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Research Paper

Genetic insights into the African Yam Bean (*Sphenostylis stenocarpa*, (Hochstex. A. Rich.) Harms) revealed through the ribulose-1,5-bisphosphate carboxylase/oxygenase large (*rbc*L) gene

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ABSTRACT

In the present study, the genetic diversity of 47 AYB leaf samples from various regions in Nigeria was evaluated using the *rbc*L gene marker. Phylogenetic analysis classified the samples into two main groups. Principal coordinate analysis (PCoA) did not show clear clustering. The Linear Genetic Distances (LGD) between the gene sequences varied from 3.60551 to 9.797959, indicating significant genetic diversity. Analysis of molecular variance (AMOVA) revealed that 99% of the genetic variation was within populations. Of the 66 *rbc*L gene sites examined, 54 were polymorphic, resulting in 45 haplotypes with a haplotype diversity of 0.998 ± 0.005 and a nucleotide diversity of 0.22741 ± 0.0003. A total of 118 single-nucleotide polymorphisms (SNPs) were identified in the *rbcL* gene of African yam bean (AYB). Among these, 105 mutations (89%) were nonsynonymous, indicating functional genetic variation, while 13 mutations (11%) were synonymous. The transversion-to-transition mutation ratio was 74:44, corresponding to 63% and 37%, respectively. These results underscore the need for more focused breeding initiatives to maximize AYB's agricultural and nutritional benefits.

***Key words*:** Diversity, Gene sequence, Phylogenetics, Polymorphism, rbcL

INTRODUCTION

In Africa, particularly in sub-Saharan countries, food and nutrition insecurity are significant challenges, exacerbated by the global impacts of climate change. Despite the potential of certain crops to address these issues, many remain underused, neglected, or inadequately improved (Aremu and Ibirinde 2012, Osuagwu *et al.* 2019, Adewale *et al.* 2008, Edem *et al.* 2025). One such crop is the African yam bean (AYB), a legume belonging to the Fabaceae family (Abdulkareem *et al.* 2015). Exploring the genetic foundation of underutilized crops like AYB is crucial for unlocking their potential to enhance food security and combat malnutrition. To fully understand the genetic structure of AYB, both in its cultivated forms and landraces, comprehensive genetic diversity assessments are necessary. These should include a range of methods, such as morphological, biochemical, cytogenetic, and molecular analyses (Udensi *et al.* 2021, Edem *et al.* 2024, Edu *et al.* 2025). Understanding AYB's genetic variation is not only vital for effective breeding but also for advancing molecular improvements (Osuagwu and Edem 2020). Knowledge of genetic diversity is key to optimizing the conservation and use of plant genetic resources, forming the foundation for successful breeding programs (Udensi *et al.* 2015).

The limitations of traditional morphological, biochemical, and other marker techniques have driven the shift toward DNA-based markers (Kumar *et al.* 2024). These molecular tools are advantageous because they are not influenced by environmental factors and do not require prior pedigree information (Kumar *et al.* 2024, Ekerette *et al.* 2024). DNA markers provide a more accurate and cost-effective approach to assessing genetic diversity, enabling breeders to rapidly identify unrelated individuals from a large pool of genotypes (Govindaraj *et al.* 2015). Various molecular marker systems have been employed to explore genetic diversity in AYB, including Random Amplified Polymorphic DNA (RAPD) (Molyib *et al.* 2008, Adewale and Odoh 2013), Amplified Fragment Length Polymorphisms (AFLPs) (Ojuedere *et al.* 2014), and Simple Sequence Repeats (SSRs) (Shitta *et al.* 2015).

In recent years, DNA sequencing has become a key tool for assessing population dynamics and

genetic diversity in crops, offering a direct and reliable approach (Olomitutu *et al.* 2022, Kumal 2024, 2022, Udensi *et al*. 2022). These molecular analyses often utilize DNA derived from various cellular compartments, such as the chloroplast (cpDNA), nucleus (nuclear DNA), or mitochondria (mtDNA) (Edu *et al.* 2024). Notably, cpDNA, located in the cytoplasm, is particularly useful for identifying genetic variation within populations due to its higher mutation rate compared to nuclear DNA, resulting in greater genetic diversity in these regions (Shitta *et al.* 2022, Camus *et al.* 2022, Dong *et al.* 2022, Gaudeul *et al.* 2014). Among the chloroplast markers, the *ribulose bisphosphate carboxylase large* (*rbcL*) gene is widely used in genetic diversity studies because of its effectiveness in analyzing evolutionary patterns, universality, ease of amplification, and alignment.

The *rbcL* gene, located in chloroplast DNA (cpDNA), encodes the large subunit of ribulose-1, 5-bisphosphate carboxylase/oxygenase (RuBisCO), and has proven valuable for investigating the phylogenetic relationships of flowering plants at both the species and genus levels (Chase *et al.* 1993, Gielly and Taberlet 1994). This gene has been widely used in evolutionary studies, phylogenetics, biogeography, population genetics, and systematics due to its ability to be easily replicated and its minimal divergence across closely related species (Müller *et al.* 2006).

Sphenostylis stenocarpa faces the risk of genetic erosion in its landraces due to insufficient conservation and breeding efforts. The genetic diversity within this species remains poorly understood. Gaining insight into genetic variation and its patterns is essential for developing effective breeding strategies. However, there has been limited research on the genetic diversity of the genus *Sphenostylis*. Additionally, there is a lack of comprehensive studies on the rbcL gene marker in *Sphenostylis stenocarpa*. This study aims to fill this gap by investigating the genetic diversity and relatedness of 47 AYB accessions of *Sphenostylis stenocarpa* using *rbcL*markers.

MATERIALS AND METHODS

Study location

The research was conducted at the International Institute of Tropical Agriculture (IITA) in Ibadan, Oyo State, Nigeria.

Sample collection

For this study, 47 AYB seed accessions were collected from various regions, including Cross River, Ebonyi, and Plateau States, alongside samples from the International Institute of Tropical Agriculture (IITA) (Table 1).

Table 1. The accessions of *Sphenostylis stenocarpa* used in this study, along with their respective origins and ecological zones.

S/N	Accessions	Origin	Ecological Zone
1	Idomi	Cross River	Forest
2	Obubra	Cross River	Forest
3	JOS31	Plateau	Savanna
4	JOS30	Plateau	Savanna
5	Abakaliki	Ebonyi	Forest
6	Ekoli-Eda	Ebonyi	Forest
7	TSs-602	Nigeria	Savanna
8	TSs-625	Nigeria	Savanna
9	TSs-561	Nigeria	Savanna
10	TSs-224	Unknown	Unknown
11	TSs-592	Nigeria	Forest
12	TSs-168	Unknown	Unknown
13	TSs-593	Nigeria	Forest
14	TSs-438	Nigeria	Savanna Woodland
15	TSs-69	Nigeria	Forest
16	TSs-42	Nigeria	Forest
17	TSs-591	Nigeria	Forest
18	TSs-111	Nigeria	Forest
19	TSs-581	Nigeria	Forest
20	TSs-84	Nigeria	Forest
21	TSs-571	Nigeria	Forest
22	TSs-128	Nigeria	Swampy with Trees & Grasses
23	TSs-138	Nigeria	Savanna
24	TSs-46	Nigeria	Savanna
25	TSs-68	Ghana	Forest
26	TSs-55	Nigeria	Savanna Woodland
27	TSs-155	Nigeria	Forest
28	TSs-65	Zaire	Forest
29	TSs-66	Bangladesh	Forest
30	TSs-93	Nigeria	Forest
31	TSs-120	Nigeria	Forest
32	TSs-98	Nigeria	Forest
33	TSs-87	Nigeria	Swampy Area
34	TSs-67	Bangladesh	Savanna
35	TSs-445	Nigeria	Savanna
36	TSs-8	Nigeria	Savanna
37	TSs-38	Nigeria	Forest
38	TSs-130	Nigeria	Forest
39	TSs-61	Nigeria	Savanna
40	TSs-77	Ghana	Savanna
41	TSs-45	Nigeria	Savanna
42	TSs-116	Nigeria	Savanna
43	TSs-12	Nigeria	Savanna
44	TSs-153	Nigeria	Savanna
45	TSs-19	Nigeria	Savanna
46	TSs-133	Nigeria	Savanna
47	TSs-150	Nigeria	Forest

Genomic DNA extraction

A modified CTAB-based approach was utilized for DNA extraction. The extraction buffer was freshly prepared with the following components: 200 mM Tris-HCl (pH 7.5), 50 mM EDTA (pH 8.0), 2 M NaCl, 2% CTAB, and 1% beta-mercaptoethanol, with volumes adjusted to accommodate 47 samples. To preserve its reducing activity, beta-mercaptoethanol was added immediately before use. Leaf samples (0.01–0.1 g) were freeze-dried and finely ground using a GenoGrinder-2000 set at 500 strokes per minute for four minutes. A cryoblock was employed to prevent sample degradation during grinding. The pulverized tissue was collected via centrifugation at 3500 rpm for five minutes. Subsequently, 600 µL of freshly prepared CTAB extraction buffer was added, and a further two-minute grinding step was performed to ensure complete homogenization. Samples were incubated at 60°C in a water bath for 30 minutes with gentle rocking (20–30 rpm) and periodic inversion to facilitate cell lysis.

Following incubation, the tubes were allowed to cool in a fume hood for 5–10 minutes, mixed gently, and centrifuged at 3500 rpm for 10 minutes. The upper aqueous phase (~500 µL) was carefully transferred to fresh tubes, followed by the addition of an equal volume (500–600 µL) of chloroform: isoamyl alcohol (24:1) mixture. The solution was gently rocked for 5–10 minutes to minimize DNA shearing, then centrifuged at 3500 rpm for another 10 minutes. The aqueous layer was transferred to a new tube, and the chloroform: isoamyl alcohol wash was repeated to improve DNA purity.

DNA precipitation was achieved by adding 600 µL of ice-cold isopropanol, followed by gentle inversion for five minutes. For enhanced precipitation, samples were optionally stored at -20°C for one hour before centrifugation at 3500 rpm for 20 minutes. The resulting DNA pellet underwent two washes with 70% ethanol, with each wash followed by centrifugation for 15 minutes. The pellet was subsequently air-dried in a fume hood or incubated at 37°C for 15–30 minutes.

The dried DNA was dissolved in 200 µL of low-salt TE buffer (10 mM Tris, 0.1 mM EDTA, pH 8.0), and 3–5 µL of RNase A was introduced to eliminate RNA contamination. Samples were incubated at 37°C for two hours or left overnight at 4°C, followed by an additional two-hour incubation at 37°C to ensure complete RNA digestion. DNA integrity was evaluated using 0.8% agarose gel electrophoresis, with ethidium bromide staining and visualization through a gel documentation system.

Quantification was carried out using a NanoDrop spectrophotometer, ensuring that A260/280 and A260/230 ratios fell within the acceptable ranges of ~1.8 and 1.8–2.2, respectively. DNA concentration was standardized to 200 ng/µL based on NanoDrop readings, and quality was verified by loading 2 µL of the adjusted sample onto a 0.8% agarose gel. Final working dilutions of DNA were prepared as required for downstream applications.

PCR amplification of the rbcLgene region

The *rbcL* gene region was amplified through polymerase chain reaction (PCR) using a reaction mixture with a total volume of 25 µL. This mixture comprised 12.5 µL of NEB OneTaq 2X Master Mix with Standard Buffer, 2 µL of genomic DNA (10–30 ng/µL), 0.5 µL of each primer rbcL-F535 (CTTTCCAAGGCCCGCCTCA) and rbcL-R705 (CATCATCTTTGGTAAAATCAAGTCCA) at a concentration of 10 µM, and 9.5 µL of nuclease-free water. The thermal cycling conditions were optimized, beginning with an initial denaturation step at 94°C for 5 minutes, followed by 35 amplification cycles. Each cycle consisted of denaturation at 94°C for 45 seconds, annealing at 52°C for 45 seconds, and extension at 72°C for 1 minute. A final extension phase at 72°C for 10 minutes was included to ensure complete amplification.

To assess the amplification success, the PCR products were subjected to 1.5% agarose gel electrophoresis and stained with ethidium bromide for visualization.

Purification and sequencing of PCR products

Following PCR amplification, the resulting products were purified using the ExoSAP enzymatic method to eliminate residual primers and nucleotides. Sequencing was performed using the ABI 3500 genetic analyzer, following the manufacturer's recommended protocol to ensure accuracy and reliability.

Data analysis

Chromas version 2.6.4 (Technelysium, Australia) was utilized for editing and viewing chromatogram-based gene sequences. Multiple sequence alignment, phylogenetic analysis, genetic distance computation, and selection type estimation were carried out using MEGA version 6.0.6. GenAlex version 6.4 was employed for calculating

pairwise Euclidean distances, performing Principal Coordinate Analysis (PCoA), and conducting Analysis of Molecular Variance (AMOVA). DnaSP version 5.1 (Librado and Rozas, 2009) was used to evaluate DNA polymorphisms, including nucleotide and haplotype diversity, along with other related genetic diversity parameters. Single-nucleotide polymorphisms (SNPs) and their mutations in aligned sequences were analyzed using CodonCode Aligner version 6.06. These tools were applied in adherence to standard methodologies to ensure accuracy and reliability in the analysis.

RESULTS AND DISCUSSION

Phylogenetic analysis of the rbcL gene in 47 African yam bean accessions

The phylogenetic analysis of the *rbcL* gene for African yam bean (AYB), as shown in Figure 1, revealed that the accessions were not clustered based on geographic origin. Instead, there was a notable intermixing of accessions from different locations within each subcluster. The phylogenetic tree identified two main groups (G1 and G2) with a low dissimilarity coefficient of 0.05, suggesting high genetic similarity among the sampled accessions. Group 1 (G1) comprised 21 accessions, including four local accessions from Obubra, Abakaliki, and Jos, along with 15 IITA collections of Nigerian origin (e.g. TSs153, TSs561, TSs150, and TSs45), one germplasm collection from Ghana (TSs68), and one from Bangladesh (TSs66). Group 2 (G2) contained 26 accessions, including two local accessions from Idomi and Ekoeda, 21 IITA collections from Nigeria (e.g. TSs591, TSs571, TSs128, and TSs87), two collections of unknown origin (TSs168 and TSs224), one from Ghana (TSs77), one from Bangladesh (TSs67), and one from the Democratic Republic of Congo (Zaire) (TSs65).

The clustering observed in this study contrasts with earlier findings, such as those by Aina (2021), who reported distinct phylogenetic groupings based on geographic origin among African yam bean populations. In this analysis, the significant intermixing of accessions suggests the potential for higher gene flow or shared ancestry across different locations. Further more, the clustering was not influenced by the location of adoption or breeding background of the IITA accessions. Supporting this observation, Sarhan *et al.* (2016) highlighted the utility of *rbcL* gene sequences in elucidating sequence divergence and genetic variation in plants. For instance, high bootstrap values were observed, such as 99% for Nigeria-TSs581 and Bangladesh-TSs67, and 94% for Nigeria-TSs445 and TSs130, indicating strong sequence reliability. Bootstrap values for Bangladesh-TSs66, Nigeria-TSs602, and TSs445 were slightly lower at 78%, reinforcing the hypothesis that clustering was independent of geographic origin or breeding background.

This finding aligns with Singh *et al.* (2019), who observed that the *rbcL* gene is more effective in resolving phylogenetic relationships at the genus or family level rather than at the species level. Similarly, the patterns observed in this study suggest that genetic relationships among AYB accessions may transcend geographic boundaries, providing insights into the species' genetic structure.

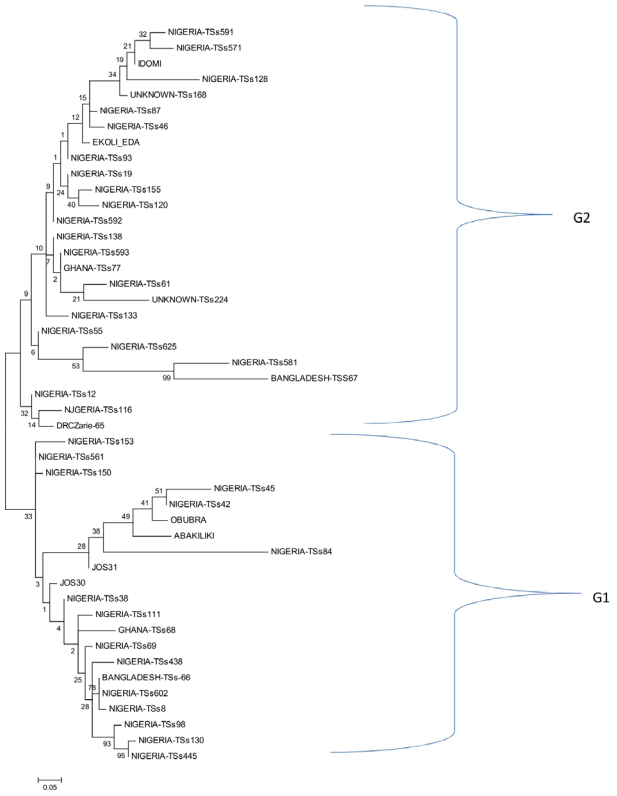

Fig. 1. Maximum likelihood-based phylogenetic tree of the 47AYB *rbc*L gene.

Principal coordinate analysis (PCoA) of the 47 AYB accessions for the rbcL gene

The principal coordinate analysis (PCoA) results did not reveal a discernible clustering pattern among the accessions. However, local accessions from Ekoli-Edda, Obubra (Cross River), Abakaliki, and Jos appeared to cluster with certain accessions from the IITA germplasm, such as TSs61, TSs224, TSs130, TSs58, TSs84, TSs67, TSs91, TSs98,

TSs625, and TSs571 (Figure 2). The first three axes of the PCoA explained 10.83%, 8.51%, and 7.05% of the total variation, respectively, with a cumulative percentage variation of 26.38% (Table 2).

Although the PCoA did not demonstrate distinct clustering, the grouping of local accessions with IITA germplasm samples suggests a potential genetic relationship that transcends geographic boundaries. This finding is consistent with the idea that the genetic structure of African yam bean (AYB) is not strongly influenced by geographical origin. Similar conclusions were drawn by Ortiz *et al*. (2023), who reported minimal geographic structuring in the genetic diversity of *Macrophomina phaseolina* isolated from soybeans and dry beans across the United States. The relatively modest percentage of variation (26.38%) captured by the first three axes indicates that factors other than geographic location may play a more significant role in determining genetic diversity within AYB populations.

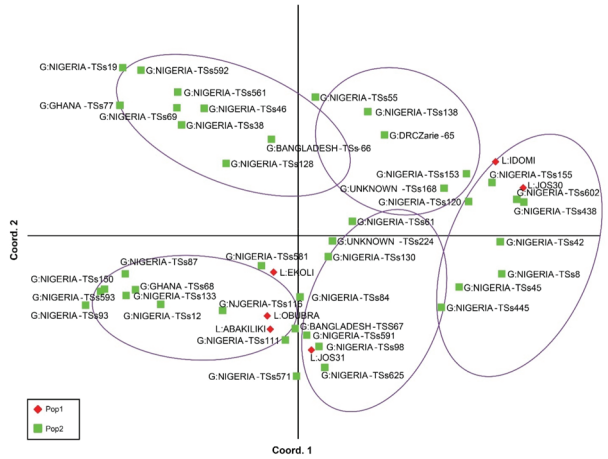

Fig. 2. Principal coordinate analysis (PCoA) of the *rbc*L gene of 47 AYB accessions

Table 2. Percentage of variation explained by the first 3 axes of the PCoA of the *rbc*L gene in 47 AYB accessions

Percentage of variation explained by the first 3 axes			
Axis	**1**	**2**	**3**
%	10.83	8.51	7.05
Cum %	10.83	19.33	26.38

Linear genetic distance (LGD) and analysis of molecular variance in 47 AYB accessions of the rbcL gene

The linear genetic distances (LGD) among the *rbcL* gene sequences of the 47 African yam bean (AYB) accessions ranged from 3.0000 to 9.0000. The narrowest distance, 3.60551, was observed between TSs592 and TSs19, while the widest distance, 9.797959, occurred between TSs111 and TSs19 (Table S1). Analysis of Molecular Variance (AMOVA) revealed that 99% of the genetic variation existed within populations, with only 1% observed among populations (Figure 3; Table 3).

The LGD between Nigeria-TSs561 and Nigeria-TSs69 was 4.582576, representing the closest genetic similarity within the study. However, the observed high within-population variation (99%) compared to the minimal among-population variation (1%) differs from previous findings, such as those by Udensi *et al*. (2022), which reported greater genetic differentiation among populations of related cowpea and pigeon pea, respectively. This difference may stem from the broader geographic sampling in his study, while our analysis focused on specific germplasm collections.

The limited genetic variation observed within the IITA germplasm and local accessions may suggest a lack of mutation-driven diversity in these populations (Salgotra and Chauhan 2023). These findings underscore the necessity for governmental and institutional efforts to introduce AYB genotypes with enhanced traits. Expanding the genetic base of AYB through targeted breeding programs could improve adaptability to diverse climatic conditions, ultimately supporting agricultural resilience and productivity.

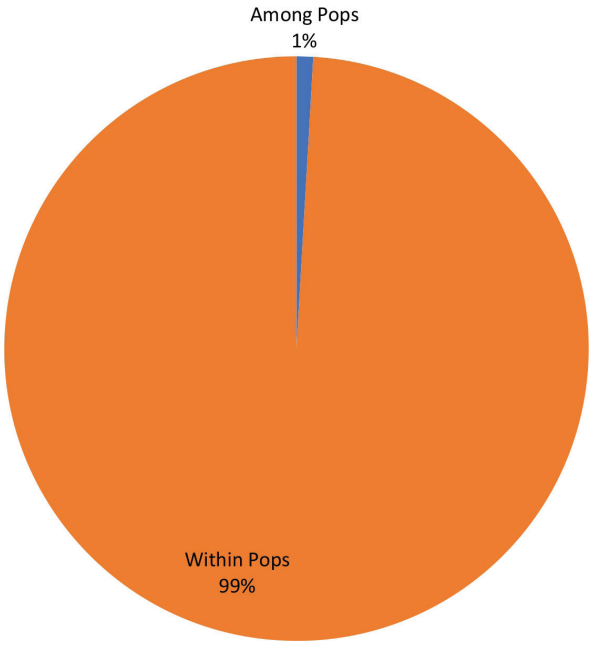

Fig. 3. Analysis of molecular variance in the *rbc*L genes of 47 AYB accessions

Table 3. Analysis of molecular variance in the *rbc*L genes of 47 AYB accessions

Source	df	SS	MS	EstVar.	%
Among Pops	1	4.733	4.733	0.040	1%
Within Pops	45	194.256	4.317	4.317	99%
Total	46	198.988		4.357	100%

Selection pressure on the rbcL gene in 47 AYB accessions from the IITA germplasm and AYB growing regions

The analysis of selection pressure on the r*bcL* sequences of African yam bean (AYB) accessions, as summarized in Table 4, revealed nine sites under positive selection, exceeding the six sites under negative selection. The nonsynonymous-to-synonymous substitution (d_N-d_S) frequency for positive selection was calculated as 13.342, compared to -8.299 for negative selection. This predominance of positive selection suggests that a significant proportion of alleles in AYB accessions are under adaptive pressure, potentially driving population structuring and speciation over time.

The high d_N-d_S ratio associated with positive selection indicates that beneficial mutations in the rbcL gene may be retained, reflecting ongoing adaptive evolution within the species. Conversely, the rate of purifying or negative selection was comparatively low, as reflected in the reduced d_N-d_S substitution rate and negative site index. These findings align with the hypothesis that adaptive evolution is influencing the *rbcL* gene in AYB, possibly enabling it to respond to environmental and biological challenges. Similar observations have been reported by Yao (2019), who documented evidence of positive selection acting on the rbcL gene in the genus *Ilex* (Aquifoliaceae). This reinforces the idea that the rbcL gene is subjected to selective pressures that contribute to its evolutionary adaptation within specific environmental contexts.

Table 4. Selection analysis of the*rbc*L gene in 47 AYB accessions from the IITA germplasm and AYB growing regions.

Selection types	d_N	Ds	d_N-ds	Site Index	P value
Positive	18.029	4.687	13.342	9.00	0.411
Negative	7.950	16.249	-8.299	6.00	0.905
Neutral	0.00	0.00	0.000	4.00	N/A

DNA polymorphisms of the rbcL gene in 47 AYB accessions from the IITA germplasm and AYB growing regions.

The DNA polymorphism analysis of the *rbcL* gene in African yam bean (AYB) accessions (Figure 2), as detailed in Table 5, examined 66 sites across 47 sequences. The analysis revealed 54 polymorphic sites and 12 monomorphic sites. A total of 45 haplotypes were identified, resulting in a high haplotype diversity of 0.998±0.005. The nucleotide diversity was calculated as 0.22741±0.0003, with an average of 15.009 nucleotide differences and a sequence conservation rate of 10.4%. Further more, the analysis identified a minimum of 10 recombination events (Table 6).

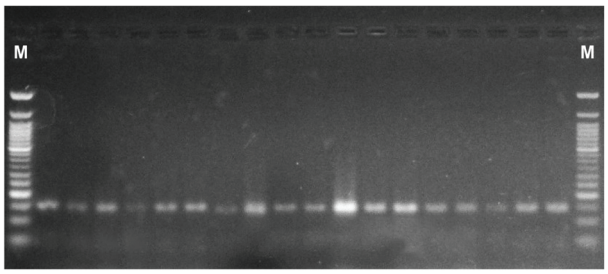

Fig. 2. Agarose gel showing PCR amplification of 47AYB accessions obtained from growing regions and IITA germplasm using *rbcL* primers. M = Molecular weight marker (100 kb PCR ladder); Lanes 1–18 = 18 AYB accessions

Haplotypes, which consist of genes inherited together, indicate that the AYB accessions share highly conserved genetic regions, reflecting their relatedness. These findings align with those of Udensi *et al.* (2022), who reported high haplotype diversity in other legumes, such as pigeon pea and cowpea (26 and 22 haplotypes, respectively). In contrast, Popoola *et al.* (2023) identified only five haplotypes with a lower haplotype diversity of 0.594 in AYB populations.

The nucleotide diversity observed in this study (0.22741±0.0003) suggests significant genetic richness within AYB populations. This contrasts with the lower nucleotide diversity of 0.00382 reported by Popoola *et al.* (2023), highlighting the variability in findings across studies. Low heterozygosity, combined with high nucleotide diversity, emphasizes the genetic complexity within the analyzed AYB accessions. These results align with those of Popoola *et al.* (2023), who documented substantial genetic diversity in other tropical legumes. The observed average nucleotide differences and low sequence conservation (10.4%)

further support the genetic variability present within AYB populations.

Table 5. DNA polymorphisms of the *rbc*L gene in 47 AYB accessions from the IITA germplasm and AYB-growing regions

Polymorphism parameters	AYB
No. of sequences	47
No of sites	66
Monomorphic sites	12
Polymorphic sites	54
Singleton variable Sites	11
Parsimony information sites	43
Number of haplotypes	45
Haplotype (gene) diversity	0.9988 ± 0.005
Nucleotide diversity	0.22741 ± 0.0003
Average number of nucleotide difference	15.009
Sequence conservation	0.104 (10.4%)
Minimum number of recombination	10

Variation in single-nucleotide polymorphisms (SNPs) in the rbcL gene of 47 AYB accessions

A total of 118 single-nucleotide polymorphisms (SNPs) were identified in the *rbcL* gene of African yam bean (AYB). Among these, 105 mutations (89%) were nonsynonymous, indicating functional genetic variation, while 13 mutations (11%) were synonymous (Table 6). The transversion-to-transition mutation ratio was 74:44, corresponding to 63% and 37%, respectively. The predominance of nonsynonymous mutations highlights the potential role of functional changes in shaping genetic diversity within the AYB accessions. Further more, the observed transversion-to-transition mutation ratio underscores a complex mutational pattern in the rbcL gene. These findings suggest that such mutational mechanisms may be conserved across plant taxa.

The analysis revealed that most of the variation in the *rbcL* gene was attributed to nonsynonymous and transversion mutations, reflecting a dynamic evolutionary process. This insight enhances our understanding of the genetic architecture of the AYB and provides valuable information for future studies on its genetic improvement.

Table 6. Single-nucleotide polymorphism (SNP) variation in the *rbc*L genes of 47 AYB accessions. SNP (single-nucleotide polymorphism); d_N (nonsynonymous); d_S(synonymous)

Position	SNPs	Amino acid change	ds/ d_N	Mutation types
1	IN>C	Xaa1Arg	d_N	Transversion
2	2G>A	Xaa1 Glu	d_N	Transversion
3	3N>G	Xaa1 Arg	d_N	Transversion
4	4N>C	Xaa2 His	d_N	Transversion
5	5A>G	Xaa 2 Ser	d_N	Transition
6	6T>C	Xaa 2 Ser	d_N	Transversion
7	7T>C	Ser 3 Arg	d_N	Transition
8	8C>A	Ser 3 Glu	d_N	Transversion
9	9A>G	Ser 3 Srg	d_S	Transition
10	10T>A	STP 4 MET	d_S	Transversion
11	11A>G	STP 4 STP	d_N	Transition
12	12A>G	STP 4 MET	d_N	Transition
13	13A>C	Thr 5 Pro	d_N	Transversion
14	14C>T	Thr 5 MET	d_N	Transition
15	15C>G	Thr 5 MET	d_N	Transversion
16	16G>C	Ala 6 Leu	d_N	Transversion
17	17C>T	Ala 6 Leu	d_N	Transition
18	18T>C	Ala 6 Leu	d_N	Transition
19	19C>A	Leu 7 Arg	d_N	Transversion
20	20T>G	Leu 7 Arg	d_N	Transversion
21	21A>G	Leu 7 Arg	d_S	Transition
22	22C>T	Pro 8 STP	d_N	Transversion
23	23C>A	Pro 8 Tyr	d_N	Transversion
24	24ADT	Pro & Pro	d_S	Transition
25	25T>G	STP 9 Trp	d_N	Transversion
26	26A>G	STP 9 Trp	d_N	Transition
27	27A>G	STP 9 Trp	d_N	Transition
28	28T>C	Phe 10 del	d_N	Transition
29	29T>C	Phe 10 Ser	d_N	Transition
30	30C>G	Phe 10 Leu	d_N	Transversion
31	32T>C	Leu 11 Ser	d_N	Transition
32	33A>C	Leu 11 Ser	d_N	Transversion
33	34G>C	Ala 12 Pro	d_N	Transversion
34	35C>G	Ala 12 Gly	d_N	Transversion
35	36G>T	Ala 12 Ala	d_S	Transition
36	37A>G	Ile 13 del	d_N	Transition
37	38T>G	Ile 13 del	d_N	Transversion
38	40A>G	Asn 14 Gly	d_N	Transition
39	41A>G	Asn 14 Arg	d_N	Transition
40	42C>G	Asn 14 Arg	d_N	Transversion
41	43C>A	Pro 15 Thr	d_N	Transversion
42	44C>T	Pro 15 Leu	d_N	Transition
43	45C>G	Pro 15 Pro	d_S	Transversion
44	46T>G	Try 16 Gly	d_N	Transversion
45	47A>G	Try 16 Gly	d_N	Transition
46	48T>C	Try 16 Tyr	d_S	Transition
47	49T>A	Leu 17 STP	d_N	Transversion
48	50T>A	Leu 17 STP	d_N	Transversion
49	51A>G	Leu 17 STP	d_N	Transition
50	52G>A	Xaa 18 Asn	d_N	Transition
51	53A>G	Xaa 18 Arg	d_N	Transition
52	54N>C	Xaa 18 Arg	d_N	Transversion
53	55N>C	Xaa 19 Leu	d_N	Transversion
54	56T>G	Xaa 19 Leu	d_N	Transversion
55	57A>T	Xaa 19 Leu	d_N	Transversion
56	58A>G	Lys 20 Gly	d_N	Transition
57	59A>G	Lys 20 Ser	d_N	Transition
58	60G>T	Lys 20 Asn	d_N	Transition

Position	SNPs	Amino acid change	ds/ d_N	Mutation types
59	61C>G	STP 21 Gly	d_N	Transversion
60	62G>C	STP 21 Ser	d_N	Transversion
61	63A>G	STP 21 Trp	d_N	Transition
62	64T>G	Ser 22 Ala	d_N	Transversion
63	65C>G	Ser 22 Gly	d_N	Transversion
64	67T>C	Xaa 23 Gln	d_N	Transition
65	69N>A	Xaa 23 STP	d_N	Transversion
66	70C>T	Xaa 24 STP	d_N	Transition
67	71V>G	Xaa 24 Pro	d_N	Transversion
68	72G>C	Xaa 24 Tyr	d_N	Transversion
69	73G<C	Gly 25 Leu	d_N	Transversion
70	74G<T	Gly 25 Leu	d_N	Transversion
71	75G>A	Gly 25 Leu	d_N	Transition
72	76A>G	Arg 26 Gly	d_N	Transition
73	77G>C	Arg 26 Thr	d_N	Transversion
74	78A>G	Arg 26 Thr	d_N	Transition
75	79A>C	Thr 27 del	d_N	Transversion
76	80C>T	Thr 27 Ile	d_N	Transition
77	81C>G	Thr 27 Thr	d_S	Transversion
78	82A>C	MET 28 Leu	d_N	Transversion
79	83T>G	MET 28 Arg	d_N	Transversion
80	84G>A	MET 28 Ile	d_N	Transition
81	87T>A	Leu 29 Val	d_N	Transition
82	88G>A	Glu 30 Asn	d_N	Transversion
83	89A>T	Glu 30 Val	d_N	Transversion
84	90G>T	Glu 30 Val	d_N	Transversion
85	91C>G	Arg 31 Gly	d_N	Transversion
86	92G>A	Arg 31 Gln	d_N	Transition
87	93G>A	Arg 31 Arg	d_S	Transversion
88	94T>G	Phe 32 Val	d_N	Transition
89	99G>C	MET 33 Ile	d_N	Transversion
90	100T>A	Ser 34 Asn	d_N	Transversion
91	101C>A	Ser 34 Asn	d_N	Transversion
92	103G>C	Val 35 Leu	d_N	Transversion
93	104T>C	Val 35 del	d_N	Transition
94	105C>T	Val 35 Val	d_S	Transition
95	106T>A	Phe 36 Asn	d_N	Transversion
96	107T>A	Phe 36 Asn	d_N	Transversion
97	109G>T	Val 37 Leu	d_N	Transversion
98	110T>C	Val 37 Ala	d_N	Transition
99	113A>T	Ghu 38 Val	d_N	Transition
100	114G>T	Glu 38 Asp	d_N	Transversion
101	115G>C	Xaa 39 Pro	d_N	Transversion
102	116M>A	Xaa 39 Asp	d_N	Transversion
103	118T>A	Leu 40 MET	d_N	Transversion
104	119T>A	Leu 40 Lys	d_N	Transversion
105	122A>G	Ser 41 Lys	d_N	Transition
106	123G>T	Ser 41 Gly	d_N	Transversion
107	123G>T	Ser 41 Gly	d_N	Transversion
108	124T>G	Ser 41 Ile	d_N	Transversion
109	125T>G	Ser 41 Arg	d_N	Transversion
110	126T>C	Leu 42 Gly	d_N	Transversion
111	127A>C	Leu 42 Gly	d_N	Transversion
112	128C>G	Leu 42 Gly	d_N	Transversion
113	129C>T	Pro 43 Val	d_N	Transversion
114	130A>C	Pro 43 Pro	d_S	Transversion
115	131A>T	Lys 44 Leu	d_N	Transversion
116	132A>T	Lys 44 Leu	d_N	Transversion
117	134A>T	Xaa 45 Xaa	d_S	Transversion
118	135T>A	Val 35 Leu	d_S	Transversion

SNP(Single nucleotide polymorphism); d_N(Non-synonymous); d_S(synonymous)

CONCLUSION

The genetic analysis of African yam bean (*Sphenostylis stenocarpa*) utilizing the ribulose-1,5-bisphosphate carboxylase/oxygenase (rbcL) gene sequence has provided critical insights due to its high conservation and moderate variability, which reveals genetic differentiation among different accessions of *Sphenostylis stenocarpa* populations, suggesting regional adaptation and possible subspecies or ecotypes of this underutilized legume. Our study reveals substantial genetic variation within populations, a key factor for understanding the adaptability and resilience of AYB across diverse environmental conditions. The application of the rbcL gene marker has proven to be a robust and reliable tool for evaluating the genetic relatedness and diversity of AYB accessions, by identifying genetically related accessions that are considered for selection during breeding,which is essential for advancing genetic improvement efforts in this crop. To enhance breeding outcomes, we recommend prioritizing the selection of genetically distant accessions, such as TSs111 and TSs19, and conducting thorough screening for introgression. This approach will be pivotal in optimizing breeding strategies and facilitating the genetic enhancement of AYB. The selection of these TSs11 and TSs19 for breeding may introduce novel traits into breeding populations, resulting in heterosis (hybrid vigor), leading to improved growth rates, yield, stress tolerance, and disease resistance.

REFERENCES

Abdulkareem KA, Animasaun DA, Oyedeju S and Olabanji OM. 2015. Morphological characterization and variability study of African yam beans *Sphenostylis stenocarpa* (Hoscht. Ex. A. Rich) Harms). Global Journal of Pure and Applied Sciences **21**: 21-27.

Adewale BD and Odoh NC. 2013. A review on genetic resources, diversity, and agronomy of African yam bean (*Sphenostylis stenocarpa* (Hochst. Ex A. Rich.) Harms): A potential future food crop. Sustainable Agriculture Research **2**(1): 5-13.

Adewale BD, Kehinde OB, Odu BO and Dumet DJ 2008. The potentials of African yam bean (*Sphenostylis stenocarpa* Hochst. ex. A. Rich) harms in Nigeria: Character distribution and genetic diversity. In: Smart J and Haq N (Eds.), New Crops and Uses: Their Role in a Rapidly Changing World, RPM Print & Design. Pp. 265-276.

Aina A, Garcia-Oliveira AL, Ilori C, Chang PL, Yusuf M, Oyatomi O, Abberton M and Potter D. 2021. Predictive genotype-phenotype relations using genetic diversity in African yam bean (*Sphenostylis stenocarpa* (Hochst. ex. A. Rich) Harms). BMC Plant Biology **21**(1): 547.

Aremu CO and Ibirinde DB. 2012. Biodiversity studies on accessions of African yam bean (*Sphenostylis stenocarpa*). International Journal of Agricultural Resources **7**: 78-85.

Camus MF, Alexander-Lawrie B, Sharbrough J and Hurst GDD. 2022. Inheritance through the cytoplasm. Heredity (Edinburgh) **129**(1): 31-43.

Chase MW, Soltis DE, Olmstead RG, Morgan D, Les DH, Mishler BD, Duvall MR, Price RA, Hills HG, Qiu YL, Kron KA, Rettig JH, Conti E, Palmer JD, Manhart JR, Sytsma KJ, Michaels HJ, Kress WJ, Karol KG, Clark WD, Hedren M, Gaut BS, Jansen RK, Kim KJ, Wimpee CF, Smith JF, Furnier GR, Strauss SH, Xiang QY, Plunkett GM, Soltis PS, Swensen SM, Williams SE, Gadek PA, Quinn CJ, Eguiarte LE, Golenberg E, Learn GH, Graham SW, Barrett SCH, Dayanandan S and Albert VA. 1993. Phylogenetics of seed plants: An analysis of nucleotide sequences from the plastid gene *rbcL*. Annals of the Missouri Botanical Garden **80**: 528-534.

Dong S, Zhou M, Zhu J et al. 2022. The complete chloroplast genomes of *Tetrastigma hemsleyanum* (Vitaceae) from different regions of China: Molecular structure, comparative analysis, and development of DNA barcodes for its geographical origin discrimination. BMC Genomics **23**: 620.

Edem UL, Osuagwu AN, Edu NE, Emeagi LI, Agbor RB, Nsungo NE, Uno UU and Ekerettee EE. 2024. Assessing genetic diversity in squash pumpkin (*Cucurbita moschata*) through computational analysis of plastid genes.Tropical Journal of Natural Product Research **8**(11): 9310–1914.

Edem UL, Osuagwu AN, Edu NE, Phillip JO, Nelson AY, Emeagi LI, Iheanetu CN and Dennis SU. 2025. Genetic potential and phytochemical diversity of African yam bean (*Sphenostylis stenocarpa*): A gateway to nutritional security and crop improvement. Tropical Journal of Phytochemistry & Pharmaceutical Sciences **4**(1): 9–15.

Edu NE, Edem UL, Osuagwu AN, Ojua EO, Aibuedefe OE, Oyohosuho PJ, Emeagi LI and Iheanetu CN. 2024. Phylogenetic analysis and protein structure characterization of the *matK* gene in *Sphenostylis stenocarpa* and related legumes. Journal of Underutilized Legumes **7**(1): 12–21.

Edu NE, Uzoma LG, Edem UL, Obua PA, Isorshe IJ and Ogbaji HO. 2025. Phytochemical profiling and bioactive compound variation in *Jatropha* landraces from Nigeria: Implications for agricultural and medicinal applications. Tropical Journal of Pharmaceutical Research **24**(3): 383–391.

Ekerette EE, Etukudo OM, Efienokwu JN, Etta HE, Henry II, Ekpo PB, Edu EN, Agbor RB, Edem UL and Ikpeme EV. 2024. Evaluation of genetic variation in *Oreochromis* tilapia species from South-South Nigeria using mitochondrial DNA hypervariable region. Tropical Journal of Natural Product Research **8**(9): 8527–8536. Gaudeul M,

Gielly L and Taberlet P. 1994. The use of chloroplast DNA to resolve plant phylogenies: Noncoding versus *rbcL* sequences. Molecular Biology and Evolution **11**: 769–777.

Govindaraj M, Vetriventhan M and Srinivasan M 2015. Importance of genetic diversity assessment in crop plants and its recent advances: An overview of its analytical perspectives. Genetics Research International **2015**: 431487.

Kumar R, Das SP, Choudhury BU, Kumar A, Prakash NR, Verma R, Chakraborti M, Devi AG, Bhattacharjee B, Das R, Das B, Devi HL, Das B, Rawat S and Mishra VK. 2024. Advances in genomic tools for plant breeding: Harnessing DNA molecular markers, genomic selection, and genome editing. Biological Research **57**(1): 80.

Librado P and Rozas J 2009. *Dna SP v5*: A software for comprehensive analysis of DNA polymorphism data. Bioinformatics **25**: 1451-1452.

Müller KF, Borsch T and Hilu K. 2006. Phylogenetic utility of rapidly evolving DNA at high taxonomical levels: Contrasting *matK*, *trnT-F*, and *rbcL* in basal angiosperms. Molecular Phylogenetics and Evolution **41**(1): 99–117.

Olomitutu OE, Abe A, Oyatomi OA, Paliwal R and Abberton MT. 2022. Assessing intraspecific variability and diversity in African yam bean landraces using agronomic traits. Agronomy **12**: 884-893.

Ortiz V, Chang H-X, Sang H, Jacobs J, Malvick DK, Baird R, Mathew FM, Estévez de Jensen C, Wise KA, Mosquera GM and Chilvers MI 2023. Population genomic analysis reveals geographic structure and climatic diversification for *Macrophomina phaseolina* isolated from soybean and dry bean across the United States, Puerto Rico, and Colombia. Frontiers in Genetics **14**: 1103969.

Osuagwu AN andEdem UL. 2020. Evaluation of genetic diversity in aerial yam (*Dioscorea bulbifera* L.) using simple sequence repeats (SSR) markers. Agrotechnology **9**(5): 2168-9881.

Osuagwu AN, Edem UL and Kalu SE. 2019.Evaluation of genetic diversity in aerial yam (*Diocorea bulbifera* L.) using qualitative and quantitative morphological

traits. Global Scientific Journal **7**(11): 368-390.

Popoola O, Eruemulor DI, Ojuederie OB and Oyelakin AS 2023. Dataset on estimate of intra-specific genetic variability of African yam bean (*Sphenostylis stenocarpa* (Hochst. ex A. Rich.) Harms.) based on *rbcL* gene marker. Data in Brief **47**: 108944

Salgotra RK and Chauhan BS. 2023. Genetic diversity, conservation, and utilization of plant genetic resources. Genes (Basel) **14**(1): 174.

Sarhan S, Hamed F and Al-Youssef W. 2016. The *rbcL* gene sequence variations among and within *Prunus* species. Journal of Agricultural Science and Technology **18**: 1105–1115.

Shitta NS, Abberton MT, Adenubi A, Adewale DB and Oyatomi O. 2015. Analysis of genetic diversity of African yam bean using SSR markers derived from cowpea. Characterization and Utilization **6**: 1–7.

Shitta NS, Unachukwu N, Edemodu AC, Abebe AT, Oselebe HO and Abtew WG. 2022. Genetic diversity and population structure of an African yam bean (*Sphenostylis stenocarpa*) collection from IITA GenBank. Scientific Reports **15**: 44-47.

Udensi OU, Okon E, Ikpeme EV, Onung OO and Ogban FU. 2015. Assessing genetic diversity in cowpea (*Vigna unguiculata* L. Walp.) accessions obtained from IITA, Nigeria using random amplified polymorphic DNA (RAPD). International Journal of Plant Breeding and Genetics **10**: 12-22.

Udensi UO, Emeagi IL, Daniel VE, Tentishe TL, Ghosh S and Achilonu CC. 2022. Insight to the genetic diversity of pigeon pea (*Cajanus cajan* (L.) Millsp.) and cowpea (*Vigna unguiculata* (L.) Walp.) germplasm cultivated in Nigeria based on *rbcL* gene region. Genetic Resource and Crop Evolution **5**: 99–107.

Udensi UO, Emeagi IL, Thomas TL, Ghosh S and Achilonu CC. 2021. Genetic polymorphism and lineage of pigeon pea (*Cajanus cajan* (L.) Millsp.) inferred from chloroplast and nuclear DNA gene regions. Arabian Journal for Science and Engineering **46**: 5285–5297.

Yao X, Tan YH, Yang JB, Wang Y, Corlett RT and Manen JF. 2019. Exceptionally high rates of positive selection on the *rbcL* gene in the genus *Ilex* (Aquifoliaceae). BMC Evolutionary Biology **19**(1): 192.