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ORIGINAL RESEARCH ARTICLE

Genetic Diversity Analysis and Molecular Characterization of some Groundnut (*Arachis hypogaea* L.) Genotypes using SSR-based Molecular Markers

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ABSTRACT

The cultivated groundnut (*Arachis hypogaea* L.) is very important for food security and agriculture around the world, especially in sub-Saharan Africa. Understanding this crop's molecular genetic diversity is critical for developing efficient breeding techniques and implementing germplasm conservation programs. This study used 10 polymorphic Simple Sequence Repeat (SSR) markers to evaluate genetic variation among 14 cultivated groundnut genotypes, comprising 5 local Nigerian landraces and 9 improved varieties. A total of 71 polymorphic bands were identified across the genotypes, with the number of alleles per locus ranging from 4 to 13, yielding an average of 7.10 alleles per locus. The markers displayed considerable polymorphism, as evidenced by polymorphic information content (PIC) values ranging from 0.67 to 0.98, yielding a mean PIC of 0.89. There was also notable gene diversity (mean = 0.71), suggesting significant allelic variation within the population. Marker FWGNF135 exhibited the greatest allelic richness (13 alleles), while Fw-Gnf3810 achieved the highest PIC value (0.98), underscoring their strong ability to differentiate among genotypes. Shannon's information index had an average value of 1.50 across the loci, further supporting the extensive genetic diversity represented by the marker set. The Analysis of Molecular Variance (AMOVA) demonstrated significant genetic differentiation among the genotypes (ΦPT = 0.162, P = 0.020). The majority of overall genetic variation (75%) was linked to differences within individuals, while 16% was found among populations and 9% within populations, suggesting moderate population differentiation and considerable within-individual diversity. Three unique genetic clusters have been identified by UPGMA analysis, which demonstrated considerable molecular divergence between local and enhanced germplasm and unambiguously distinguished the BAHAUSA (VAR11) landrace from the improved SAMNUT cultivars. The findings show that valuable genetic diversity is retained in farmer-maintained populations, and that SSR markers were highly effective in characterizing this variation and guiding strategic parent selection to improve yield and climatic resilience in groundnut breeding.

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INTRODUCTION

Cultivated peanut (*Arachis hypogaea* L.) is a globally significant grain legume crop that serves multiple purposes, providing essential food, vegetable oil, feedstock, and ground cover (Zheng et al. 2018; Abdurrasheed et al., 2024). It is an allotetraploid (2n = 4x = 40, AABB) member of the *Arachis* section and a significant crop grown in more than 100 nations worldwide (Hong et al. 2021). Although an anticipated 54 million tonnes of groundnuts were produced worldwide in 2021, productivity in Africa is still lower, with average yields of 885.6 kg/ha (Daba et al. 2023). Nigeria leads Africa and is the world's third-largest producer of groundnuts (FAO, 2021). In addition to being a significant source of edible oil, groundnuts are essential to the lives of small-scale farmers in Northern Nigeria (Ajeigbe et al. 2015). Despite this, many West African

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countries struggle to meet domestic demand, resulting in economic deficits and dependence on imports (Daudi et al. 2021). Increasing groundnut yield in Nigeria is crucial for both food security and reducing the region's dependency on imports (Daudi et al. 2021; Lokossou et al., 2022). The peanut (*Arachis hypogaea*) was introduced to Nigeria at the beginning of the sixteenth century. Small-seeded runners and bunch types of various seed and pod types make up the main grain output of the wide variety of locally adapted cultivars that are cultivated there now (Ajeigbe et al. 2015).

The main prerequisite for every plant breeding process intended to produce superior cultivars is genetic diversity (Tesfaye, 2021). The assessment of dissimilarity based on phenotypic features was the traditional method of determining this variety (Kameswara, 2007). However, the expression of morphological markers is largely dependent on the environment, posing considerable challenges for precise variance measurement. Molecular markers, on the other hand, are highly effective tools for selecting desirable agronomic traits because they are independent of environmental influences, sufficiently abundant, and directly based on the plant's genotype (Pandey et al., 2014; Daudi et al., 2021). Given these advantages, using high-throughput molecular methods is critical for assessing genetic variation and population structure in groundnut genetic resources (Daudi et al., 2021). Since its introduction into Nigeria in the sixteenth century, groundnuts have evolved into a broad spectrum of locally adapted landraces that coexist with improved cultivars such as the SAMNUT series. Despite this history, the genetic potential of these particular Nigerian collections is essentially untapped. As a result, the use of molecular markers is now required for successful screening and use of West African germplasm (Daba et al., 2025; Olasan et al., 2023).

High-throughput molecular technologies are essential for precisely characterizing genetic resources in today's breeding operations. Groundnut genetic variation studies have used a variety of molecular markers, including Restriction Fragment Length Polymorphism (RFLP), Random Amplified Polymorphic DNA (RAPD), Amplified Fragment Length Polymorphism (AFLP), and Single Nucleotide Polymorphisms (SNPs) (Dwivedi et al. 2017; Pandey et al. 2014; Vishwakarma et al. 2017; Daudi et al. 2021). Cost, automation compatibility, genome coverage, and ease of application all affect technique selection (Desmae et al., 2019). Simple Sequence Repeat (SSR) markers are preferred over other technologies because they are co-dominant, highly repeatable, and capable of identifying large levels of polymorphism (Pandey et al., 2014; Daudi et al., 2021). According to Nadaf et al. (2019), microsatellite markers remain a useful tool for analyzing genetic variation in cultivated crops, particularly groundnuts. Simple Sequence Repeat (SSR) markers are popular among existing technologies because they are co-dominant, highly reproducible, and capable of identifying high levels of variation over wide genome coverage (Pandey et al., 2014; Daudi et al., 2021). According to Nadaf et al. (2019), microsatellite-based markers are extremely useful for studying genetic differences in planted groundnuts. In Northern Nigeria, molecular analysis has begun to uncover different genetic partitions between inbred lines and indigenous landraces, indicating a pool of untapped alleles (Olasan et al., 2023). Despite this advancement, the majority of the extant literature focuses on larger African or Asian germplasm, leaving a gap in the genetic mapping of genotypes found in North-Western Nigeria. Given the crop's importance, it is critical to improve the varieties currently used or favored by farmers; however, the population structure of these distinct regional collections remains largely unknown. By analyzing a diversity panel comprising landraces and improved varieties collected from Katsina and Kano States, this study aims to close this gap. A collection of 10 SSR markers was selected based on their verified high Polymorphic Information Content (PIC) and wide genomic distribution to ensure a reliable yet economical evaluation, a crucial component for sustainable breeding in sub-Saharan Africa (Olasan et al., 2023). To overcome existing trade-offs in groundnut production, a more in-depth study of genotypic diversity is required. This study uses SSR markers to describe the genetic diversity of Nigerian landraces and SAMNUT lines, providing a fundamental molecular foundation for precision breeding. These findings enable crop improvement by selecting high-yielding, early-maturing, and multi-stress robust genotypes adapted to the Nigerian Savanna. Finally, this research contributes to the drive for high productivity and food security by integrating local agricultural breakthroughs with global sustainable development goals. This study aims to establish a basic genetic framework for these selected genotypes to enable future breeding and conservation efforts in the region.

MATERIALS AND METHODS

Plant Materials

The study used 14 groundnut (*Arachis hypogaea* L.) genotypes, including nine improved genotypes (SAMNUT series) from the Center for Dry Land Agriculture (CDA) at Bayero University in Kano and five local genotypes sourced from major groundnut-producing areas in Katsina and Kano states. Table 1 includes detailed descriptions and sources. The 14 genotypes were chosen based on their economic significance and prevalence in Northern Nigeria's agricultural terrain. SAMNUT variants are enhanced, high-yielding, disease-resistant lines that were unveiled to increase productivity in semi-arid environments. The locally obtained genotypes, on the other hand, are farmer-preferred landraces chosen for their long-standing adaptation to local pedoclimatic conditions and popularity among smallholder farmers.

DNA Extraction and Genotyping with SSR Markers

Plant Materials and DNA Isolation

A total of 14 A. *hypogaea* (Table 1) genotypes were sown under greenhouse conditions at the Faculty of Agriculture Research farm, Bayero University Kano, Nigeria (11.9799375^{0}N, 8.4211719^{0}E). Five seeds from each groundnut genotype were planted in plastic containers.

After 14 days of establishment, fresh, healthy leaves were collected from five randomly chosen plants per entry. These tissues were bulked before extraction, providing a stabilized representative DNA pool for each genotype.

Table 1: Genotypes and Their Sources of Collection

Germplasm/Genotype	Accession Number	**Status**	Source
G1	SAMNUT21	Released Variety	CDA
G2	SAMNUT22	Released Variety	CDA
G3	SAMNUT23	Released Variety	CDA
G4	SAMNUT24	Released Variety	CDA
G5	SAMNUT25	Released Variety	CDA
G6	SAMNUT26	Released Variety	CDA
G7	SAMNUT27	Released Variety	CDA
G8	SAMNUT28	Released Variety	CDA
G9	SAMNUT29	Released Variety	CDA
G10	MAI BARGO	Farmer Landrace	Kaura, Katsina
G11	BAHAUSA	Farmer Landrace	Bakiyawa,Katsina
G12	YARJINGILA	Farmer Landrace	Kaware, Katsina
G13	EX DAKAR	Farmer Landrace	Bakiyawa,Katsina
G14	MAI ZABUWA	Farmer Landrace	Bichi, Kano

Table 2: List of ten SSR primer pairs used for amplification of genomic DNA isolated from 14 groundnut genotypes

S/N	Marker	Forward Sequence	Reverse Sequence
1	GNF101	AATAAACCGGAGCAACCCAC	CCGACCTCGACAAGACACAT
2	GNF102	CAACCACACACACCTCAACT	AGACAATGGTGCGGATGAAG
3	GNF113	TGGCTGGCAATAAGTTGTGG	CTGGATCAAACTGGGATGGT
4	GNF124	CCCATCCAAACAAGTGAGAG	GACACATCTTGAGGTTCCAG
5	GNF135	AGAAGATGGGTGTGAGAGTG	ATGGAAGCTCAAGAGGAACC
6	GNF146	CAAGAGGAGAAGGAAAGGAG	GGATCTCACTTGCTTCAGAG
7	GNF147	TATGGGAAGGGGAAGAGTTG	GCTTCATGCTCAAGGTTCTG
8	GNF158	ATCTCCTGCTCCGACTTCTT	GCCGAGAGTTCAAGAATCGA
9	GNF169	TACGCATGCCACCTATTCGA	TCTAAATTGCTCGGAGCGGT
10	GNF3810	TGTTGCTTGGGATCCTTGTC	CACCCTCCGTATCTCATTGA

DNA Extraction and PCR

Genomic DNA was extracted from two-week-old plants using a modified CTAB method (Cuc et al., 2008), with some modifications. The DNA's purity and concentration were assessed using both a 1% agarose gel and a NanoDrop spectrophotometer, which measured absorbance at 260/280nm. The study employed ten specific SSR markers (see Table 2). Each PCR reaction was performed using 25.µl reaction mixtures that included 2.0µl of each template DNA sample, 12.5µl of CS-JADNA2X (Cleaver Scientific) PCR Master Mix (containing Taq polymerase, PCR Buffer, dNTPs, and MgCl2), 1µl of forward primer, 1µl of reverse primer, 8.5µl of nuclease-free water. The PCR process involved an initial denaturation at 94°C for 3 minutes, followed by 33 cycles of denaturation at 94°C for 30 seconds, annealing at 55°C-57°C for 30 seconds, and polymerization at 72°C for 1 minute. The final step consisted of an extension at 72°C for 5 minutes using a PCR machine from GTC965 Cleaver Scientific Ltd. PCR amplification products were resolved on a 2.5% (w/v) agarose gel stained with ethidium bromide to achieve high-resolution allele sizing. Electrophoresis was performed at 80V in 1× TAE buffer for 90 minutes. A 1-kb DNA ladder (Cleaver Scientific Ltd) was used as a molecular weight marker to identify fragment size and ensure correct scoring. A UV transilluminator was then used to visualize and photograph DNA bands.

SSR Marker Data Analysis

The SSR marker genotyping data underwent thorough analysis. The SSR markers were initially developed and screened across all genotypes using PCR analysis. Subsequently, genotypes were scored based on whether SSR bands were present or absent. Although SSRs are codominant, they were used as dominant markers in the present research to account for groundnut's complex genetic history. This method addressed difficulties in resolving multi-locus peaks and possible aneuploidy. Clear, reproducible bands were assigned a value of '1', whereas absent or unclear bands were assigned a value of '0', resulting in a standardized binary matrix for subsequent diversity analysis. The analysis involved assessing polymorphism by considering the presence or absence of bands in the data matrix. Genetic parameters, including the number of polymorphic bands, average alleles per locus, polymorphism information content (PIC), and AMOVA for each SSR locus, were calculated using GeneAlEx version 6.5 software and the method described by Gore et al. (2022). Significant levels were assessed using 999 permutations. The polymorphism information content (PIC) of primers was calculated using the formula PIC = 1 - ∑ij Pij2, where Pi and Pj are the frequencies of the ith and jth genes at a locus (Serrote *et al.*, 2020). NTSYS-pc v2.1 was used to create a genetic similarity matrix to clarify patterns of genetic variation among groundnut genotypes (Gore et al., 2022). The Unweighted Pair Group Method with Arithmetic Mean (UPGMA) was used to analyze genetic linkages and produce a dendrogram that shows the diversity within the germplasm panel in a hierarchical manner. According to Mafakheri et al. (2017), this clustering method was used to describe the genetic relatedness between the improved varieties and landraces.

RESULTS

Genetic parameters of the SSR Markers

A total of 10 pairs of SSR primer sets (Table 2) were used to evaluate genetic diversity and estimate genetic polymorphism in 14 groundnut genotypes using the co-dominant marker system. These markers revealed a great level of diversity in the study. Altogether, 10 pairs of SSR primers used in this study were polymorphic; a total of 71 polymorphic bands were amplified by these SSR markers Table 3. The distinct primers presented different banding patterns. In this study, not all markers noticeably revealed fixed amplification fragments. Many markers failed to amplify in some genotypes, while others produced clear, distinct bands. SAMNUT25, SAMNUT26, SAMNUT27, and SAMNUT28 showed consistent non-amplification. The presence of null alleles within these genotypes is indicated by this pattern, which may be caused by sequence differences at primer annealing sites or by the absence of the target loci in particular genomic backgrounds. From the studied primers, the least number of alleles per locus went from 4 alleles for fwGNF146 & CgbGF3810 each to the maximum number of 13 alleles for fwGNF135. The average number of alleles in this study is 7.10. A total of 71 bands were detected across all populations, all of which were polymorphic (not present in at least one of the 14 genotypes used in this study). The allele frequency per maker ranged from 0.286 to 0.929, with an average of 0.507, indicating allelic variation. There is also variation in gene diversity, ranging from 0.137 (primer fwGNF135) to 0.918 (primer fwGNF146), with an average of 0.705. Large Variations were also observed among the markers in polymorphic information content (PIC) values, ranging from 0.673 (fwGNF101) to 0.975 (cgbGNF3810), with a mean of 0.885.

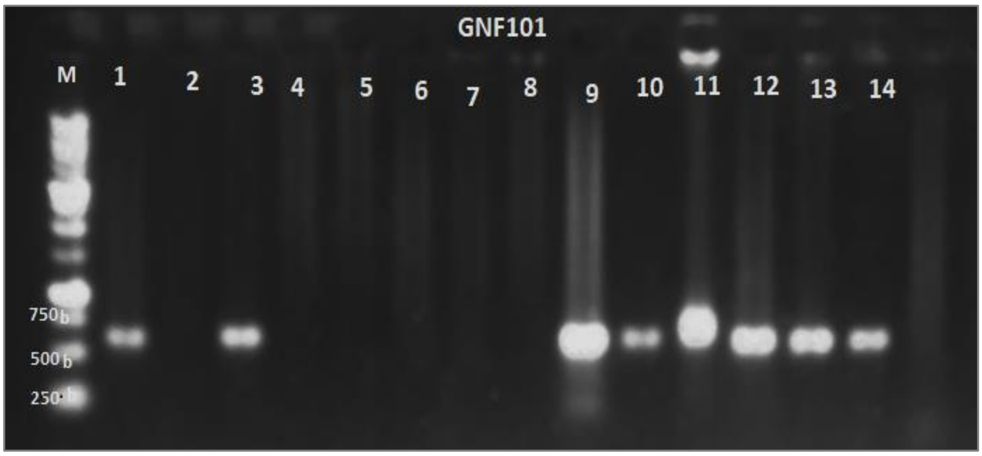

Plate 1: PCR-SSR analysis with the use of fwGNF101 on 14 groundnut genotypes

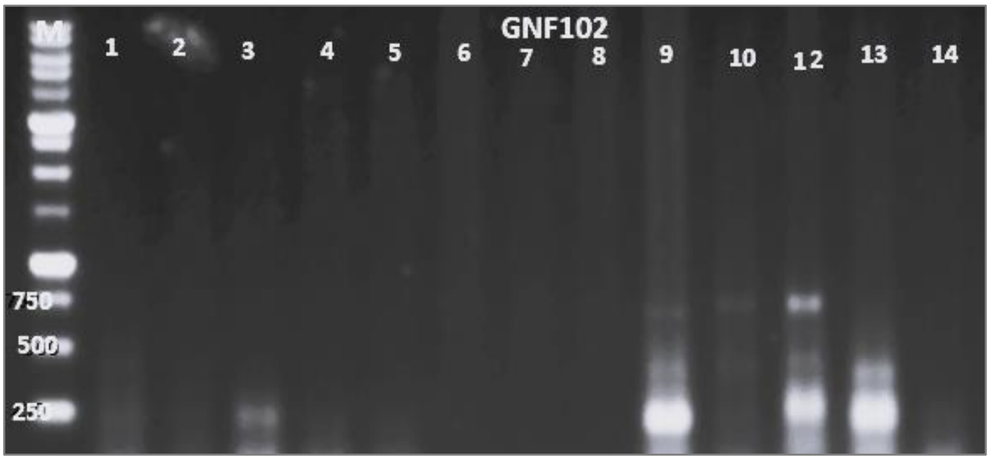

Plate 2: PCR-SSR analysis with the use of fwGNF102 on 14 groundnut genotypes

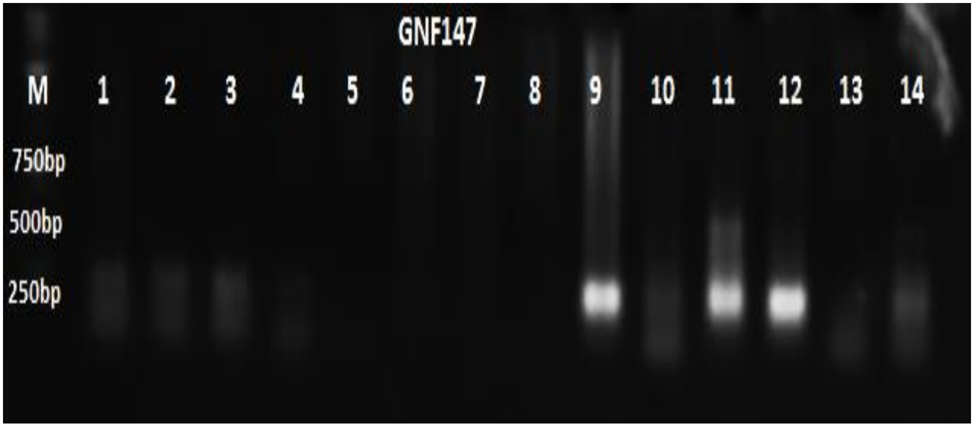

Plate 3: PCR-SSR analysis with the use of fwGNF147 on 14 groundnut genotypes.

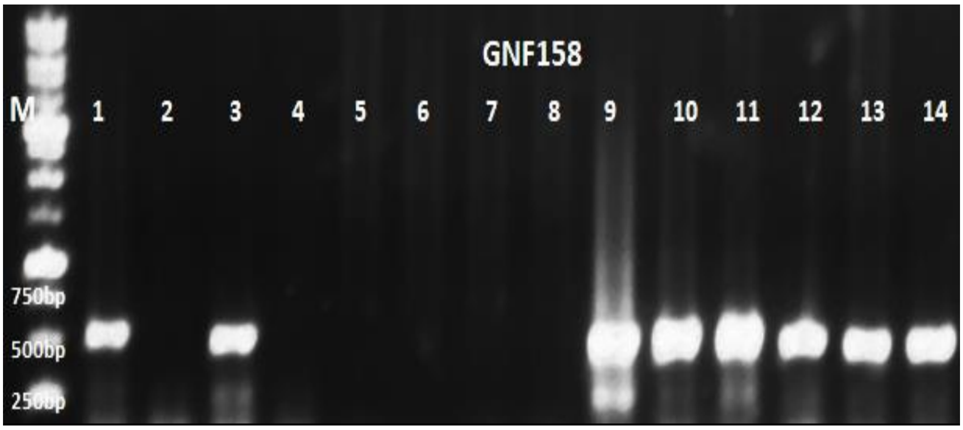

Plate 4: PCR-SSR analysis with the use of fwGNF158 on 14 groundnut genotypes

Table 3: Genetic diversity parameters of 14 groundnut genotypes based on 10 SSR marker

Locus/Primer	Allele size	No. of alleles	Allele frequency	Shannon Index (I)	Gene diversity (H_e)	PIC
FW-GNF101	580	8.00	0.571	2.079	0.674	0.673
FW-GNF102	250-755	6.00	0.429	1.243	0.816	0.948
FW-GNF113	322-500	6.00	0.429	1.040	0.816	0.949
FW-GNF124	230-513	10.00	0.714	1.386	0.490	0.827
FW-GNF135	220-509	13.00	0.929	1.995	0.137	0.783
FW-GNF146	250-518	4.00	0.286	1.099	0.918	0.974
FW-GNF147	250-312	5.00	0.357	1.386	0.873	0.957
FW-GNF158	457-600	9.00	0.643	2.076	0.587	0.834
FW-GNF169	457-780	6.00	0.428	1.609	0.817	0.934
GB-GNF3810	550-916	4.00	0.286	1.083	0.918	0.975
Total		71	------	--------		-----
Mean		7.10	0.507	1.500	0.705	0.885

Table 4: Analysis of molecular variance (AMOVA) for the groundnut genotypes of 10 SSR markers

Source	D f	SS	MS	Est. Var.	% of variation	Statistics	Fixation indices (Φ_{ST})	P - Value
Among Population	1	5.837	5.837	0.314	16%	Φ_{PT}	0.162	0.020*
Among Individual	12	21.556	1.796	0.166	9%	Φ_{IS}	0.102	0.229
Within Individual	14	20.500	1.464	1.464	75%	Φ_{IT}	0.247	0.054
Total	27	47.893	-	1.945	100%		-	

*Significant at $p < 0.05$based on 999 computations

Analysis of molecular variance (AMOVA) for the groundnut genotypes of 10 SSR markers

Table 4 shows the hierarchical partitioning of genetic diversity using the Analysis of Molecular Variance (AMOVA). Total molecular variation was assessed at three levels: among populations, among individuals within populations, and within individuals. A significant portion of genetic variance (75%) was contained within individuals (intra-individual variation). The substantial internal genetic diversity seen is likely due to the allotetraploid nature of Arachis hypogaea, which allows SSR markers to detect differences between homeologous sub-genomes or residual heterozygosity within the lines.

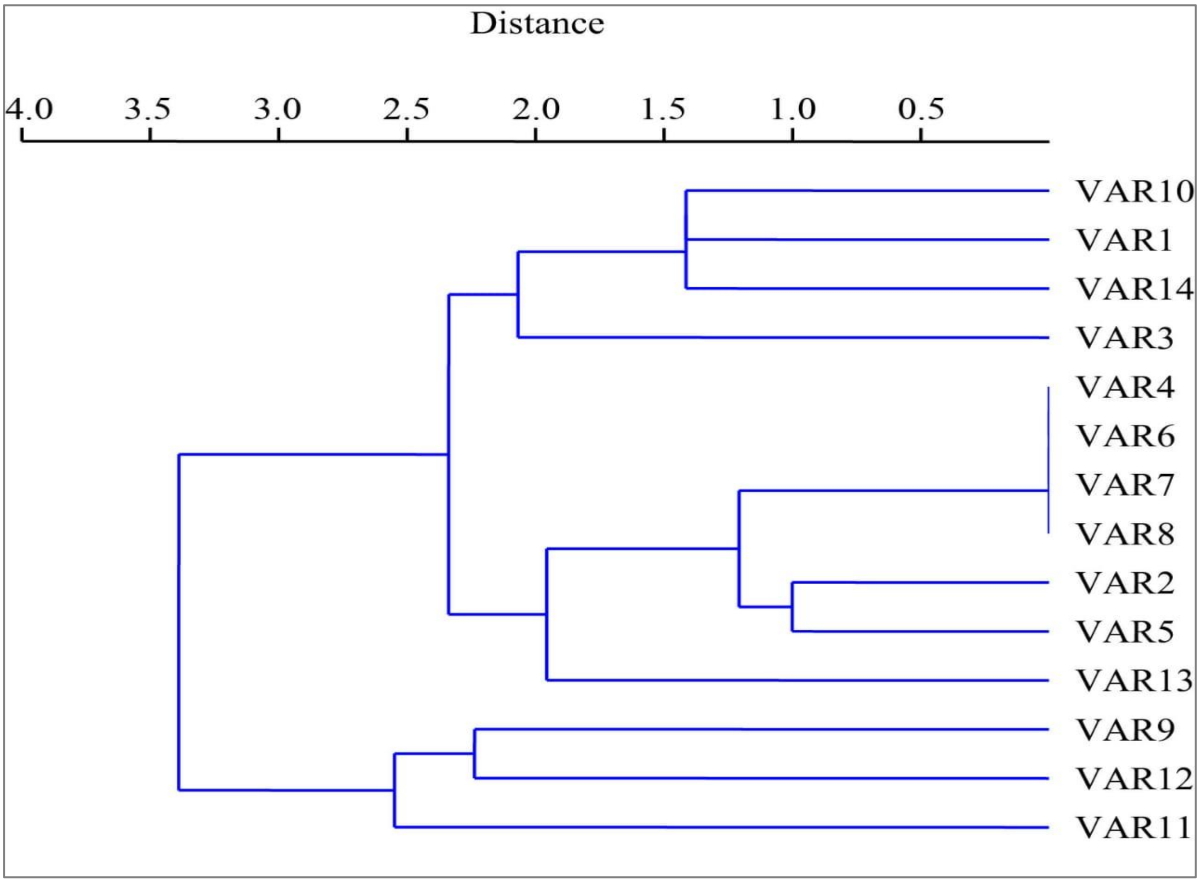

Figure 1: Dendogram showing Euclidean genetic distance among 14 groundnut genotypes obtained using SSR markers

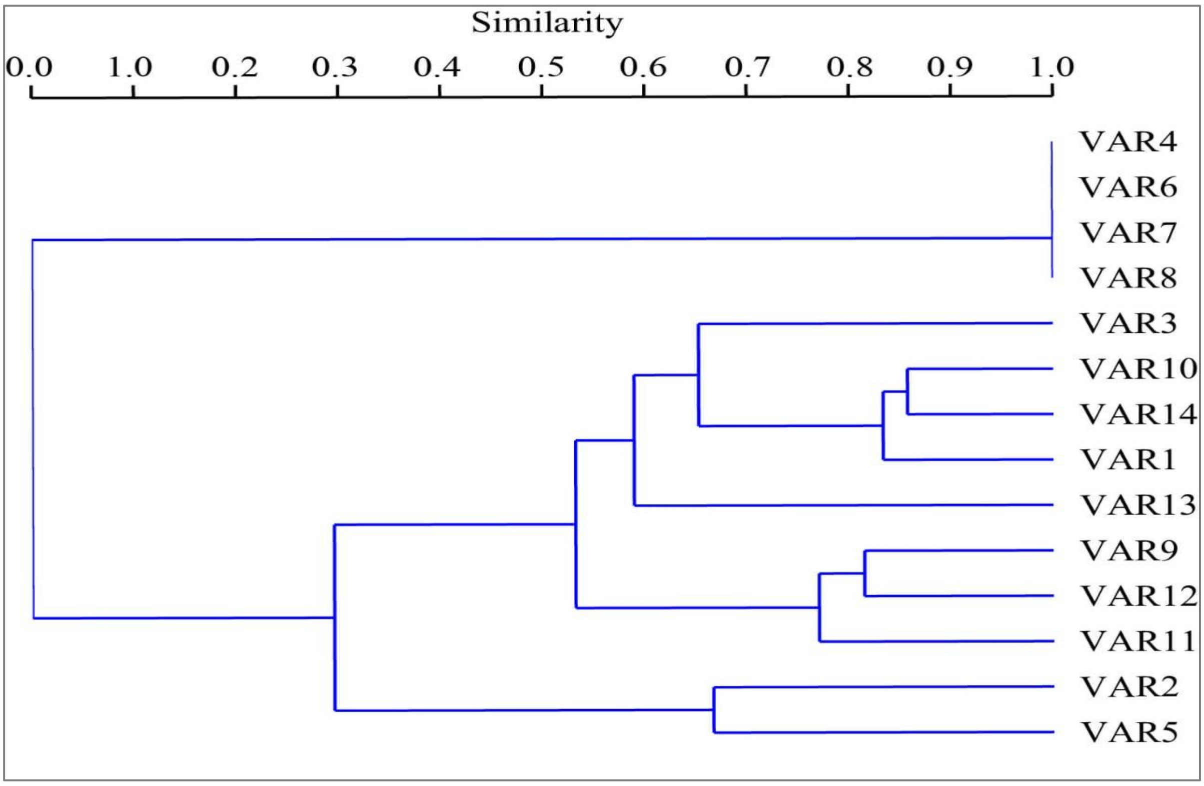

Figure 2: Dendogram showing Euclidean genetic similarity among 14 groundnut genotypes obtained using SSR markers

A moderate but statistically significant degree of genetic differentiation between the Improved (SAMNUT) and "Landrace" groups is indicated by the 16% genetic variance among populations, the associated fixation index (ΦPT = 0.162), and a significant P-value (P = 0.020). This implies that local landraces retain a unique genetic identity even when breeding operations have used a variety of parents. The least amount of variance (9%) came from variations among certain genotypes within their respective populations. This low proportion suggests that individuals in the Landrace and Improved groups exhibit a comparatively high level of genetic homogeneity at the SSR loci examined.

Plates 1 and 2 are the separation of alleles on 1% agarose gel stained with ethidium bromide and imaged under UV light. PCR products were amplified by groundnut SSR primers fwGNF101 (Plate 1) and fwGNF102 (Plate 2). The M is the molecular ladder marked with band size in base pairs, and the numbers 1-14 represent the groundnut genotypes. 1= SAMNUT21, 2=SAMNU22, 3= SAMNUT23, 4=SAMNUT24, 5=SAMNUT25, 6=SAMNUT26, 7=SAMNUT27, 8=SAMNUT28, 9=SAMNUT29, 10=MAIBARGO, 11=BAHAUSA, 12=YARGINLA, 13=EXDAKAR and 14 is MAIZABUWA

Plate 3 & 4 is the separation of alleles on 1% agarose gel stained with ethidium bromide and imaged under UV light. PCR products were amplified by groundnut SSR primers fwGNF147 (Plate 3) and fwGNF158 (Plate 4). The M is the molecular ladder marked with band size in base pairs, and the numbers 1-14 represent the groundnut genotypes. 1= SAMNUT21, 2=SAMNU22, 3= SAMNUT23, 4=SAMNUT24, 5=SAMNUT25, 6=SAMNUT26, 7=SAMNUT27, 8=SAMNUT28, 9=SAMNUT29, 10=MAIBARGO, 11=BAHAUSA, 12=YARGINLA, 13=EXDAKAR and 14 is MAIZABUWA.

The Euclidean clustering method- Bray-Curtis dissimilarity and similarity using the weighted pair group method with arithmetic mean (UPGMA) obtained from molecular Data

Based on the binary (1/0) SSR marker data, the dendrogram in Figure 1 and 2 shows the genetic distances among the 14 groundnut genotypes. The UPGMA dendrogram, which uses genetic distance coefficients, efficiently partitioned the 14 groundnut genotypes into three major clusters at a threshold of roughly 2.5-3.0, indicating high germplasm variation. Cluster I is a compact group divided into Subcluster IA: VAR1(SAMNUT21), VA10 (MAIBARGO), and VAR14 (MAIZABUWA), which has the shortest spatial distance and highest relatedness, and Subcluster IB: VAR3 (SAMUT23), which is its closest relative. Cluster II is a larger grouping divided into Subcluster IIA; which contains the genetically identical VAR4(SAMUT24), VAR6(Samut26), VAR7 (SAMUT27), and VAR8 (SAMUT28) and Subcluster IIB, which pairs VAR2(SAMUT22), and VAR5(SAMUT25), with VAR13(EXDAKAR). Cluster III, on the other hand, is the most divergent group, branching out at a distance of 2.8. It is further divided into Subcluster IIIA; VAR9(SAMUT29), and VAR12(YARGINLA), and Subcluster IIIB VAR11(BAHAUSA), the latter being the panel's most genetically distinct genotype. The greatest observed distance of 3.6 between the main nodes highlights significant evolutionary divergence, suggesting that the divergent genotypes in Cluster III, particularly VAR11(BAHAUSA), are promising candidates for boosting hybrid vigor.

Using the UPGMA approach, the 14 groundnut genotypes were partitioned into three separate genetic groups at a similarity threshold of 0.60, with coefficients ranging from 0.30 to 1.00. Cluster I is the most homogeneous group, characterized by Subcluster IA, with VAR4 (SAMNUT24), VAR6 (SAMNUT26), VAR7 (SAMNUT27), and VAR8 (SAMNUT28) exhibiting near-perfect similarity (~1.00). This group is a major genetic outlier relative to the remainder of the collection. Cluster II shows moderate genetic relatedness and is further divided into two groups: Subcluster IIA, which comprises VAR1 (SAMNUT21) and the local landraces VAR10 (MAIBARGO) and VAR14 (MAIZABUWA), distinguished by a strong internal linkage of 0.85, and Subcluster IIB, where VAR3 (SAMNUT23) and VAR13 (EXDAKAR) join the cluster at a lower threshold of 0.65. Cluster III has high internal diversity and is divided into Subcluster IIIA, which contains VAR9 (SAMNUT29), VAR11 (BAHAUSA), and VAR12 (YARGINGILA) with roughly 0.75 similarity, and Subcluster IIIB, which contains VAR2 (SAMNUT22) and VAR5 (SAMNUT25). The strong reciprocal similarity within Subcluster IIIB shows a common pedigree, despite the group splitting from the main population at a lower similarity of 0.30. Overall, our data suggest a highly organized population in which the most divergent genotypes, particularly those within Cluster III, such as VAR11 (BAHAUSA), offer the greatest promise for widening the genetic base.

DISCUSSION

Genetic diversity is the most important resource for crop improvement since it provides the necessary variability for cultivar selection and improvement. In groundnut breeding, molecular characterization of germplasm is an important step for identifying genetically distinct parental lines for trait improvement and linkage mapping. Simple Sequence Repeats (SSRs) remain a popular molecular tool because of their high polymorphism, co-dominant inheritance, repeatability, and genome-wide distribution (Roomi et al., 2014). In line with SSR-based diversity observed in groundnut and other legume crops across Africa, the polymorphism identified in this study indicates quantifiable genetic variation among the assessed groundnut accessions (Mace et al., 2007; Mondal & Badigannavar, 2010; Pandey et al., 2024). Given that the domestication and polyploid origin of cultivated groundnut have historically limited its overall genetic base, recent studies highlight moderate polymorphism and significant intra-population variation across the continent (Mofokeng et al., 2021; Diallo et al., 2024). Farmer-managed seed systems, which encourage gene flow and preserve heterogeneity within local germplasm pools through regular exchange of planting material across communities, also affect this variation in the Nigerian and wider West African environment. According to Mahamane et al. (2024), the identified diversity serves as a crucial reservoir for improving yield stability, disease resistance, and adaptability to the changing climatic circumstances that are currently affecting West African groundnut production systems.

The SSR markers used in this study revealed more alleles than in previous studies (Pramanik et al., 2019), similar to the findings of Mafakheri et al. (2017), who identified 4 to 14 alleles per locus, with an average of 8.45 alleles per primer. The presence of more alleles per locus is an important criterion for selecting a marker for assessing genetic variability (Gore et al. 2022). The allele frequency per marker indicates that allelic diversity exists between genotypes. This is consistent with the findings of Gore et al. (2022), who reported a main allele frequency range of 0.27-0.90, and Odongo et al. (2015), who reported an average allele frequency of 0.76. This shows that there is good allelic variation, which is necessary for assessing genetic diversity (Daudi et al., 2021). Variation in the number of alleles per locus across publications suggests that the diversity and composition of the genotypes used significantly influence the allelic estimate.

The mean gene diversity (He) of 0.705 observed in this study is significantly higher than the values of 0.11, 0.59, and 0.626 reported by Ren et al. (2014), Wang et al. (2011), and Daudi et al. (2021), respectively, indicating the fact that there were indeed multiple variations of the genes in this population considering that it included diversified genotypes such as released varieties and traditional landraces. The substantial gene diversity indicates that the SSR markers utilized were highly polymorphic (Daudi et al., 2021). This increased diversity indicates that the present panel has a broad genetic foundation, most likely due to the intentional inclusion of both improved SAMNUT cultivars and geographically diverse Nigerian landraces. According to Conde et al. (2023), in recent studies on West African groundnut, the integration of distinct germplasms with different selection histories is a major driver of high allelic variation. The considerable gene diversity suggests that the SSR markers used proved highly polymorphic and useful for germplasm characterization. Using molecular markers with high polymorphism information content and gene diversity improves the resolution of genetic variations among closely related groundnut genotypes (Daudi et al., 2021; Reddy et al., 2022; Kalyani & Sasidharan, 2021). These results demonstrate that gene diversity remains a crucial factor in determining marker efficiency, and that greater diversity and PIC values typically translate into greater discriminatory power in diversity analyses.

The mean Polymorphic Information Content (PIC) of 0.88 attained in this study highlights the markers' improved ability to differentiate across genotypes. These findings are consistent with recent genetic diversity assessments in Arachis hypogaea by Daudi et al. (2021), Reddy et al. (2022), and Kalyani & Sasidharan (2021), all of which emphasize the significance of high polymorphic information content (PIC) values and gene diversity indices for establishing robust molecular baselines. Studies using SSR, RAPD, and ISSR markers revealed that primers with high PIC and gene diversity successfully distinguish between closely related genotypes, enabling precise evaluation of genetic differences among landraces and improved cultivars. Zhao et al. (2014) reported that even among geographically dissimilar cultivars, high PIC markers improve the visibility of genetic linkages, suggesting their utility for germplasm characterization and for improving breeding program efficiency. Differences in PIC values correspond to differences in the markers and genotypes utilized in research. The PIC values of all markers employed in the present study were more than 0.5, indicating their potential for identifying the supplied groundnut accessions collections (Nashath et al., 2024). However, it demonstrates that each study's germplasm has sufficient genetic variety that may be used to enhance groundnuts. The panel comprised both closely related and divergent genotypes, since genetic variability arises from differences in an individual's genetic makeup. Additionally, it demonstrates that the markers effectively differentiated between genotypes, which is essential in genetic research to assess the degree of genetic diversity in the gene pool (Daudi et al. 2021). Higher PIC values and multiple-locus amplification primers have been suggested for phylogenetic research, breeding applications, and the characterization and assessment of germplasm.

Analysis of Molecular Variance (AMOVA) based on allelic variants identified by SSR markers showed that the greatest amount of genetic variation (75%) was distributed within individuals, followed by variation among populations (16%) and among individuals within populations (9%), with a significant degree of population differentiation ($\Phi PT = 0.162$; $p = 0.020$). The majority of variation is preserved within populations and individuals; this distribution shows moderate but significant genetic structure within the assessed groundnut populations. According to Uba et al. (2021), the high genetic diversity within the individual population, as measured by AMOVA, can be attributed to natural adaptation, extensive seed exchange between farmers and environments, or the population's common origin. A similar prevalence of within-population and within-individual variance (above 70%) has been documented in African groundnut germplasm, indicating shared ancestry and gene flow (Mace et al., 2007; Abady et al.,2019; Mofokeng et al., 2021; Daudi et al., 2021). The moderate fixation index ($\Phi ST \approx 0.16$) in this study suggests some degree of genetic divergence, potentially influenced by geographic separation, selection pressure, or breeding history. This could suggest that the genotypes studied here are substantially more different than the highly homogenized seed systems commonly observed in various West African producing zones. Moderate differentiation and significant intra-population variation have been linked to farmer-managed seed systems and regular interchange of planting materials, which increase gene flow and limit strong population stratification (Pandey et al., 2024; Diallo et al., 2024).

Additionally, the prevalence of within-individual variation is compatible with *Arachis hypogaea's* self-pollinating behavior and its complex allotetraploid nature, which results in limited but noticeable differentiation over time (Mondal & Badigannavar, 2010). This study builds on the findings of Banla et al. (2020), who reported an AMOVA and found that the majority of the genetic variation observed was due to differences between individuals, suggesting that the variation was less influenced by collection sources or population structure. This trend is similar in groundnut germplasm, where populations exhibit the lowest genetic structure, but the majority of exploitable variation is within individuals (Daudi et al., 2021; Abady et al., 2021).

The high within-population and within-individual diversity provides an excellent reservoir of alleles for selection, whereas the significant among-population differentiation suggests the crossing of genotypes from different populations could broaden the genetic base for marker-assisted improvement and multi-stress resilience breeding programs.

The UPGMA dendrogram revealed significant genetic structuring among the 14 groundnut genotypes, which included nine improved SAMNUT varieties (SAMNUT21-SAMNUT29) and five farmer-preferred landraces (MAIBARGO, BAHAUSA, YARGINGILA, EXDAKAR, and MAIZABUWA), with a maximum Euclidean distance of 3.6, confirming substantial allelic divergence within the panel. The division into three major clusters at a threshold of 2.5-3.0 indicates both similar breeding history and local farmer selection. The close grouping of several improved varieties (e.g., SAMNUT24, SAMNUT26, SAMNUT27, and SAMNUT28) suggests derivation from closely related parental stocks, consistent with reports that African groundnut improvement programs frequently rely on a relatively narrow genetic base due to repeated recycling of elite lines (Bertioli et al., 2016; Pandey et al., 2024). Certain landraces (e.g., MAIBARGO and MAIZABUWA) cluster with improved lines, indicating historical gene flow or introgression between formal breeding materials and farmer-managed germplasm, a pattern previously noted in West African research, where informal seed exchange systems promote admixture (Ajeigbe et al., 2015; Frimpong et al., 2015). In contrast, the substantial divergence in Cluster III, particularly BAHAUSA (VAR11), demonstrates the survival of unique allelic reservoirs among traditional types. Similar results have been reported in recent African SSR-based diversity studies, in which unique landraces formed separate clusters from improved cultivars and were recommended as key parents to expand the breeding pool (Conde et al., 2023). This observation supports the findings of Younis et al. (2020). In self-pollinated crops like groundnut, crosses between genetically distant parents from different UPGMA clusters have been linked to increased heterotic expression, greater transgressive segregation, and better recovery of complex traits like drought tolerance and aflatoxin resistance (Pandey et al., 2024). Thus, the considerable inter-cluster diversity observed, particularly between the SAMNUT lineage and BAHAUSA, provides a strong scientific basis for targeted hybridization to broaden the genetic base and strengthen climatic resilience in sub-Saharan African groundnut breeding programs.

On the other hand, the observed genetic architecture, as evidenced by a wide range of similarity coefficients (0.30-1.00), indicates a high level of genetic diversity within this groundnut panel, which is required for effective trait introgression in West African breeding programs. The near-perfect similarity within Subcluster IA (improved varieties SAMNUT24, SAMNUT26, SAMNUT27, and SAMNUT28) indicates a significant narrowing of the genetic base or potential redundant nomenclature within the regional seed system, a phenomenon frequently documented in Nigerian germplasm where intensive directional selection for specific traits like early maturity leads to "pedigree-narrowing" (Ajeigbe et al., 2015).

In contrast, the integration of farmer-preferred landraces with improved varieties in Subcluster IIA (SAMNUT21 with MAIBARGO and MAIZABUWA) and Subcluster IIB (SAMNUT23 with EXDAKAR) suggests a shared selection history or the potential to use these local genotypes as founding parents in the development of the SAMNUT series. The most notable crop improvement findings are the divergence of Cluster III, specifically the clustering of the landrace BAHAUSA (VAR11) with SAMNUT29 and YARGINGILA, and the early branching of the SAMNUT22/SAMNUT25 pair at a low similarity of 0.30. Selecting parents from widely separated UPGMA or cluster groups maximizes heterosis and increases the likelihood of recovering superior transgressive segregants for complex traits such as drought tolerance and pathogen resistance (Upadhyaya et al., 2003; Pandey et al., 2024). Besides, the genotype partitioning is consistent with recent molecular characterizations of African groundnut collections (Pandey et al., 2024), confirming that strategic hybridization between divergent lineages in Cluster III and stable, improved lines in Cluster II is a strategic imperative for broadening the genetic base to meet evolving sub-Saharan environmental pressures. The genotypes of each subgroup are closely connected (Younis et al., 2020).

CONCLUSION AND RECOMMENDATION

The genetic analysis of 14 groundnut genotypes revealed a robust allelic reservoir, AMOVA effectively partitioned the majority of this genetic variation within genotypes, which is critical for increasing yield, while UPGMA clustering further structured the population into three distinct groups, addressing a critical research gap by providing a comparative molecular baseline between some Nigerian farmer-preferred landraces and improved SAMNUT lines. Clusters I and II revealed strong genetic similarity between improved varieties and local landraces, such as MAIBARGO and MAIZABUWA, whereas Cluster III identified the landrace BAHAUSA (VAR11) as a notable genetic outlier, with a maximum evolutionary distance of 3.6. While these findings highlight significant diversity, the study recognizes the limitations of gel-based fragment analysis and suggests moving to high-throughput sequencing to achieve deeper allelic segregation. To maximize heterosis and broaden the genetic base for climate-resilient traits in sub-Saharan breeding programs, it is recommended to combine these molecular analyses with multi-locational phenotypic evaluations and to target strategic crosses between the divergent landrace lineage and stable SAMNUT lines.

COMPLIANCE WITH ETHICAL STANDARDS

Declarations

The authors declare that they have no conflicts of interest.

Data Availability

The information is available from the authors upon request.

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