved in plant. The GC range observed in this study is similar to the 43.77% to 44.11% reported by Omonhinminet al. (2023) on Moringaoleiferaaccessions but slightly higher than the 28.5% to 42.00% range reported by Parvathyet al. (2022) using rbcL sequences.

In this study, single nucleotide polymorphisms (SNPs) revealed six variable or polymorphic sites (representing 1.09% of the total sequence) (Table 4 and Figure 2). The six mutational evens include five indels (insertion and deletion) and one substitutions (transversion). Deletions of A were observed at positions 5 (13 accessions), 8 (4 accessions) and 18 (27 accessions) of the rbcL sequence, whereas deletion of C and G were observed at 9th (27 accessions) and 385th positions (10 accessions) of the sequence respectively. Also, transversion of A to C was recorded at the 11th position (4 accessions) of the sequence (Table 4 and Figure 2). This low frequency of variable sites observed in this study further supported high degree of conservation within the rbcL gene among the sesame accessions studied. This pattern of mutation observed in this study suggests potential hotspots for indels within the rbcL gene of Sesamumindicum which could serve as possible markers for further analysis of genetic diversity within this species. Although, the 6 SNPs observed in this study is notably lower than the 22 indels and 4 transversions events reported byDevi and Chrungoo (2017) after nucleotide sequences alignment of rbcL sequence of Chenopodium quinoa. The higher number of indel and transversion reported in their study compared to ours may be attributed to the fact that whole

rbcL gene of approximately 1183 bp was considered.Dossouet al. (2023) identified indels as the major cause of variations in the genes responsible for Lignans synthesis in sesame.

Interestingly, all the 20 standard amino acids were found in the rbcL protein (Table 5). However, cysteine (Cys) having the least occurrence (1.36%) in this study was absent in accessions R7, R14, R15, R16, R20, R22 and R24. Generally, the sequence is rich in Leucine (12.14%) and Arginine (9.89%). This complete absence of cysteine in some accessions is connected to the earlier reported SNPs that resulted in codon changes, leading to substitution of cysteine with other amino acids. This finding is consistent with the report of Liu et al. (2018) that the codons for the production of amino acid cysteine are the least frequent universal amino acid in Chrysanthemum carinatum and Kalimerisindica.

Table 6 showed that 34 (excluding methionine and tryptophan) were preferred codons. This result also revealed that termination codon was highly biased towards UGA but UAG was not favoured while UAA was moderately used. The observed strong bias towards UGA as a termination codon in this study is consistent with the earlier report of Liuet al. (2005) that the UGA is favoured over UAA and UAG in eukaryotes. From Figure 3 it was observed that 43.75% of the codons did not show codon bias, 25% were highly preferred codons, and 18.17% exhibited low codon bias usage while 12.50% of the codons were moderately used. The wide variations observed in codon bias pattern suggests that some codons are used more frequently (highly biased) than others for encoding the same amino acid. According to Bahiri-Elitzur and Tuller (2021**)** codon bias occurs as a result of specific codons favoured by the abundant tRNAs in the organism to ensure efficient translation of mRNA into protein.Our study also revealed underlying preference for codons ending in A and U at the third base position.Parvathyet al. (2022) reported predominance of A- and U-ending codons in seven species of Citrus and FAD7 gene of dicots. Wang et al. (2024) also reported that the chloroplast genomes of dicotyledons generally prefer to use codons ending with A/U.

The Neighbor Joint (NJ) analysis separated the 29 accessions into two main clusters (Figure 4). This suggests a potential separation into two distinct lineages within the studied Sesamumindicumsamples. Interestingly, a specific pattern of SNPs (deletion of A at position 8 and transversion of C for A at position 11) was observed among the four accessions in Cluster II. This reveals possible relationships between the SNPs and the evolutionary patterns of the studied sesame samples. Therefore, accessions that shared the same mutation types might have diverged from a common ancestor more recently. Our study also revealed that accessions lacking cysteine in their rbcL

sequence are scattered across the three sub-clustered which suggests divergent evolution of those samples. The failure of accessions to cluster according to geographical origin and grouping of accessions from the six geo-political zones in the first sub-cluster of cluster 1 indicates that geographical origin does not correlate with genetic diversity since genotypes from different geographical origins clustered together in the same group. This finding is consistent with the report of Bhattacharjeeet al. (2020) who observed grouping of sesame accessions from different geographical regions in India into the same cluster. This grouping pattern observed in this study may be facilitated by exchange of seeds among sesame farmers across different zones in Nigeria.

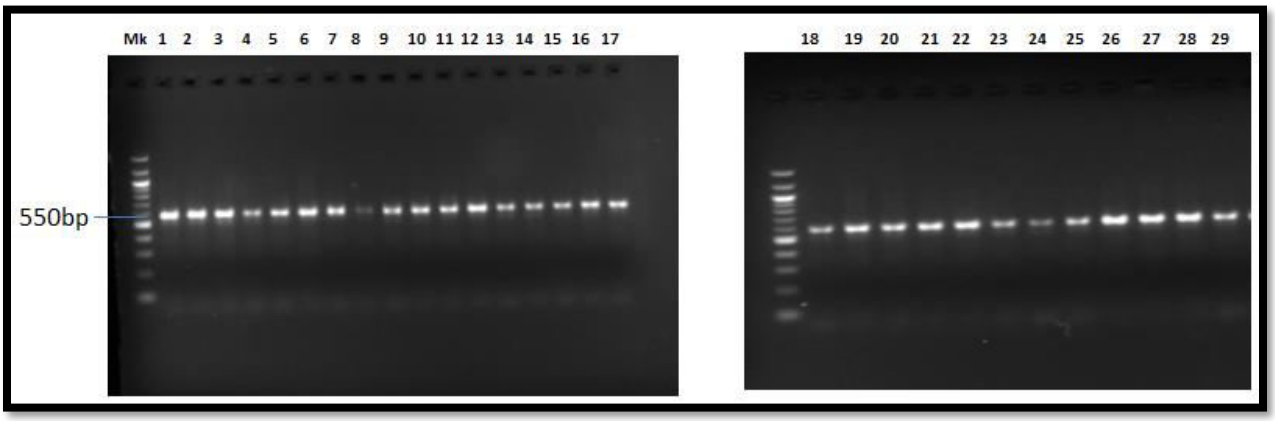

Plate 1: PCR amplification for the twenty nine Sesamumindicum accessions using of rbcL marker.

Table 1: Details of Sesamumindicumaccessions collection and NCBI GenBank submission codes.

S/N	Accession No.	**Area of collection**	**State of Collection**	**Collection Geopolitical Zone**
1	R1	E8 (Improved Variety)	Abeokuta (Ogun)	South West
2	R2	Yandev Y55 (Improved Variety)	Abeokuta (Ogun)	South West
3	R3	NCRI BEN 01M (Improved Variety)	Badeggi (Niger)	North Central
4	R4	NCRI BEN 04E (Improved Variety)	Badeggi (Niger)	North Central
5	R5	NCRI BEN 05E (Improved Variety)	Badeggi (Niger)	North Central
6	R6	Otukpo	Benue	North Central

7	R7	Akwanga	Nasarawa	North Central
8	R8	Offa	Kwara	North Central
9	R9	Yelwa	FCT	North Central
10	R10	Gombi	Adamawa	North East
11	R11	Kashere	Gombe	North East
12	R12	Jalingo	Taraba	North East
13	R13	Maiduguri	Borno	North East
14	R14	Dutse	Jigawa	North West
15	R15	Dutsin-ma	Kastina	North West
16	R16	Bodinga	Sokoto	North West
17	R17	Zaria	Kaduna	North West
18	R18	Olokoro	Abia	South East
19	R19	Abakaliki	Ebonyi	South East
20	R20	Umuode	Enugu	South East
21	R21	Umudioka	Anambra	South East
22	R22	Kwale	Delta	South South
23	R23	Elele	Rivers	South South
24	R24	Igbogene	Balyesa	South South
25	R25	Jattu	Edo	South South
26	R26	Ede	Osun	South West
27	R27	Ijebu-ode	Ogun	South West
28	R28	Ognomosho	Oyo	South West
29	R29	Iyin-Ekit	Ekiti	South West

Table 2: rbcL BLAST outcome for the 29 Sesamumindicum accessions

Sample ID	Scientific Name	Max Score	Total Score	Query Cover	E value	Percentage Identity (%)
1	Sesamumindicum	974	974	100%	0	98.73
2	Sesamumindicum	1003	1003	100%	0	99.82
3	Sesamumindicum	1009	1009	100%	0	100.00
4	Sesamumindicum	1009	1009	100%	0	100.00
5	Sesamumindicum	998	998	100%	0	99.63
6	Sesamumindicum	1009	1009	100%	0	100.00
7	Sesamumindicum	998	998	100%	0	99.63
8	Sesamumindicum	1003	1003	100%	0	99.82
9	Sesamumindicum	1009	1009	100%	0	100.00
10	Sesamumindicum	1009	1009	100%	0	100.00

11	Sesamumindicum	974	974	100%	0	98.73
12	Sesamumindicum	1003	1003	100%	0	99.82
13	Sesamumindicum	1009	1009	99%	0	100.00
14	Sesamumindicum	992	992	100%	0	99.45
15	Sesamumindicum	998	998	100%	0	99.63
16	Sesamumindicum	992	992	100%	0	99.45
17	Sesamumindicum	1009	1009	100%	0	100.00
18	Sesamumindicum	1009	1009	100%	0	100.00
19	Sesamumindicum	1003	1003	100%	0	99.82
20	Sesamumindicum	998	998	99%	0	99.63
21	Sesamumindicum	1009	1009	99%	0	100.00
22	Sesamumindicum	1009	1009	99%	0	100.00
23	Sesamumindicum	992	992	100%	0	99.45
24	Sesamumindicum	1003	1003	100%	0	99.82
25	Sesamumindicum	1003	1003	100%	0	99.82
26	Sesamumindicum	1005	1005	100%	0	99.82
27	Sesamumindicum	1009	1009	99%	0	100.00
28	Sesamumindicum	1009	1009	100%	0	100.00
29	Sesamumindicum	1009	1009	100%	0	100.00

Table 3: Rbcl sequence length and proportion of nucleotide in the Sesamumindicum

Accession Number	Geopolitical Zone	A (%)	T (%)	G (%)	C (%)	GC (%)	Sequence Length
R1	South West	28.86	27.95	21.6	21.6	43.19	551
R2	South West	28.88	28.15	21.94	21.02	42.96	547
R3	North Central	28.75	28.21	21.98	21.06	43.04	546
R4	North Central	28.75	28.21	21.98	21.06	43.04	546
R5	North Central	28.94	28.21	21.79	21.06	42.86	546
R6	North Central	28.75	28.21	21.98	21.06	43.04	546
R7	North Central	28.94	28.21	21.79	21.06	42.86	546
R8	North Central	28.70	28.15	21.94	21.21	43.14	547
R9	North Central	28.75	28.21	21.98	21.06	43.04	546
R10	North East	28.75	28.21	21.98	21.06	43.04	546
R11	North East	28.86	27.95	21.60	21.60	43.19	551
R12	North East	28.88	28.15	21.94	21.02	42.96	547
R13	North East	28.70	28.15	21.94	21.21	43.14	547

R14	North West	28.57	28.21	21.79	21.43	43.22	546
R15	North West	28.94	28.21	21.79	21.06	42.86	546
R16	North West	28.57	28.21	21.79	21.43	43.22	546
R17	North West	28.75	28.21	21.98	21.06	43.04	546
R18	South East	28.75	28.21	21.98	21.06	43.04	546
R19	South East	28.88	28.15	21.94	21.02	42.96	547
R20	South East	28.88	28.15	21.76	21.21	42.96	547
R21	South East	28.70	28.15	21.94	21.21	43.14	547
R22	South South	28.57	28.21	21.79	21.43	43.22	546
R23	South South	28.70	28.15	21.94	21.21	43.14	547
R24	South South	28.57	28.21	21.79	21.43	43.22	546
R25	South South	28.88	28.15	21.94	21.02	42.96	547
R26	South West	28.94	28.21	21.98	21.06	43.04	546
R27	South West	28.83	28.28	21.9	20.99	42.88	548
R28	South West	28.70	28.15	21.94	21.21	43.14	547
R29	South West	28.75	28.21	21.98	21.06	43.04	546

Table 4: Monomorphic and polymorphic sites in the Sesamumindicum accessions

Total Sequence Length	Aligned (Monomorphic) Sites	Variable (Polymorphic) Sites	Details of Variable Sites		
551 kb	545 kb (98.91%)	6 kb (1.09%)	Locations of Variable Site	Mutation Observed	Accessions Affected
			5th	Deletion of A	R3, R4, R6, R8, R9, R10, R13, R17, R18, R21, R23, R28 and R29
			8th	Deletion of A	R14, R16, R22 and R24
			9th	Deletion of C	All except accession R1 and R11
			11th	Transversion (A to C)	R14, R16, R22 and R24
			18th	Deletion of A	All except accession R1 and R11

			385th	Deletion of G	R1, R5, R7, R11, R14, R15, R16, R20, R22 and R24.

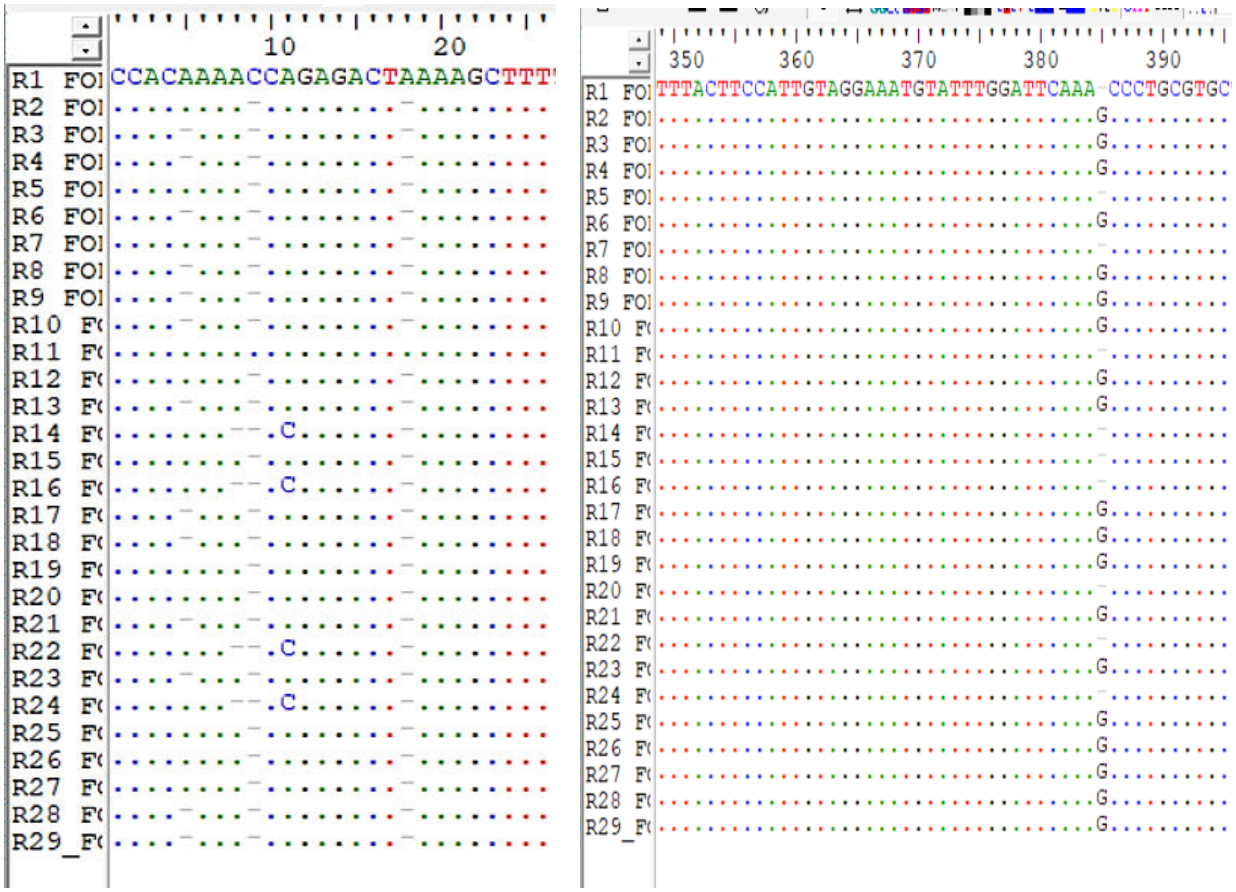

Figure 2: Multiple alignment of the rbcL sequence and the mutational evens of the Sesamumindicum accessions.

Acc. No.	Phe (%)	Leu (%)	Ile (%)	Met (%)	Val (%)	Ser (%)	Pro (%)	Thr (%)	Ala (%)	Tyr (%)	His (%)	Gln (%)	Asn (%)	Lys (%)	Asp (%)	Glu (%)	Cys (%)	Trp (%)	Arg (%)	Gly (%)
1	6.94	14.45	3.47	2.89	3.47	1.73	7.51	7.51	2.31	4.62	4.05	4.62	5.20	1.16	4.62	5.20	2.31	1.16	10.40	6.36
2	1.16	8.14	6.40	5.23	6.98	17.44	4.65	15.12	2.91	3.49	1.16	1.74	5.81	1.74	1.16	0.58	0.58	1.16	9.30	5.23
3	7.98	15.95	3.68	3.07	3.68	1.23	9.82	3.07	4.29	0.61	3.68	4.29	6.75	2.45	3.07	1.84	2.45	1.84	11.66	8.59
4	7.98	15.95	3.68	3.07	3.68	1.23	9.82	3.07	4.29	0.61	3.68	4.29	6.75	2.45	3.07	1.84	2.45	1.84	11.66	8.59
5	4.14	8.88	4.14	4.14	8.28	14.20	5.92	11.83	5.33	2.37	1.18	1.78	7.10	2.37	4.14	0.59	0.00	1.18	7.10	5.33
6	7.98	15.95	3.68	3.07	3.68	1.23	9.82	3.07	4.29	0.61	3.68	4.29	6.75	2.45	3.07	1.84	2.45	1.84	11.66	8.59
7	4.14	8.88	4.14	4.14	8.28	14.20	5.92	11.83	5.33	2.37	1.18	1.78	7.10	2.37	4.14	0.59	0.00	1.18	7.10	5.33
8	7.98	15.95	3.68	3.07	3.68	1.23	9.82	3.07	4.29	0.61	3.68	4.29	6.75	2.45	3.07	1.84	2.45	1.84	11.66	8.59
9	7.98	15.95	3.68	3.07	3.68	1.23	9.82	3.07	4.29	0.61	3.68	4.29	6.75	2.45	3.07	1.84	2.45	1.84	11.66	8.59
10	7.98	15.95	3.68	3.07	3.68	1.23	9.82	3.07	4.29	0.61	3.68	4.29	6.75	2.45	3.07	1.84	2.45	1.84	11.66	8.59
11	6.94	14.45	3.47	2.89	3.47	1.73	7.51	7.51	2.31	4.62	4.05	4.62	5.20	1.16	4.62	5.20	2.31	1.16	10.40	6.36
12	1.16	8.14	6.40	5.23	6.98	17.44	4.65	15.12	2.91	3.49	1.16	1.74	5.81	1.74	1.16	0.58	0.58	1.16	9.30	5.23
13	7.9	15.9	3.6	3.0	3.6	1.23	9.8	3.07	4.2	0.6	3.6	4.2	6.7	2.4	3.0	1.8	2.4	1.8	11.6	8.5

	8	5	8	7	8		2		9	1	8	9	5	5	7	4	5	4	6	9
14	6.55	8.33	4.17	4.17	7.14	11.90	6.55	10.12	5.36	2.38	0.60	2.98	6.55	2.98	4.17	0.60	0.00	1.79	7.74	5.95
15	4.14	8.88	4.14	4.14	8.28	14.20	5.92	11.83	5.33	2.37	1.18	1.78	7.10	2.37	4.14	0.59	0.00	1.18	7.10	5.33
16	6.55	8.33	4.17	4.17	7.14	11.90	6.55	10.12	5.36	2.38	0.60	2.98	6.55	2.98	4.17	0.60	0.00	1.79	7.74	5.95
17	7.98	15.95	3.68	3.07	3.68	1.23	9.82	3.07	4.29	0.61	3.68	4.29	6.75	2.45	3.07	1.84	2.45	1.84	11.66	8.59
18	7.98	15.95	3.68	3.07	3.68	1.23	9.82	3.07	4.29	0.61	3.68	4.29	6.75	2.45	3.07	1.84	2.45	1.84	11.66	8.59
19	1.16	8.14	6.40	5.23	6.98	17.44	4.65	15.12	2.91	3.49	1.16	1.74	5.81	1.74	1.16	0.58	0.58	1.16	9.30	5.23
20	4.14	8.88	4.14	4.14	8.28	14.20	5.92	11.83	5.33	2.37	1.18	1.78	7.10	2.37	4.14	0.59	0.00	1.18	7.10	5.33
21	7.98	15.95	3.68	3.07	3.68	1.23	9.82	3.07	4.29	0.61	3.68	4.29	6.75	2.45	3.07	1.84	2.45	1.84	11.66	8.59
22	6.55	8.33	4.17	4.17	7.14	11.90	6.55	10.12	5.36	2.38	0.60	2.98	6.55	2.98	4.17	0.60	0.00	1.79	7.74	5.95
23	7.98	15.95	3.68	3.07	3.68	1.23	9.82	3.07	4.29	0.61	3.68	4.29	6.75	2.45	3.07	1.84	2.45	1.84	11.66	8.59
24	6.55	8.33	4.17	4.17	7.14	11.90	6.55	10.12	5.36	2.38	0.60	2.98	6.55	2.98	4.17	0.60	0.00	1.79	7.74	5.95
25	1.16	8.14	6.40	5.23	6.98	17.44	4.65	15.12	2.91	3.49	1.16	1.74	5.81	1.74	1.16	0.58	0.58	1.16	9.30	5.23
26	1.16	8.14	6.40	5.23	6.98	17.44	4.65	15.12	2.91	3.49	1.16	1.74	5.81	1.74	1.16	0.58	0.58	1.16	9.30	5.23

27	1.16	8.14	6.40	5.23	6.98	17.44	4.65	15.12	2.91	3.49	1.16	1.74	5.81	1.74	1.16	0.58	0.58	1.16	9.30	5.23
28	7.98	15.95	3.68	3.07	3.68	1.23	9.82	3.07	4.29	0.61	3.68	4.29	6.75	2.45	3.07	1.84	2.45	1.84	11.66	8.59
29	7.98	15.95	3.68	3.07	3.68	1.23	9.82	3.07	4.29	0.61	3.68	4.29	6.75	2.45	3.07	1.84	2.45	1.84	11.66	8.59
Mean (%)	5.72	12.14	4.38	3.82	5.49	8.01	7.55	8.15	4.15	2.00	2.39	3.24	6.46	2.27	3.08	1.47	1.36	1.55	9.89	6.89

Table 5: Amino acid composition of the Sesamumindicum accessions from the RBCL sequences

Table 6: Relative Synonymous Codon Usage for rbcL Sequences of Sesamumindicum

Amino acids	Codon	Count	RSCU	Amino acids	Codon	Count	RSCU	Amino acids	Codon	Count	RSCU	Amino acids	Codon	Count	RSCU
Ala	GCG	1.66	0.83	His	CAU	2.03	1.69 *	Thr	ACG	0.45	0.16	Trp	UGG	5.00	1.00 *
Ala	GCA	1.24	0.62	His	CAC	0.38	0.31	Thr	ACA	3.07	1.08 *	Arg	AGG	4.83	1.61 *
Ala	GCU	2.59	1.29 *	Lys	AAG	3.10	0.59	Thr	ACU	5.17	1.81 *	Arg	AGA	4.72	2.23 *
Ala	GCC	2.48	1.24 *	Lys	AAA	7.31	1.40 *	Thr	ACC	5.17	0.95	Arg	CGG	0.62	0.29
Cys	UGU	4.59	1.34 *	Leu	UUG	3.59	1.04 *	Val	GUG	1.45	0.59	Arg	CGA	2.90	1.37 *
Cys	UGC	2.24	0.65	Leu	UUA	4.55	1.32 *	Val	GUA	2.97	1.21 *	Arg	CGU	1.66	0.78
Phe	UUU	3.21	1.00 *	Leu	CUG	4.38	1.27 *	Val	GUU	4.17	1.70 *	Arg	CGC	0.93	0.44
Phe	UUC	3.17	1.00 *	Leu	CUA	2.86	0.83	Val	GUC	1.21	0.49	Ser	AGU	1.72	0.75
Gly	GGG	2.69	1.02 *	Leu	CUU	3.55	1.03 *	Tyr	UAU	3.90	0.82	Ser	AGC	2.74	1.21 *
Gly	GGA	3.76	1.42 *	Leu	CUC	1.69	0.49	Tyr	UAC	5.62	1.18 *	Ser	UCG	0.83	0.36
Gly	GGU	2.76	1.04 *	Glu	GAG	2.66	0.75	Pro	CCG	2.24	0.88	Ser	UCA	1.52	0.66
Gly	GGC	1.38	0.52	Glu	GAA	4.41	1.25 *	Pro	CCA	1.45	0.57	Ser	UCU	3.41	1.48 *

Ile	AUA	2.28	0.74	Asn	AAU	2.52	0.73	Pro	CCU	4.76	1.87 *	Ser	UCC	3.52	1.53 *
Ile	AUU	3.55	1.15 *	Asn	AAC	4.41	1.27 *	Pro	CCC	1.69	0.67	End	UGA	2.90	1.42 *
Ile	AUC	3.41	1.11 *	Gln	CAG	1.17	0.39	Asp	GAU	4.10	1.31 *	End	UAG	0.69	0.34
Met	AUG	3.03	1.00 *	Gln	CAA	4.83	1.61 *	Asp	GAC	2.14	0.69	End	UAA	2.55	1.24 *

RSCU: Relative Synonymous Codon Usage, RSCU values greater than in asterisk (*).

Categories of Relative synonymous codon usage (RSCU):

- Lack of bias (RSCU < 1.0)
- Low bias (1.0 < RSCU< 1.2)
- Moderately biased (1.2 < RSCU< 1.3)
- Highly biased (RSCU > 1.3)

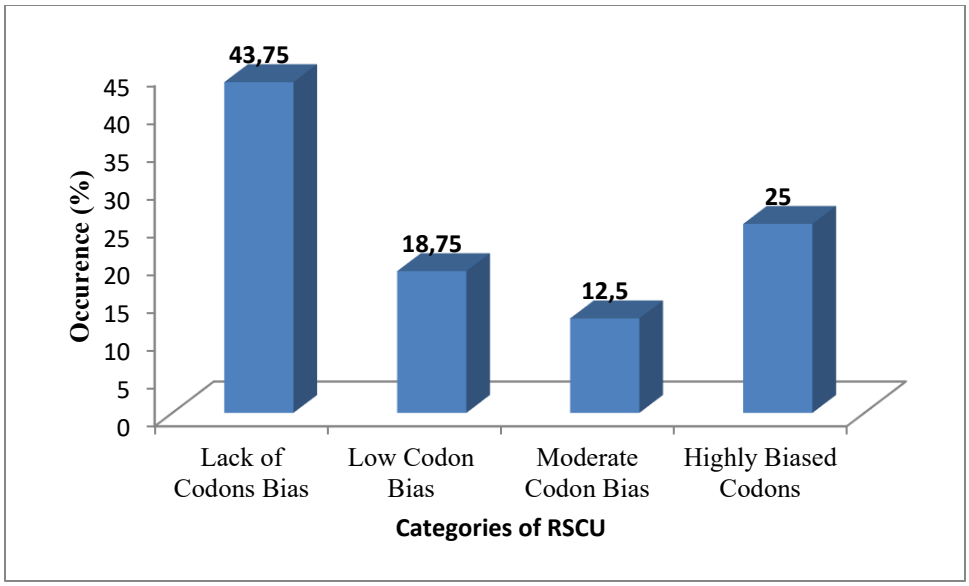

Figure 3: Frequency of Relative Synonymous Codon Usage (RSCU) in Sesamumindicum

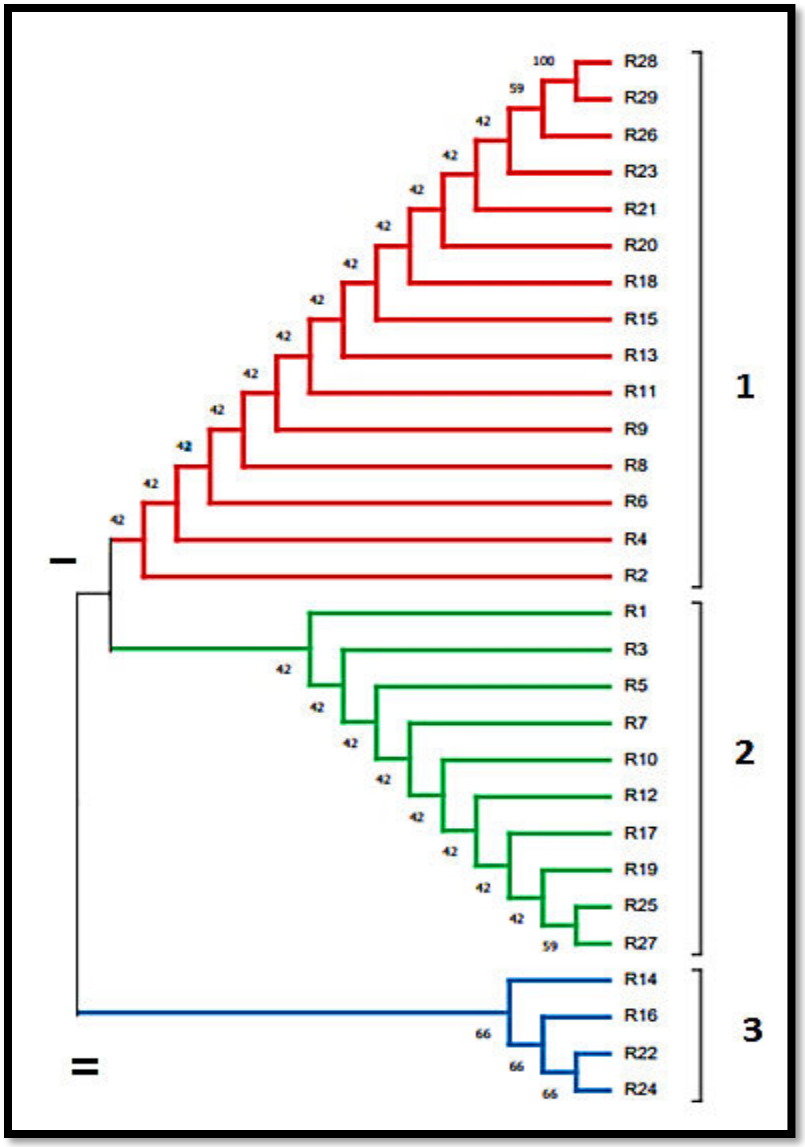

Figure 4: Relationships among the Sesamumindicum accessions based on sequences from the RBCL marker.

4.0. Conclusion

This study revealed that the rbcL sequence of the 29 accessions closely matched the sequence of Sesamumindicumin the database. The low frequency of variable sites in this study reveals high level of conservation within the rbcL gene in the studied samples. This study also revealed relationship between the observed SNPs and evolutionary patterns of the sesame. Accessions from different geographical origins clustered together which suggests that there is no relationship between geographical origins of samples and genetic diversity. This therefore suggests

exchange of sesame seeds among farmers across the six geopolitical zones in Nigeria.

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References

1. Abbas, S., Sharif, M. K., Sibt-e-Abbas, M., FikreTeferra, T., Sultan, M. T. and Anwar, M. J. (2022). Nutritional and therapeutic potential of sesame seeds. J. of Food Qual. 22, 1-9.
2. Ajibade, Y. E., Opaluwa, H. I., Adio, M. O., Gbadamosi, A. O. and Abdullahi, S. (2023). Analysis of sesame production among indigenous and non-indigenous farmers in Yagba East, Kogi State, Nigeria. American Int J. of Agric Stud. 7(1).32-40.
3. Alake, C. O., Ayo-Vaughan, M. A. and Ajani, O. O. (2010). Estimate of variability for yield and its characters in Nigerian sesame (Sesamumindicum) genotypes. Jl of AgricSciand Environ. 10(1), 72-85.
4. Alege, G. O., Akinyele, B. O., Ayodele, S. M. and Ogbode, A. V. (2011).Taxonomic importance of the vegetative and pod characteristics in three Nigerian species of sesame. Afr J. of Plant Sci. 5(3), 213-217.
5. Alege, G.O. (2019): Characterization of Nigerian Sesame (SesamumindicumL.) using Random Polymorphic DNA (RAPD) marker. J of Biotech Res. 5(9), 77-84.
6. Animasaun, D. A., Oyedeji, S., Musa, L. B., Adedibu, P. A. and Adekola, O. F. (2022). Performance and genetic diversity of some sesame (Sesamumindicum L.) accessions based on morpho-agronomic traits and seed proximate composition in Kwara State of Nigeria.ActaagricSlovenica. 118(1), 1-15.
7. Antil, S., Abraham, J.S., Sripoorna, S., Maurya, S., Dagar, J., Makhija, S., Bhagat, P., Gupta, R., Sood, U., Lal, R. and Toteja, R. (2023). DNA barcoding, an effective tool for species identification: a review. MolBiol Reports. 50(1), 761-775.
8. Bahiri-Elitzur, S. and Tuller, T. (2021).Codon-based indices for modeling gene expression and transcript evolution. Comp and Structural Biotech J. 19, 2646 - 2663.
9. Bhattacharjee, M., Prakash, S. H., Roy, S., Soumen, S., Begum, T. and Dasgupta, T. (2020). SSR-based DNA fingerprinting of 18 elite Indian varieties of sesame (Sesamumindicum L.). The Nucleus, 63: 67-73.
10. Camus, M. F., Alexander-Lawrie, B., Sharbrough, J. and Hurst, G. D. (2022).Inheritance through the cytoplasm. Heredity, 129(1): 31-43.
11. Chac, L. D. and Thinh, B. B. (2023). Species identification through DNA barcoding and its applications: A review. Bio Bulletin. 50(6), 1143-1156.

12. Chen, T., Wang, B., Yang, Y., Han, B. and Jiang, H. (2024). Sesame use in Turpan during the Tang dynasty: Evidence from the Astana Cemetery. J. of Arch Sci. Report. 53, 104.
13. Devi, R. J. and Chrungoo, N. K. (2017).Evolutionary divergence in Chenopodium and validation of SNPs in chloroplast rbcL and matk genes by allele-specific PCR for development of Chenopodium quinoa-specific markers. The Crop J., 5(1), 32-42.
14. Dossou, S.S.K., Song, S., Liu, A., Li, D., Zhou, R., Berhe, M., Zhang, Y., Sheng, C., Wang, Z., You, J. and Wang, L. (2023). Resequencing of 410 sesame accessions identifies SINST1 as the major underlying gene for lignans variation. Int J. of Mol Sci. 24(2), 1055.
15. Durodola, F.A., Adubi, A.O. and Azeez, M.A. (2023). Assessment of genetic diversity among Nigerian Sesame (Sesamumindicum L.) accessions Using Inter Simple Sequence Repeats Markers. ActaSci Malaysia, 7(2), 66-74.
16. Falusi, O. A., Yahaya, S. A., Gado, A. A., Daudu, O. A. Y., Akinbo, O. A. and Teixeira da Silva, J. A. (2015). Morphological evaluation of selected sesame (Sesamumindicum L.) genotypes from five states in Northern Nigeria.Afr JAgric Res. 10(37), 3657-3661.
17. HL-Agro (2022).Sesame Seed Market Report 2021 and Beyond, HL Annual report of Agro-Products Pvt Ltd. India.hlagro.com
18. Jakusko, B. B., Usman, B. D. and Mustapha, A. B. (2013).Effect of row spacing on growth and yield of sesame (Sesamumindicum L.) in Yola, Adamawa State, Nigeria. J of Agric and Vet Sci. 2(3): 36-39.
19. Jian, S, Chen F, Qin P, Xie H, Peng G, Li Y. and Guo, X. (2022).The specific DNA barcodes based on chloroplast genes for species identification of Theaceae plants. Physiol MolBiol Plants.28(4), 837-848.
20. Kumaraswamy, H.H., Ramya, K.T., Mandal, S.N., Ratnakumar, P., Jawahar-Lal, J., Pushpa, H.D., Ramesh, K., Rathnakumar, A.L., Duraimurugan, P. and Sakthivel, D. (2023). Biotechnological Approaches for Genetic Improvement of Sesame (Sesamumindicum L.). In Smart Plant Breeding for Field Crops in Post-genomics Era Singapore: Springer Nature Singapore, 343-368Pp.
21. Laczkó, L., Jordán, S. and Sramkó, G. (2022). The RadOrgMiner pipeline: Automated genotyping of organellar loci from RADseq data. Meth in Ecology and Evol. 13(9), 1962-1975.
22. Li, H., Xiao, W., Tong, T., Li, Y., Zhang, M., Lin, X., Zou, X., Wu, Q. and Guo, X. (2021).The specific DNA barcodes based on chloroplast genes for species identification of Orchidaceae plants. Sci Reports, 11(1), 1424.
23. Liu, Q. (2005). Comparative analysis of base biases around the stop codons in six eukaryotes. Biosystems, 81(3): 281-289.

24. Liu, X., Zhou, B., Yang, H., Li, Y., Yang, Q., Lu, Y. and Gao, Y. (2018). Sequencing and analysis of Chrysanthemum carinatumSchousb and Kalimerisindica. The complete chloroplast genomes reveal two inversions and rbcL as barcoding of the vegetable. Molecules, 23(6): 1358.
25. Madina, P. (2020). Performance of ten (10) varieties of sesame (Sesamumindicum) grown in Billiri, Gombe State, Nigeria. Fudma J of Sci. 4(3), 453-456.
26. Merheb, M., Matar, R., Hodeify, R., Siddiqui, S.S., Vazhappilly, C.G., Marton, J., Azharuddin, S. and Al Zouabi, H. (2019).Mitochondrial DNA, a powerful tool to decipher ancient human civilization from domestication to music, and to uncover historical murder cases. Cells, 8(5):433.
27. Nweke, F. N., Ubi, B. E. and Kunert, K. (2011).Simple Sequence Repeat Polymorphisms in Nigerian Sesame Cultivars and its Relationship with Morpho-Agronomic Traits. Journal of Crop Improvement, 25(5): 572-596.
28. Nweke, F. N., Ubi, B. E. and Kunert, K. (2012).Application of microsatellite polymorphisms to study the diversity in seed oil content and fatty acid composition in Nigerian sesame (Sesamumindicum L.) accessions. AfrJ of Biotech. 11, 8820-8830.
29. Nweke, F. N., Uwa, C. U., Egutah, A. S., Ibe, T. I., Nwose, R. N., Uchewa, E. N. and Usman, A. (2019). Agro-Morphological Characteristics of Sesame (Sesamumindicum L.) from the Different States of Nigeria. World ApplSci J. 37(5), 425-429.
30. Odesola, K. A., Kayode, A. E., Igwe, D. O. and Ojuederie, O. B. (2020). Molecular analysis of sesame (Sesamumindicum L.) seeds sampled from Northern Nigeria. J of Underutilized Leg., 2(1), 22-32.
31. Oduoye, O.T., Oluwasanya, O.A., Arikawe, O.O., Sunday, A., Ayekun, O.A., Oyenpemi, O.S., Jandong, E.A., Uguru, M.I. and Okechukwu, E.C. (2020).Genetic variation via SSR polymorphic information content and ecological distribution of Nigerian sesame.Afr. J. of Biotech. 19(4), 165-170.
32. Omonhinmina C. A., Olomukoroa, E.E., Onuselogua, C.C., Popoolaa, J.O. and Oyejide, B.S.O. (2023).Intra-specific genetic variability dataset on rbcL gene in Moringaoleifera Lam. (Moringaceae) in Nigeria.Data in Brief, 30: 1-9.
33. Oriel, R. C., Elizur, A. and Sicherer, S. H. (2024).Comprehensive diagnosis, management, and treatment of sesame allergy. The J. of Allergy and Clinical Immunology: In Practice, 12(3): 590-597.
34. Parvathy, S. T., Udayasuriyan, V. and Bhadana, V. (2022).Codon usage bias. MolBiol reports, 49(1), 539-565.
35. Pere, K., Mburu, K., Muge, E. K., Wagacha, J. M. and Nyaboga, E. N. (2023). Molecular discrimination and phylogenetic relationships of Physalis species based on ITS2 and rbcL DNA barcode sequence. Crops, 3(4), 302-319.

36. Rani, K., Kumar, M., Razzaq, A., Ajay, B. C., Kona, P., Bera, S. K., &Wani, S. H. (2023). Recent advances in molecular marker technology for QTL mapping in plants. QTL Mapping in Crop Impr.10, 1-15.
37. Shehu, H. (2023). Effect of induced ageing on viability and nutrient level of sesame seed accessions (M. Tech., Thesis), Department of Biochemistry, Federal University of Technology Minna, Nigeria, 147Pp.
38. Singletary, K. W. (2022). Sesame: Potential Health Benefits. Nutrition Today, 57(5): 271-287.
39. Tamura K., Stecher G., and Kumar S. (2021). MEGA 11: Molecular Evolutionary Genetics Analysis Version 11. Molecular Biology and Evolution
40. Ukaan, S. and Ogbonna, P. (2012).Genetic variability and character association in sesame (Sesamumindicum L.) accessions. Int J. of Plant Breeding, 6(2), 139-143.
41. Wang, L. L., Li, Y., Zheng, S. S., Kozlowski, G., Xu, J. and Song, Y. G. (2024). Complete chloroplast genomes of Four Oaks from the section Cyclobalanopsis improve the phylogenetic analysis and understanding of evolutionary processes in the Genus Quercus. Genes, 15(2), 230.
42. Wei, X., Gong, H., Yu, J., Liu, P., Wang, L. and Zhang, Y. (2017).SesameFG: An integrated database for the functional genomics of sesame. Scientific reports, 7 (1), 1–10.
43. Xu, H. H. and Tabita, F. R. (1996).Ribulose-1, 5-bisphosphate carboxylase/oxygenase gene expression and diversity of Lake Erie planktonic microorganisms. Appl and Environ Microbiology, 62(6): 1913-1921.
44. Yeasmin, S., Chowdhury, T. A., Rana, G. M., Rahman, M. M. and Ferdousi, L. (2021). Characteristics of sesame (Sesamumindicum L.) seed meal grown in the Northern Region of Bangladesh. Biomed. J. Sci. Tech. Res, 38(1): 29944-29949.
45. Yunusa, M. M., Usman, M., Nangere, M. G. and Usman, B. D. (2019). Yield of sesame varieties and soil properties as influenced by tillage and fertilizer in sudan savanna zone of Nigeria. Int J of Innovative Agric and Biology Res. 7(4), 30-39.
46. Zhang, H., Langham, D. R., and Miao, H. (2021).Economic and academic importance of sesame. The Sesame Genome, 21: 1-18.
47. Zhigila, D. A., Fatsuma, B. J. S. and Suleiman D. A. (2015). Morphometric study of accessions of Sesamumindicum L. collected from Nigeria. Afr J. of Plant Sci. 15), 56-64.