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Linking inter simple sequence repeat-based genetic diversity with nutritional traits in Nigerian sesame (*Sesamum indicum L.*) for biofortification, conservation and breeding applications

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Abstract

Background: A simultaneous population increase and erratic climatic condition call for urgent innovative approaches in food production worldwide. Modern biotechnology provides effective ways to increase crop yield, nutritional quality and resistance against pests or other abiotic stresses. Sesame (Sesamum indicum L.) is a widely cultivated essential oil seed crop primarily grown in the northern Nigeria.

Purpose: Limited information exists on the genetic diversity of Nigerian Sesame germplasm and how genetic variation influence nutritional quality, particularly mineral and vitamin composition.

Methods: In this study, genetic variability of Nigerian sesame genotypes using ISSR markers and its relationship to minerals and vitamins composition was investigated. The vitamins (B1, B2, B3, B4, B6) and Folic acid were analysed using titrimetric methods of Harbone (1983), while atomic absorption spectrophotometric method were used for the determination of minerals (Fe, Cu, Ca, Mg, Mn and Zn).

Results: The results of the analysis of the molecular variance (AMOVA) showed 39% among individual and 61% within individual. The analysis revealed 25 alleles among the 10 genotypes and the average number of alleles per locus was 5, the lowest and highest numbers of alleles were 3 and 9. The polymorphic information content (PIC) ranged from 0.31 (UBC 840) to 0.87 (UBC 811) with an average of 0.59. The 10 genotypes assessed were clustered into four groups. Group1 consisted of DOM 012, NAK 017, MAR 016, and AGAI 039, while group II had DUM 043 and NAS 034 varieties. Similarly, group III consisted of LAP 041, GUM 024, NGB/019 and JIG 074.

Conclusion: The PIC value of 0.59 found showed that ISSR primers were informative and has the ability to differentiate the sesame genotypes. Sesame seeds, were found to contain the following minerals in descending order Mg (6.193±1.938) > Ca (5.425±1.494) > Fe (3.278±0.719) > Zn (1.297±0.664) > Cu (0.726±0.278) > Mn (0.332±0.097) in ppm. Mg, Ca, Fe and zinc and vit.E, vit.B_5 and folic acid were found to relatively high in all the cultivars with NGB/019 having the highest concentrations of Mg and DUM 043 with the highest composition of vit.E. Hence, these important varieties could be made available to farmers for propagation and sustainability.

Keywords: Polymorphism, diversity, vitamin, genetic, proximate, and mineral

Introduction

Sesame (*Sesamum indicum L*) have a lengthy history of domestication primarily due to the fact that they are dependable source of edible oil. Sesame appears to have been first domesticated in Asia or India Abbas *et al.* (2020). In some parts of Nigeria, the oil seed crop is farmed, although production has been declining for a number of reasons, including low yields, the use of landraces and the absence of high yielding cultivars by farmers (Adhikari *et al.,* 2017). Production has lately increased as a result of using molecular techniques to address genetic variability and for higher productivity, using a properly selected and developed variety of sesame (Ajibola *et al.,* 1993). Sesamum indicum possesses a distinctive fragrance reminiscent of nuts, complemented by a luscious, creamy flavour resembling that of milk and butter. The seeds have been employed for an extended period as consumable oilseeds and foodstuffs. Traditional health practitioners in South-Western Nigeria have recommended the decoction or infusion of various parts (roots, leaves, seeds) of this plant for treating a range of ailments, such as bruised skin, catarrh, eye pains, inflamed mouth membranes, chicken pox, measles, and taenia capitis infestations. Additionally, it can be used as a hair shampoo. The plant has been subjected to phytochemical analysis, which identified the presence of lignans, phenolic acids, flavonoids, saponins, and alkaloids (Adhikari *et al*., 2017). Sesame oil is a significant and ancient oilseed crop that has been widely utilised for several millennial. It is valued for its stable oil content and its nutritious protein, which is abundant in methionine, tryptophan, and valine (Nwalo, 2015) [23]. Sesame seeds have gained significant attention in recent research due to their antioxidant and anticarcinogenic properties. This has led to a rise in their use in health food items that promote liver and heart health, as well as help prevent tumours. Sesame seeds are rich in dietary fibre, vitamin B1, protein and serve as an outstanding supply of magnesium, phosphorus, iron, zinc, calcium, manganese, and copper (Ahmad *et al*., 2016).

Molecular Marker Studies in Sesame

In a conventional breeding process, entire genomes are combined by crossbreeding, and the most favourable recombinants are selected from the different segregation outcomes (Elleuch *et al*., 2012) [19]. Linkage drags, characterised by the tight coupling of undesirable loci with desired loci, might provide additional challenges in achieving the intended goal. This strategy needs several

crosses, generations, and meticulous phenotypic selection, making it a tedious and time-consuming process (Enyoh, 2019). The utilisation of marker-assisted selective breeding can significantly enhance breeders' capacity to identify the most advantageous gene combinations for desired traits. By incorporating novel and commercially valuable traits from wild relatives, landraces, and other sources, it is possible to introduce fresh genetic diversity and track variations in DNA sequences within and between species (Enemor *et al.*, 2019) [11]. Studies on diversity can be conducted using a variety of techniques, including morphological, analytical, biochemical, and molecular markers Tomimori *et al.* (2010). The main method used to evaluate genetic variations between sesame genotypes is morphology study. Multiple studies utilising morphological markers have demonstrated that sesame populations exhibit a significant degree of genetic diversity. The genetic divergence of Sesame (Sesamum indicum L.) landrace has been examined based on qualitative and quantitative characteristics (Nwalo, 2015) [23]. A study conducted in Pakistan analysed 105 sesame accessions collected from different environments and found significant variation in terms of morphological and agronomic traits. (Tashiro *et al*., 2007). However, because of the substantial influence of environmental factors and the high dependence of morphological markers on the culture circumstances, morphological markers are limited in their ability to estimate genetic diversity (Shivhare and Satsangee, 2012). Studying isozymes, or allelic variations of enzymes, is a component of biochemical research. This method, which makes use of enzymatic processes, is effective for determining the allele frequencies for particular genes (Sharma *et al*., 2020). Sesame cultivars have been the subject of isozyme studies for research on genetic variation. The main drawbacks of biochemical markers, which are comparable to morphological markers is that they are few in number and affected by the environment or a plant's stage of development (Sharma *et al*., 2016)

Inter Simple Sequence Repeats (ISSR) Marker

Sequences of DNA that are 100 to 3000 base pairs in length are known as inter simple sequence repeat (ISSR)-PCR. Sections of microsatellites facing in different directions sandwich these segments (Zhang *et al*., 2018) [7]. The polymerase chain reaction (PCR) is used to amplify ISSRs by using primers that are microsatellite core sequences and by inserting a small number of nucleotides that have been carefully chosen as anchors into nearby non-repetitive areas (16-18 bp). At the same time, anywhere from ten to sixty fragments are formed from various places, separated by gel electrophoresis, and then assessed according to whether or not there are fragments of a certain size (Morris *et al*., 2021). In order to create multi-locus markers, ISSR analysis methods employ polymerase chain reactions that utilise microsatellite sequences as primers. Directed amplification of minisatellite-region DNA and single primer amplification reaction are two methods that use a single primer with a core motif exclusive to a minisatellite (Mbadiwe, 2018) [21]. Table 1 shows the most often used ISSR primers, which have been used in a large number of research papers. The great genetic variability of ISSR markers makes them useful tools for studying evolution, gene identification, genome mapping, genetic diversity, and phylogeny (Nwalo, 2015) [23]. Sequences that are appropriate for DNA fingerprinting can be amplified by ISSR-PCR. It is not possible to differentiate individuals using this method since ISSRs can be preserved or non-conserved. Phylogeographical studies and, perhaps, species definitions benefit much from it, though. While ISSR does not have as much sequence variety as SSR-PCR, it is still greater than the level of sequence diversity found in actual gene sequences (Nwalo, 2015) [23]. Also, one technique can produce primers for the other; this is called a symbiotic interaction between microsatellite sequencing and ISSR sequencing. The ISSR Primers that are often used, together with the annealing temperature, sequences, and amplification efficiency that were optimised for them, are included in Table 1.

Table 1: List of Some ISSR Primers with their Annealing Temperature, Respective Sequences and Amplification Efficiency used during Optimization

Primer	Annealing Temperature (°C)	Primer Sequence	Amplification Efficiency
UBC-812	47	(GA)8A	Excellent
UBC-826	47	(AC)8C	No band
UBC-834	47	(CA)8AG	Excellent
UBC-835	48	(AG)8YC	Very good
UBC-841	48	(GA)8YC	Excellent
UBC-844	48	(AG)8YT	Excellent
UBC-848	48	(CA)8RG	No band
UBC-851	49	(GT)8YG	Poor
UBC-852	48	(AC)8T	Poor
UBC-854	49	(TC)8RG	Excellent
UBC-857	49	(AC)8AYG	Excellent
UBC-860	52	(TG)8RA	Poor
UBC-864	48	(CA)8RT	Very poor
UBC-865	47	(CCG)6	Poor
UBC-873	45	(GACA)4	No band
UBC-866	55	(CTC)6	Good
UBC-880	48	(GGAGA)3	Excellent
UBC-881	49	(GGTG)3	No band

Adapted from jbiolres.biomedcentral.com

Methods

1. Greenhouse Experimentation

A total of ten (10) distinct accessions of sesame were gathered from the National Cereals Research Institute (NCRI) in Badeggi, Niger State, Nigeria. The experiment was carried out in the greenhouse at AE-FUNAI using a blend of clay-loam soil that was created using animal excrement. The Diammonium phosphate (DAP) fertiliser was administered at a dosage of 100g per 10 buckets, along with antibiotics and antifungal agents. This was done at a temperature range of 25-37oC, pH level of 5-8, and a water treatment volume of 50-70cm3. The mixture was then put into ten well-labelled buckets with perforations. About five seeds of each genotype were scattered on the soil medium in each labeled bucket and were covered with grasses and an average daily temperature of 29.8°C. Water was sprinkled on daily for 21 days before all of them were vegetative grown for harvesting. It was raised 21 days because all of them do not have the same growth potential. After planting, some accessions germinated between 3 - 9 days of planting.

2. DNA Extraction Using SDS Extraction Protocol

Approximately 100mg of Silica gel was placed on the leaf samples on a clean mortar and 1ml of preheated DNA extraction buffer containing 20g SDS/l, 150 mMNaCl, 100 mMTris/HCl, 25 mM EDTA, (pH 8.0) and then pulverized using pestle until a homogenous mixture was obtained. The

ground samples were transferred into a properly labelled 2mL tube and subjected to incubation at a temperature of 650°C for a duration of 20 minutes. To homogenise the material, the tubes were occasionally inverted, causing agitation. The tubes were extracted and let to cool for 2 minutes. Then, 200µl of ice-cold 5M Potassium acetate was added. The protein was precipitated by incubating it on ice for 20 minutes. A further 500µl of chloroform Isoamylalcohol (24:1) was introduced and mixed delicately to induce further precipitation of protein and lipids. The sample was spun in a centrifuge at 10,000 RPM for 10 minutes to separate the components. Carefully, the resultant liquid was transferred from the sediment to freshly marked tubes. After cooling to a low temperature, 500µl of isopropanol was carefully added and stirred. The DNA was allowed to precipitate for one hour after the mixture was placed in an incubator set at -20oC. This was followed by 10 minutes of centrifugation at 10,000 rpm for the mixture. The liquid part was drained entirely. In order to clean the DNA pellet, a 70% ethanol solution, with a volume of around 400 microliters, was applied. Centrifugation was applied to the sample for 10 minutes at a speed of 10,000 rpm. After removing all of the liquid above the sediment, the solid residue was left to dry in the air until the ethanol smell went away. The DNA was then re-suspended using 60 ul of a low-salt Tris/EDTA solution, which included 10 mM Tris and 1 mM EDTA. Furthermore, 2 microliters of RNase A were included in the mixture before being incubated at 37 degrees Celsius for 35 minutes.

2.1 Gel Electrophoresis for DNA

Precisely 1 gramme of agarose was measured and combined with 100 millilitres of 1xTAE in a flask that is safe to use in a microwave. The agarose was heated in a microwave for 3-5 minutes until it entirely dissolved. The resulting solution was then allowed to cool down to around 60 °C, which is a temperature at which it may be easily touched with the hand. This cooling process took 5 minutes. The DNA can be observed using ultraviolet (UV) light when a volume of approximately 10µL of EZ vision DNA stain forms a compound with it. Crystallisation of the agarose was achieved by allowing it to cool at room temperature in a gel tray while the well comb was in place. Every DNA sample was treated with buffer and left to set. After that, the electrophoresis apparatus known as the gel box was used to place the agarose gel. To ensure that the gel was fully submerged, the container was filled with 1xTAE solution. The samples were injected into different wells of the gel, with a molecular weight ladder put in the first lane. After that, the gel spent an hour in an electric field that was 115 volts strong. The gel was removed from the gel box once the power source was turned off and the electrodes were disconnected. The last step was to use a UV transilluminator to see the DNA fragments or PCR results.

2.2 Polymerase Chain Reaction (PCR)

The following components were used to make the PCR mixture: 12.5µL of Taq 2X master mix (M0270) from New England Biolabs, 2µL of 10µM primer for each of the five primers listed in table 2, 2µL of DNA template, and 8.5µL of nuclease-free water to reach the final volume. Using a denaturation stage at 94˚C for 5 minutes, followed by 40 cycles of denaturation at 94˚C for 30 seconds, annealing at 55˚C for 30 seconds, and elongation at 72˚C for 45 seconds, was the thermal cycling approach. After 7 minutes of being kept at 72˚C, the temperature was lowered to 10˚C.

2.3 Data Scoring and Analysis

A data matrix was generated by assigning a score of 1 for the existence of ISSR bands in gels and 0 for their absence. The software Power-marker version 3.5 was utilised to compute the genetic diversity, shared allele, and polymorphic information content (PIC). The dendrogram was constructed utilising the neighbour joining approach with the utilisation of MEGA X. Table 2 below displays the five ISSR primers together with their corresponding sequences.

Table 2: List of Primers Used with their Respective Sequences

Primer	Primer Sequence
UBC 840	GAGAGAGAGAGAGAGAYT
UBC 811	GAGAGAGAGAGAGAGAC
UBC 855	ACACACACACACACACYT ((AC)8YT)
UBC 864	ATGATGATGATGATGATG
UBC 842	GAGAGAGAGAGAGAGAY

Minerals and Vitamins Analysis

Sesame seeds were subjected to analytical screening to determine the levels of minerals (iron, copper, calcium, magnesium, manganese, and zinc) and vitamins (thiamine, riboflavin, niacin, pantothenic acid, pyridoxine, and folic acid) using established protocols. The quantitative determination of vitamins was conducted using Harbone's titrimetric methods (1983), whereas the determination of minerals was carried out using the atomic absorption spectrophotometric method as specified by AOAC (1990).

Minerals Analysis of Sesame Seeds

Utilising the FS240AA Atomic Absorption Spectrophotometer in accordance with the AOAC (1990) protocol, the mineral analysis was carried out. Atomic absorption spectrometers work by transferring the material into a flame for atomization. Afterwards, the light beam from the spectrometer is guided into a monochromator, which in turn leads to a detector that detects the quantity of light absorbed by the atomized element in the flame. A metal source lamp was used to reduce the possibility of interference from other spectra or radiation since metals have a different absorption wavelength (AOAC, 1990). The quantity of the element in the sample is proportionate to the energy absorbed by the flame at the characteristic wavelength. Furthermore, a 2g sample was added to a digestion flask along with 20 ml of an acid mixture (650 ml concentrated HNO3, 80 ml perchloric acid, and 20 ml concentrated H2SO4) to facilitate wet digestion. Once a clear digest was achieved, the mixture was cooked in the digestion flask. In order to make the solution reach the 50 ml mark, distilled water was added. Atomic Absorption Spectroscopy (AAS) was then used to analyse the digest.

Vitamins Content Determination (AOAC, 1990)

a. Vitamin B_1

A sample weighing approximately 1g was placed into a conical flask and subsequently dissolved in 100ml of deionized water. The mixture was vigorously agitated and then subjected to heat for a duration of 5 minutes. Afterward, it was allowed to cool and subsequently filtered. The filtrate was transferred into a cuvette, and the

absorbance of the vitamins was measured using a spectrophotometer at their respective wavelengths. For vitamin B1, the wavelength used was 401nm.

b. Vitamin B_2

A sample weighing approximately 1g was measured and dissolved in 100ml of deionized water in a conical flask. The mixture was vigorously agitated and then subjected to heat for a duration of 5 minutes. Afterward, it was allowed to cool and subsequently filtered. The liquid that passed through the filter was transferred into a small container called a cuvette. The absorbance of the vitamins in the liquid was measured using a spectrophotometer, which detects the specific wavelengths of light absorbed by the vitamins.
The wavelength of Vitamin B2 is 242nm.

Calculations:

$$\text{Concentration (mg\%)} = \frac{\text{A x DF x cuvettevolume}}{\text{E}}$$

Where A = absorbance
E = extinction coefficient = 25 for B1 and B
DF = dilution factor

c. Vitamin B_3 (Nicotinamide)

A quantity of 5 grammes of the sample was dissolved in 20 millilitres of anhydrous glacial acetic acid and gently heated. A volume of approximately 5 millilitres of acetic anhydride was added and thoroughly mixed. Then, 2 drops of crystal violet solution were added as an indicator. The resulting mixture was titrated with a solution of 0.1 molar perchloric acid until it reached a greenish blue hue.
Calculation:

$$\text{Vitamin B3} = \frac{\text{titre value x 0.0122}}{0.1}$$

d. Vitamin B5

Approximately 0.2 grammes of the samples were placed into the separator. 5 ml of water was added to the separator, followed by thorough mixing. Subsequently, 5 ml of chloroform was used to extract the mixture. The aqueous layer was removed and the remaining chloroform was transferred into a dry 50 ml volumetric flask by passing it through anhydrous sodium sulphate. The flask was then filled up to the 50 ml mark with chloroform. After the samples were prepared, approximately 0.5ml of both the sample and blank solution were transferred into a test tube. Each test tube included 2 millilitres of a 0.2% phenyl hydrazine solution, prepared by mixing hydrochloric acid and alcohol in a 1:5 volume-to-volume ratio. The sample was further heated on a water bath until it reached a nearly dry state, and then cooled to room temperature. A solution mixture of ammonia and alcohol in a 1:1 ratio, totalling approximately 15 ml, was added to each test tube. The absorbance was measured at a wavelength of 635 nm relative to a blank sample.

e. Vitamin B_6

Approximately 0.1 of the sample was dissolved in a solution containing 1ml of anhydrous glacial acetic acid and 1ml of a 0.1m mercury II acetate solution.
About1ml of 0.1M perchloric acid was added. The absorbance read at 542nm against blank. Standard B6 concentration was expressed in mg/l.

f. Vitamin E

This was determined using the futter Mayer coulometric approach in conjunction with the expertise of vitamin chemists (Kirk and Sawyer, 1991). A quantity of 1 gramme of the sample was combined with 10 millilitres of ethanoic sulphuric acid and subjected to gentle boiling under reflux for a duration of 30 minutes. The substance was passed into a separating funnel and treated with precisely three portions of 30 millilitres of diethyl ether. After each treatment, the layer containing the recovered ether was separated. The ether extract was then transferred into a desiccator and dried for 30 minutes. Finally, it was evaporated at room temperature until completely dry. The desiccated extract was dissolved in 10ml of absolute ethanol. Approximately 1 millilitre of the dissolved extract and an equivalent volume of a standard vitamin E solution were put into separate tubes. Following the sequential addition of 5 millilitres of absolute alcohol and 1 millilitre of strong nitric acid solution, the mixtures were left undisturbed for a duration of 5 minutes. The resulting absorbance values were then measured using a spectrophotometer at a wavelength of 410 nanometres, with the blank reagent set as the reference point.

g. Determination of Folic Acid

5 grammes of the sample was dissolved in a solution containing 5 millilitres of anhydrous glacial acetic acid, 6 millilitres of mercury II acetate solution, and 2 drops of crystal violet as an indicator. A solution containing approximately 0.1 moles of perchloric acid was utilised to perform a titration until a green hue was observed, indicating the end point.
Each millilitre of 0.1 molar perchloric acid is equal to 0.02056 grammes of folic acid.

Biochemical Data Analysis

The values generated from titrating the analytes against the titrants using standard titrimetric method for each of the vitamins and mineral determination using atomic absorption spectrophotometer (AAS) were analysed and compared using Duncan post hoc test and values presented in mean ± standard deviation (mean± SD, ppm).

Results

Banding Pattern and Polymorphism of ISSR Markers.

Among the 10 genotypes, a total of 25 alleles were found. The average number of alleles per locus was 5, with the lowest and greatest numbers of alleles being 3 and 9, respectively. The range of polymorphic information content (PIC) values varied from 0.3142 (UBC 840) to 0.8680 (UBC 811), with an average PIC value of 0.5854.

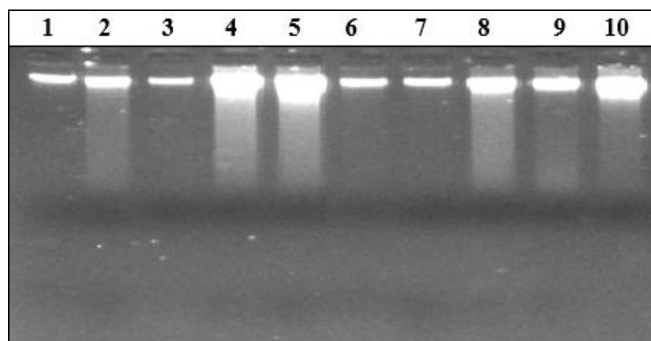

Fig 3: Gel Image of High Molecular Weight DNA Extracted from the Sesame Samples

Keys

NAK 017=1 DUM 043 =6
DOM 012=2 AGAI 039 =7
LAP 041=3 MAR 016 =8
GUM 024 =4 NGB/019 =9
NAS 034 =5 JIG 074 =10

Fig. 3, represent the gel image of high molecular weight DNA extracted from the ten sesame genotypes five primers of ISSR marker. The total numbers of alleles are altogether 25 alleles, with average PIC value 0.5854.

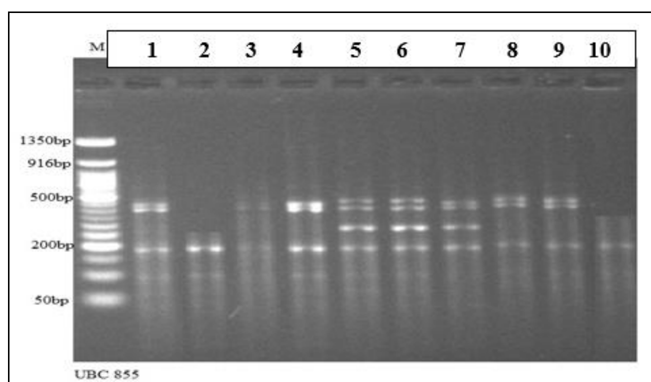

Fig 4: Bands of Amplified DNA of Different Varieties of Nigerian Sesame Using UBC: 855 ISSR Marker

Keys

NAK 017=1 DUM 043 =6
DOM 012=2 AGAI 039 =7
LAP 041=3 MAR 016 =8
GUM 024 =4 NGB/019 =9
NAS 034 =5 JIG 074 =10

The Fig. 4, represent the gel image of high molecular weight DNA extracted from the ten sesame genotypes using UBC 855 primer of ISSR marker. With approximate band size range of 200bp to 500bp dominated at 200bp with 5 major alleles and PIC value of 0.6756

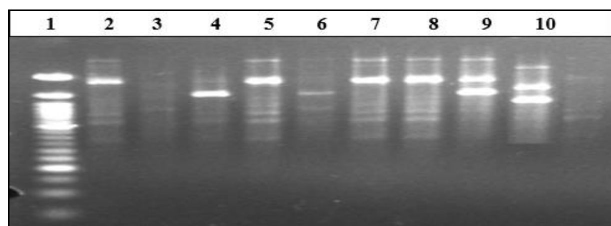

Fig 5: Bands of Amplified DNA of Different Varieties of Nigerian Sesame Using UBC: 811: ISSR Marker

Keys

NAK 017=1 DUM 043 =6
DOM 012=2 AGAI 039 =7
LAP 041=3 MAR 016 =8
GUM 024 =4 NGB/019 =9
NAS 034 =5 JIG 074 =10

Fig 5: represent the gel image of high molecular weight DNA extracted from the ten sesame genotypes using UBC 811 primer of ISSR marker. With 9 major alleles and PIC value of 0.8680

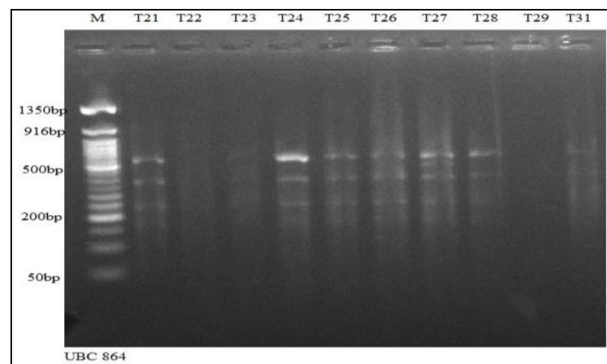

Fig 6: Bands of Amplified DNA of Different Varieties of Nigerian Sesame Using UBC: 864 ISSR Marker

Keys

NAK 017=1 DUM 043 =6
DOM 012=2 AGAI 039 =7
LAP 041=3 MAR 016 =8
GUM 024 =4 NGB/019 =9
NAS 034 =5 JIG 074 =10

The above Fig. 6, represent the gel image of high molecular weight DNA extracted from the ten sesame genotypes using UBC 864 primer of ISSR marker. With approximate band size range of 250bp to 500bp dominated at 250bp with 5 major alleles and PIC value of 0.5700

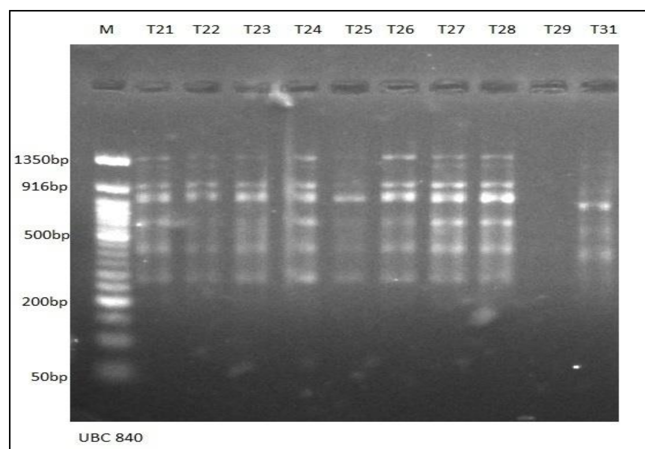

Fig 7: Bands of Amplified DNA of Different Varieties of Nigerian Sesame Using UBC: 840: ISSR Marker

Keys

NAK 017=1 DUM 043 =6
DOM 012=2 AGAI 039 =7
LAP 041=3 MAR 016 =8
GUM 024 =4 NGB/019 =9
NAS 034 =5 JIG 074 =10

This DNA bands represent the gel image of high molecular weight DNA extracted from the ten sesame genotypes using UBC 840 primer of ISSR marker. With approximate band size range of 200bp to 500bp dominated at 200bp with 3 major alleles and PIC value of 0.3142.

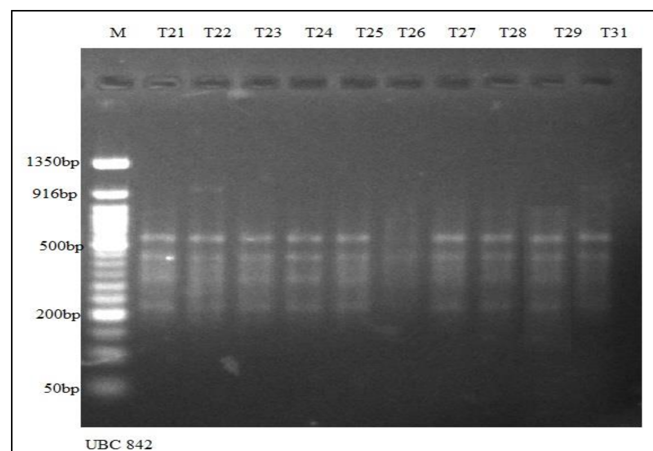

Fig 8: Bands of Amplified DNA of Different Varieties of Nigerian Sesame Using UBC: 842: ISSR Marker

Keys

NAK 017=1	DUM 043 =6
DOM 012=2	AGAI 039 =7
LAP 041=3	MAR 016 =8
GUM 024 =4	NGB/019 =9
NAS 034 =5	JIG 074 =10

This DNA bands represent the gel image of high molecular weight DNA extracted from the ten sesame genotypes using UBC 842 primer of ISSR marker. The total numbers of 3 alleles and PIC value of 0.4992

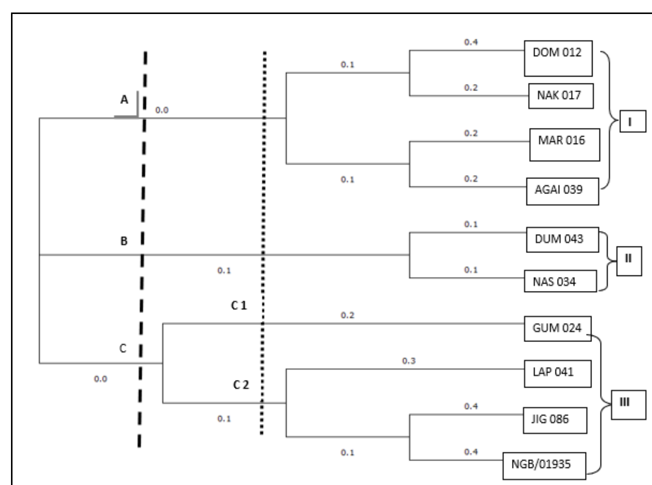

Fig 9: Dendrogram of Nigerian Sesame Genotypes with five ISSR markers using neighbor joining method

According to dendrogram and cluster analysis in a (Fig 10), above we three main groups, group I contained two subgroups, the subgroup (A) in group I consist of two genotypes DOM 012, NAK 017, while subgroup (B) in group I contained MAR 016, and AGAI 039. Group II contained two genotypes DUM 043 and NAS 034; whereas group III consisted of two subgroups, subgroup c1 contains one genotype GUM 024 and subgroup c2 contained three clusters, LAP 041, NGB/019 and JIG 074

Table 3: Genetic Diversity Parameters Among the Subpopulation of Nigerian Sesame

Marker	Allele Frequency	Sample Size	Allele No.	Gene Diversity	PIC
UBC 864	0.60	10.00	5.00	0.60	0.57
UBC 811	0.20	10.00	9.00	0.88	0.87
UBC 840	0.80	10.00	3.00	0.34	0.31
UBC 842	0.60	10.00	3.00	0.56	0.50
UBC 855	0.40	10.00	5.00	0.72	0.68
Mean	0.52	10.00	5.00	0.62	0.59

The summary statistics produced included the frequency of alleles, the number of alleles, gene diversity, and the PIC value. These statistics were calculated using the genetic analysis software PowerMarker (Liu & Muse, 2005) [20]. A total of 25 alleles were detected across the 10 sesame genotypes. The average number of alleles per locus was 5, with the minimum and maximum counts of alleles being 3 and 9, respectively. The range of polymorphic information content (PIC) varied from 0.31 (UBC 840) to 0.87 (UBC 811), with an average value of 0.59.

Table 4: Matrix of ISSR Dissimilarity Among Nigerian Sesame Seeds.

Sample	NAK017	DOM012	LAP041	GUM024	NAS034	DUM043	AGAI039	MAR016	NGB/019	JIG074
NAK017	0.00									
DOM012	0.60*	0.00								
LAP041	0.80**	0.80**	0.00							
GUM024	0.40	0.80**	0.60*	0.00						
NAS034	0.60*	0.80**	0.60*	0.40	0.00					
DUM043	0.40	0.80**	0.60*	0.40	0.20	0.00				
AGAI039	0.60*	0.80**	0.80**	0.60*	0.40	0.40	0.00			
MAR016	0.40	0.80**	0.80**	0.40	0.60*	0.60*	0.40	0.00		
NGB/019	0.80	1.00***	0.80**	0.60*	0.80**	0.80**	1.00***	0.80**	0.00	
JIG074	1.00***	1.00***	0.80**	0.80**	0.80**	0.80**	1.00***	1.00***	0.80**	0.00

* Moderate correlation ** Strong correlation *** Perfect correction

Table 5: The Minerals Content of the Nigerian Sesame Seeds in (mean ± SD, ppm)

Sample	Copper	Iron	Zinc	Manganese	Magnesium	Calcium
NAK 017	0.4846 ± 0.0042b	2.5697 ± 0.0239c	1.2883 ± 0.0067f	0.2673 ± 0.0413abc	6.1080 ± 0.1741d	4.7447 ± 0.1163c
DOM 012	0.7623 ± 0.0297e	4.2797 ± 0.0239g	1.3490 ± 0.0010g	0.4220 ± 0.0165e	5.0363 ± 0.0586c	5.2750 ± 0.0182e
LAP 041	0.8803 ± 0.0239f	3.2613 ± 0.0280d	0.9370 ± 0.0010d	0.5523 ± 0.0086f	6.3483 ± 0.0049b	6.3450 ± 0.0049h
GUM 024	0.0593 ± 0.0059a	3.3630 ± 0.0010e	2.8460 ± 0.0165j	0.3167 ± 0.0107cd	7.0260 ± 0.0338e	5.7807 ± 0.0222g
NAS 034	0.9477 ± 0.0058g	4.3160 ± 0.0010h	1.5390 ± 0.0338h	0.2137 ± 0.0124a	4.0050 ± 0.0010b	7.2870 ± 0.0010j
DUM 043	0.5460 ± 0.0173c	3.5100 ± 0.0010f	1.0600 ± 0.0010e	0.4090 ± 0.0010e	3.0533 ± 0.0577a	5.3480 ± 0.0173f
AGAI 039	0.7693 ± 0.0173g	2.3540 ± 0.0010a	2.0897 ± 0.0059i	0.3217 ± 0.0297cd	8.0853 ± 0.1088f	6.8107 ± 0.0569i
MAR 016	0.7693 ± 0.0231e	4.3730 ± 0.0010i	0.7523 ± 0.0586b	0.2457 ± 0.0559ab	7.0310 ± 0.0355e	6.8320 ± 0.0165i
NGB/019	0.8570 ± 0.0116f	3.3427 ± 0.0462e	0.9620 ± 0.0044d	0.4443 ± 0.0560e	9.0393 ± 0.0586g	1.9870 ± 0.0010a
JIG 074	0.6660 ± 0.0165d	2.5100 ± 0.0010b	0.5930 ± 0.0010a	0.3070 ± 0.0010bcd	8.0020 ± 0.0010f	4.2980 ± 0.0010b
JIG 086	0.9477 ± 0.0586g	3.3540 ± 0.0010e	0.8697 ± 0.0404c	0.3387 ± 0.0289d	0.0433 ± 0.0404e	4.9963 ± 0.0030d

The results above are presented as mean ± standard deviation. Values with different superscripts down the group are significantly different from each other at $p<0.05$. Duncan post hoc test was used for the multiply comparison.

Inferences drawn from the above (table: 5) shows that magnesium (Mg), calcium (Ca) and iron (Fe) are relatively higher while Zinc (Zn), Copper (Cu) and manganese (Mn) are relatively lower in the samples.

Table 6: Correlation Coefficient (r) Matrix of Minerals in Nigerian Sesame Seeds

	Copper	Iron	Zinc	Manganese	Magnesium	Calcium
Copper	1					
Iron	0.12	1				
Zinc	-0.5	-0.1	1			
Manganese	0.09	-0.09	-0.15	1		
Magnesium	0.02	-0.43	0.05	-0.24	1	
Calcium	0.11	0.3	0.31	-0.33	-0.47	1

The Pearson correlation coefficients for all the Minerals observed from the analytical result is presented in (Table 6) above which showed that the emboldened values correspond to very strong correlation coefficient.

Table 7: Concentration of Vitamins in the Nigerian Sesame Seeds (mean ± SD, PPM)

Sample	Vitamin B1	Vitamin B2	Vitamin B3	Vitamin B5	Vitamin B6	Folic Acid	Vitamin E
NAK 017	0.0127 ± 0.0115cd	0.0074 ± 0.0017a	0.0074 ± 0.0006bc	0.4668 ± 0.0035f	0.0460 ± 0.0012d	0.2668 ± 0.0012h	6.9372 ± 0.0017g
DOM 012	0.0070 ± 0.0018abc	0.0172 ± 0.0006bc	0.0073 ± 0.0006bc	0.1163 ± 0.0006a	0.0334 ± 0.0012b	0.0765 ± 0.0017d	4.8861 ± 0.0115c
LAP 041	0.0053 ± 0.0006ab	0.0179 ± 0.0006bc	0.0074 ± 0.0004bc	0.5109 ± 0.0004h	0.0385 ± 0.0003c	0.0645 ± 0.0011c	7.3581 ± 0.0039h
GUM 024	0.0072 ± 0.0006abc	0.0203 ± 0.0058c	0.0063 ± 0.0012bc	1.1616 ± 0.0017j	0.0317 ± 0.0023b	0.1037 ± 0.0017g	5.1647 ± 0.0017d
NAS 034	0.0123 ± 0.0017bcd	0.0170 ± 0.0001bc	0.0060 ± 0.0001bc	0.1983 ± 0.0001b	0.0567 ± 0.0001e	0.0833 ± 0.0001f	8.1049 ± 0.0001i
DUM 043	0.0074 ± 0.0002abc	0.0176 ± 0.0002bc	0.0069 ± 0.0018bc	0.3847 ± 0.0004e	0.0393 ± 0.0004c	0.2975 ± 0.0002i	8.1538 ± 0.0002j
AGAI 039	0.0106 ± 0.0005abc	0.0168 ± 0.0004bc	0.0036 ± 0.0001a	0.2383 ± 0.0001c	0.0339 ± 0.0018b	0.0054 ± 0.0001a	2.1882 ± 0.0001a
MAR 016	0.0160 ± 0.0001d	0.0160 ± 0.0007b	0.0053 ± 0.0028ab	0.3430 ± 0.0001d	0.0578 ± 0.0018e	0.0787 ± 0.0010j	4.3310 ± 0.0011b
NGB/019	0.0064 ± 0.0001abc	0.0073 ± 0.0003a	0.0054 ± 0.0002ab	0.7454 ± 0.0002i	0.0326 ± 0.0003b	0.3752 ± 0.0003e	5.2373 ± 0.0001e
JIG 074	0.0042 ± 0.0003a	0.0164 ± 0.0002b	0.593 ± 0.0001c	0.5066 ± 0.0003g	0.0255 ± 0.0003a	0.0172 ± 0.0003b	5.9187 ± 0.0002f

The results of (Table: 7) above are presented as mean ± standard deviation. Values with different superscripts down the group are significantly different from each other at $p<0.05$. Duncan post hoc test was used for the multiply comparison. Vitamin E followed by VitaminB_5 is relatively higher in all the ten sesame accessions while vitamin B_3 appeared least in all the samples.

Table 8: Correlation Coefficient (r) Matrix of Vitamins in Nigerian Sesame Seeds

Vitamins	Folic Acid	Vit. B6	Vit. B5	Vit. B3	Vit. B2	Vit. B1	Vit. E
Folic Acid	1						
Vit. B6	-0.00	1					
Vit. B5	0.15	0.03	1				
Vit. B3	0.05	-0.12	0.15	1			
Vit. B2	-0.72	0.03	-0.08	-0.14	1		
Vit. B1	-0.22	0.77**	-0.06	-0.65	0.23*	1.00	
Vit. E	0.31*	0.40**	0.22*	0.65**	0.01	-0.19	1

Pearson correlation here, * significance at 5% level of significance; ** significance at 1% level of significance; correlation coefficient of sesame vitamins in part per million (ppm).

Discussion

This study utilised Inter Simple Sequence Repeat (ISSR) markers to evaluate the genetic diversity of Nigerian sesame genotypes. The selection of the ISSR Maker was motivated by its simplicity and efficiency, since it combines the key benefits of microsatellite (SSRs) and amplified fragment length polymorphism (AFLP), while also offering the universal applicability of random amplified polymorphic DNA (RAPD). ISSR markers exhibit a high degree of polymorphism and are valuable tools for investigating genetic diversity, phylogeny, gene tagging, genome mapping, and evolutionary biology.

Five ISSR primers were used for the amplification (Table 4, Fig 3-7). Electrophoregram of the ISSR band profile of the genotypes of Sesame profile presented in Fig s 3 to 8

strongly indicates that each of the 10 studied *Sesamum indicum L* is a distinct genotype and as such, the five ISSR genotyping has proven to be a useful tool for their discrimination. The differences observed in banding patterns among the studied varieties illustrate genetic divergence among the species of sesame over evolutionary time which could be used for their improvement. All primers utilised in this investigation exhibited polymorphism at a rate of 100%, with the exception of UBC 811. The range of polymorphic information content (PIC) was 0.31 (UBC 840) to 0.87 (UBC 811), with an average value of 0.59. The polymorphic information in Table 3 revealed that UBC 811 had a lowest major allele frequency of 0.20 compared to 0.80 of UBC 840, while UBC 842 and UBC 864 with the allele frequency of 0.60 are the second highest followed by UBC 855 with major allele frequency of 0.40. The five ISSR markers showed an average allelic frequency of 0.52 (UBC 811 = 0.20, UBC 864 = 0.60, UBC 840 = 0.80, UBC 842 = 0.60, and UBC 855 = 0.40). The average mean of allele frequency 0.52 was similar to the 0.56 reported by Zhang *et al*. (2018) [7], lower than the (0.81) that reported by Enemor *et al*. (2020) and higher than (0.35) reported by Enyoh, (2019). The UBC 811 marker, which exhibits a greater polymorphic information content (PIC), is the most informative among the five markers utilised. Therefore, it is a superior marker for evaluating the genetic diversity of sesame genotypes. The PIC quantifies the primer's capacity to distinguish between various genotypes. The primers exhibited a moderate ability to distinguish the Nigerian sesame seeds, as indicated by the PIC value of 0.59 (almost 0.6). Hence, the average polymorphic information content (PIC) values discovered in the ISSR markers utilised in this investigation suggest that these markers are sufficiently informative, and the genotypes exhibit significant genetic diversity. Polymorphic information content (PIC) refers to the ability of primers to distinguish between different genetic variations and is commonly used as a comparative measure of the level of polymorphism. In the context of plants, the PIC values typically fall between the range of zero to 0.5. This range applies to both monomorphic ISSR markers and polymorphic ISSR markers. The polymorphic ISSR markers are found in 50% of the plants while being absent in the other 50%. (Wang *et al*., 2019) [35]. Markers with values ranging from 0 to 1 and loci having PIC values close to 1 are considered more acceptable. The PIC value, which is comparable to the findings of this investigation, was documented by Anggraeni *et al*. (2022) [3]. Nevertheless, the primer's primer identification codes (PIC) utilised in this investigation are lower compared to those reported by Tyagi, S. (2021) [32] (ranging from 0.48 to 0.85), but greater than the findings acquired by Mbadiwe (2018) [21] on Nigerian sesame (ranging from 0.02 to 0.35). The discrepancies identified across the different investigations may be attributed to the populations utilised, as well as the characteristics and quantity of molecular markers employed. Additionally, the numbers of alleles were altogether, 25 alleles. The UBC 811 markers produced the highest number of alleles, with a maximum of 9, whereas UBC 840 and UBC 842 markers produced the lowest number of alleles, with only 3. The observed variation in amplified alleles by different primers can be attributed to many variables, such as the primer structure and the presence of annealing sites within the genome (Adhikari *et al*., 2010).

The following minerals (Ca, Mg, Fe, Cu, Zn, and Mn) were evaluated in ten (10) sesame genotypes. Data in table 5 revealed that Mg, Ca, Fe were detected in all sesame genotypes at a relatively higher concentration. For instance; Mg concentrations 6.1080 ± 0.1741^d, 7.0260 ± 0.0338^e, 6.3483 ± 0.0049^b, 7.0260 ± 0.0338^e, 4.0050 ± 0.0010^b, 3.0533 ± 0.0577^a, 8.0853 ± 0.1088^f, 7.0310 ± 0.0355^e, 9.0393 ± 0.0586^g, 8.0020 ± 0.0010^f, and 0.0433 ± 0.0404^e in (mean ± SD, PPM) for NAK 017, DOM 012, LAP 041, GUM 024, NAS 034, DUM 043, AGAI 039, MAR 016, NGB/019, and, JIG 074 respectively. The concentration of Mg was markedly lower than the reported values of 37.4360 ±49.42 mg/kg by Christian *et al*. (2019) [8], which is similar with the findings of Ahmad *et al*. (2016) regarding other minerals. Calcium is a crucial mineral in the human body. It has a crucial function in maintaining bone health and regulating blood pressure. The suggested maximum threshold for calcium intake is 2500mg per day for persons between the ages of 19 and 50. The daily limit for individuals aged 51 and above is 2000mg/day, as stated by Abbas *et al*. (2020). The genotype NAS 034 had the greatest mean concentration of Ca, which was 7.28700±0.0010j. The calcium concentration range observed in this investigation, 7.28700±0.0010j-1.9870±0.0010a ppm, was determined to be lower than the tolerable limit reported by Ajibola *et al*. (1993). Iron (Fe) is a vital element for the metabolic processes in the human body, serving as a catalyst. In the absence of enzymatic catalysis, biological reactions are hindered or unable to take place at low levels of temperature and pressure that are suitable for sustaining life. Iron is a crucial trace mineral necessary for the synthesis of haemoglobin. The concentration of iron derived from the sesame genotypes employed in this investigation ranged from 2.3540±0.0010a – 4.3730±0.0010i. The results of this study showed that magnesium (Mg), calcium (Ca) and iron (Fe) are relatively higher while Zinc (Zn), Copper (Cu) and manganese (Mn) are relatively lower. From the results in (Fig 3) sesame accessions curves in graph showed that magnesium (Mg highest in NGB/019) followed by calcium is relatively higher in all the sesame seeds compared to manganese and copper that is relatively lesser in Nigerian sesame accessions. There was a positive relationship between the Iron content and Copper content in the sesame plants. Specifically, the Iron content and Copper content in the sesame plants are weakly and insignificantly related with a correlation coefficient of 0.12 and p- value of 0.73 as in (Table 3). There was a weakly negative relationship between the Iron content and the Zinc content in all the accessions as appeared in the correlation Table 3. Specifically, the Iron content and the Zinc content in the plants are weakly and insignificantly related with a correlation coefficient of -0.1 and p- value of 0.77. There was a negative relationship between the Magnesium content and the Calcium content in all genotypes. However, the Magnesium content and the Calcium content in the plants are moderately and insignificantly related with a correlation coefficient of -0.47 and p- value of 0.15. From the table 3, it's evident that there was also a negative relationship between the Manganese content and Magnesium content in all the accessions. Particularly, the Manganese content and the Magnesium content in the plants are weakly and insignificantly related with a correlation coefficient of -0.24 and p- value of 0.47. However, these values remain lower

than those obtained by Cao *et al*. (2015) in 39 Nigerian sesame genotypes.

Vitamins relationship profile in all the accessions was also analysed statistically using correlation analysis. There was a positive relationship between the Vitamin E content and the Folic acid content in sample ten (10) Sesame accessions. The results of vitamins concentration presented in (fig 4) showed that vitamin E is relatively higher in all sesame accessions but highest in DUM 043. Specifically, the Vitamin E content and Folic Acid content in the crop are moderately and insignificantly related with a correlation coefficient of 0.31 and p- value of 0.35 as in (Table4). There was a positive relationship between the Vitamin B_1 content and Vitamin B_2 content in all the accessions. There was a negative relationship between the Vitamin B_2 content and Vitamin B_3 content in all the plants. Specifically, the Vitamin B_2 content and Vitamin B_3 content in the samples are weakly and insignificantly related with a correlation coefficient of - 0.14 and p- value of 0.69. There was a positive relationship between the Vitamin B_3 content and Vitamin B_5 content in genotype 1 all through to genotype 10 (Table4). Thus, the Vitamin B_3 content and VitaminB_5 content in the plants are very weakly and insignificantly related with a correlation coefficient of 0.15 and p- value of 0.65. There was a positive relationship between the Vitamin B_5 content and Vitamin B_6 content in all the accessions. Specifically, the Vitamin B_5 content and Vitamin B_6 content in the plants are very weakly and insignificantly related with a correlation coefficient of 0.03 and p- value of 0.93. However, the result of the elemental composition is relatively lower than that reported by Beshaw *et al*. (2022) [3], which may be caused by the regional differences.

Conclusion

The primers exhibited a moderate ability to distinguish the Nigerian sesame seeds, as indicated by the PIC value of 0.59 (almost 0.6). Thus, the average PIC values discovered in the ISSR markers utilised in this work validate that these specific ISSR primers, particularly UBC 811, provide sufficient information and demonstrate genetic diversity among the genotypes. The dendrogram demonstrated a distinct separation of several sesame seeds according to their mineral and vitamin compositions. For instance, NAS 034 and DUM 043 both are relatively high and similar in vitamin E content. In all, 6 essential minerals and 7 vitamins that were quantified in ten Nigerian sesame genotypes by ISSR Marker, AAS and analytical process. Mg, Ca, Fe and zinc minerals and vitamin E, vitamin B5 and folic acid were found to relatively high in all the cultivars with NGB/019 having the highest percentage of Mg and DUM 043 having the highest composition of vitamin E.

Competing interests

The author declares that they have no competing interests.

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Authors' contributions

The research design and statistical analysis of the study, including the data collection was solely done by the author; Nwankwo Samuel Chiemerie did the literature search, manuscript preparation, and editing. The author equally reviewed and approved the manuscript.

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Availability of data and materials

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Consent for publication

Not applicable.

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