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# SNP VARIATION AND PHYLOGENETIC PATTERNS OF THE CPDNA RPOA GENE IN BAMBARA GROUNDNUT (*VIGNA SUBTERRANEA* (L.) VERDC) GENOTYPES

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## Abstract

Bambara groundnut (*Vigna subterranea* (L.) Verdc), a legume native to Africa, has the potential to address food security challenges on the continent. However, it is an underutilised crop because there hasn't been enough research done on its genetic diversity, selection, and breeding, particularly with the use of genomic tools. Chloroplast DNA (cpDNA) is a short, compact, circular, and double-stranded DNA structure present in plants. Its size ranges from 83 to 292 kilobases. It exhibits uniparental inheritance, with maternal inheritance being more prevalent in angiosperms and paternal inheritance in gymnosperms. This study was aimed at assessing the single nucleotide polymorphism (SNPs) and phylogenetic analysis of rpoA gene sequences in Bambara groundnut genotypes from Nigeria, West-African Subregion, using polymerase chain reaction (PCR) and agarose gel electrophoresis. PCR amplification of the rpoA gene shows the resulting fragments observed on the gel had an approximate length of 1000 base pairs (bp). This consistent fragment length of around 1000 bp was observed across all seven different genotypes of *Vigna subterranea* that were examined in the study. A total of 442 SNP variations were detected in the 5' untranslated region (5'-UTR) region and 437 in the 3' untranslated region (3'-UTR) regions from the sequence of seven genotypes of *Vigna subterranea* rpoA gene, which represent 4.2 and 4.4%, respectively, which suggest a certain diversity within the studied genotypes. The frequency of the predominant allele varied, with GDK having the lowest frequency at 0.58, while GNR had the highest frequency at 0.89. GNR exhibited a total gene diversity of 0.480, and the Shannon diversity index reached its peak at 0.786 in the DWS genotype. Genetic distances between pairs of regions showed variability, ranging from 0.001 to 0.050. Notably, higher similarity was observed between the KSSB-KSSC and DWS-DAG genotypes. Cluster analysis based on genotypic data grouped the 7 samples into 4 clusters. Based on genotypic data, it can be concluded that there is a significant degree of genetic diversity in the germplasm genotyped that can be used by plant breeders in crop improvement programmes. This diversity can be valuable for breeding programmes aimed at developing more resilient or improved varieties of this plant species

**Keywords:** Bambara groundnut, cpDNA, Phylogenetic analysis, PCR, rpoA gene, SNPs, *Vigna subterranean*

### Introduction

Bambara groundnut (Vigna subterranea) is an underutilised crop with high nutritional value and the ability to resist pests, drought, and poor soil conditions. It has the potential to contribute to food security and enhance the consumption of underutilised legumes (Dzandu et al., 2023). Many African countries rely on agriculture and struggle to keep their economies afloat as their main crops fail to adapt (Minnaar-Ontong *et al*., 2021). Bambara groundnut, also known as the poor man's crop, is commonly grown in sub-Saharan Africa and is both drought-tolerant and underutilized. It has several medicinal benefits and is the third most produced and consumed crop after groundnuts and cowpea in semi-arid Africa (Chai *et al*., 2017; Olukolu *et al*.,

2012; Shegro *et al*., 2013), with Nigeria a major producer (Onuche, 2020).

Bambara groundnut is a nutritious food source, high in carbohydrates (63-65%), protein (18-20%), and oil (17-18%) (Ajilogba and Babalola, 2023). It is considered a balanced food, as it contains a good amount of iron and its protein contains high levels of lysine and methionine, making it a suitable food source (Biswal *et al*., 2023). Lysine is the major essential amino acid and represents 10.3% of the total essential amino acids (Adewumi *et al*., 2022). The seed is rich in minerals such as calcium, potassium, iron, and sodium, and its high potassium content can also help manage diabetes by prompting the insulin hormone (Chandra *et al*., 2017). The fresh seeds of Bambara groundnut can be boiled to make pudding (Okpuzor *et al*., 2010), and the leaves have medicinal uses as an anti-vomiting agent. Additionally, the seeds can be used as feed for animals (Khan *et al*., 2021).

Bambara groundnut did not have enhanced seed releases with better agronomic features for industrial- and small-scale cultivation. Therefore, the scarcity of high-quality seeds from improved varieties, growers' inadequate understanding of the crop's significance, and the failure to introduce high-yielding genotypes in places where it is cultivated are some of the main causes of its low production and productivity (Uba et al., 2021). Inadequate understanding of taxonomy, reproductive biology combined with the genetics of agronomic and qualitative features, pests and diseases (Uba et al., 2021), a lack of genetic improvement, and adaptation to specific agro-ecological zones (Muhammad et al., 2020) are also some of the development-restraining factors. Due to underutilization, there has been little to no effort made to improve the crop's genetic makeup, which has decreased crop output and quality.

Chloroplasts are organelles found in plant cells that play a crucial role in photosynthesis and primary metabolism. They also have the ability to synthesize phytohormones and secondary metabolites, as well as respond to environmental signals. The structure and dynamics of chloroplasts are important for their function, and recent technological advancements have provided insights into their architecture and functionality (Littlejohn et al., 2021; Kirchhoff, 2019). Chloroplast DNA is a short, circular, double-stranded DNA molecule found in plants. It is typically 83-292 kb in size and exhibits uniparental inheritance, with maternal inheritance being predominant in angiosperms and paternal inheritance in gymnosperms (Turudić et al., 2021). The chloroplast genome is widely used in plant phylogenetic and biotechnological applications and for analysing evolutionary relationships among organisms (Wanichthanarak et al., 2023). Understanding chloroplast biology is essential for comprehending plant defence responses, energy conversion, and metabolic regulation and is used to analyse the evolutionary relationships of plant species (Sonnante, 2019).

The rpoA gene coding for the subunit of DNA-dependent RNA polymerase is located in the chloroplast genome of plants; it specifies a protein of 339 amino acids with a deduced molecular mass of 36,510 Da, exhibiting 64.3 to 70.7% similarity over its entire length. It encodes the alpha subunit of DNA-dependent RNA polymerase. The α subunit of the RNA polymerase core enzyme consists of two domains connected by a flexible linker. The rpoA amino-terminus is necessary and sufficient for the dimerization of rpoA and subsequent assembly of the RNA polymerase core complex (Ortelt and Link, 2021).

Single Polymorphism (SNP) refers to a variation in a single nucleotide at a specific site in the genome.

SNPs are present in human genomes as well as in plants and microorganisms. They are widely used in genetic association studies to understand the genetic basis of various traits and phenotypes (Tesi et al., 2021). SNP markers and phylogenetics are studied to understand a crop's genetic diversity and population structure, which are crucial for crop improvement programmes and breeding efforts (Ajilogba and Babalola, 2023; Majola et al., 2023). SNP markers have been used to characterise genetic diversity and identify genomic variations that can be utilised by plant breeders for crop improvement (Ajilogba and Babalola, 2020). The study of SNP markers and phylogenetics helps with conservation strategies and effective management of the crop. These studies contribute to a better understanding of the genetic makeup of Bambara groundnut and provide valuable information for its utilisation and improvement. This study aimed at unveiling evolutionary insights into Bambara groundnut (*Vigna subterranea* (L.) Verdc) genotypes by investigating SNP variation and phylogenetic patterns of the cpDNA rpoA gene.

**Materials and Methods:**

**Plant material**

The plant material consisted of seven Bambara nut genotypes from Yobe State, Nigeria from different locations.

**Collection and Identification**

The Seven (7) *V. subterranea* genotypes used in the study were collected from different locations in Yobe State, Nigeria. The seeds were identified and authenticated at the Herbarium Section, Department of Plant Biology, Faculty of Life Sciences, Bayero University Kano, by comparing with already deposited voucher specimen No. BKHAN 509.

**Planting/Sowing**

The *V. subterranea* seeds were planted in the Department of Biochemistry Garden. A round plastic tray divided into eight sections using thread was used for sowing the seeds, where each section was used for different genotypes. The tray was filled up with soil and then pressed down until the top was level with the top of the tray, It was then moistened with water and the seeds were sowed in each of the sections. It was watered twice daily (Morning and Evening). Four days after germination, young needles were collected and cleaned with distilled water, and stored at 4 $^{o}$C in a refrigerator for cpDNA extraction.

***Chloroplast DNA extraction***

The modified high salt cpDNA isolation method described by Drummond *et al.*, (2009) was used. **Buffer A (pH 3.8)** 1.25 M NaCl, 0.25 M ascorbic acid, 10 mM sodium metabisulfite, 0.0125 M Borax, 50 mM Tris-HCl (pH 8.0), 7 mM EDTA, 1% PVP-40 (w/v), 0.1% BSA (w/v), 1 mM DTT; **Buffer B (pH 8.0)**

1.25 M NaCl, 0.0125 M Borax, 1% PVP-40 (w/v), 50 mM Tris-HCl (PH 8.0), 25 mM EDTA, 0.1% BSA (w/v), 1 mM DTT; **Buffer C** 100 mM NaCl, 100 mM Tris-HCl (PH 8.0), 50 mM EDTA, 1 mM DTT;

Both BSA and DTT were added just before the start of the experiment.

***Chloroplast isolation***

The young needles were cut into pieces and homogenized in 400 ml ice-cold buffer A for 30 seconds. The homogenate was then filtered into centrifuge bottles using two layers of Miracloth (Merck) by softly squeezing the cloth. The homogenate was then centrifuged (at 200 g, for 20 min), and the nucleus pellet and cell-wall debris were discarded. The centrifugation was repeated once again; the supernatant will include chloroplasts suspended in it. The supernatant was then

centrifuged at a higher centrifugal force of 3500 g for 20 min, and the resulting pellet which is chloroplast pellet with, 250 ml Buffer B was added to the pellet and suspended gently using a paintbrush to wash the nuclear DNAs attaching to the chloroplast cytomembrane, which was then centrifuged at 3500 g for 20 min and the supernatant discarded. The pellet was re-suspended with 250 ml buffer B again and centrifuged (at 3750 g for 20 min) to gain the purified chloroplasts.

***Chloroplast DNA isolation***

8 ml Buffer C, 1.5 ml 20% SDS, 20 ml b-Me and 30 ml Proteinase K (10 mg/ml) was added to the purified chloroplast pellet in centrifuge bottles and incubated at 55 $^{o}$C for 4 hours. The chloroplast was then fully lysed. The centrifuge bottles were then put on ice for 5 minutes, then 1.5 ml 5 M KAc (pH 5.2) was added and allowed to freeze for 30 minutes and then centrifuged at 10000 g for 15 min, the pellet was discarded. The supernatant was extracted with an equal volume of saturated phenol and chloroform: isoamyl-alcohol (24:1) and subjected to centrifugation at 10000 g for 20 min twice. An equal volume of isopropyl alcohol (10 ml) was added to the upper clear aqueous phase and centrifuged at 10000 g for 20 min. The cpDNA pellet was then washed repeatedly with ethanol (70%, 96%), air-dried, and re-dissolved in 50 ml TE buffer, and then treated with 2 µl RNAse.

**Polymerase chain reaction**

Amplification of rpoA gene was carried out as described by (Oxelman *et al*., 1997) using rpoA gene primer (Forward 5′-ATGAATTGCAAAATGCTTTTCTAGA -3′; Reverse 3′-TGGTTCGAGAGAAAGTAACAGTATC-5′) which were design according to the sequence of UTR regions. The PCR reaction was carried out using KAPATaq DNA polymerase. The Total reaction volume was 25µL, reaction mixture comprised 2µL each of the cpDNA, 2.5 µL of 10 TaqA Buffer, ~0.4M (0.85 µL) of forward and reverse primers, 1.25 mM (1.5 µL) of $MgCl_2$, 0.25 mM (0.2 µL) of dNTP mixes and 0.2 µL of Taq DNA polymerase, in ddH2O. Amplification was done using the following conditions: initial denaturation phase of 5 min at 95℃, followed by 35 consecutive cycles of denaturation each of 30 s at 94℃, hybridization of primers for 1 min at 44 – 51℃ (primer annealing) and stretch for 1 min at 72℃. A final elongation at 72℃ for 10 min followed by cooling to 10 $^{o}$C.

**Agarose gel electrophoresis**

The Amplified PCR product was visualised using the agarose gel electrophoresis as described by Mason (2015). The amplification products were subjected to electrophoresis at 150 V using 2% agarose gel in which 7.5 µl of Ethidium Bromide (BET) during 1 h 30 min in 0.5x Tris Borate EDTA (TBE) buffer. The wells were loaded with 10 µl of PCR product in the presence of a marker of molecular weight. The migration gel visualized using a UV transilluminator was and then was photographed for evaluation.

**Sequencing**

The amplified rpoA gene product was then sequenced using the method described by (Shi *et al*., 2012).

**Nucleotide polymorphisms**

The rpoA gene sequence of *V. subterranea* was used to carry out single nucleotide polymorphism using bioinformatics software as described by Landegren *et al.*, (1998).

The SNP analysis of the chloroplast DNA for the seven DNA sequencing was done by aligning the sequences using CLC Sequence viewer. All reads

that are not aligned were removed, only filtered reads were further run to identify SNPs in the chloroplast DNA sequences respectively. Homogeneous SNPs with a read depth ≥10, mapping quality ≥20, and SNP quality ≥15 were retained and heterogeneous SNPs were filtered. The SNPs of intersection among the three repeat subsamples were used to quantify the effects of the variants on genes within the population.

**Phylogenetic analysis**

The rpoA gene sequences of *V. subterranea* genotypes were aligned and phylogenetic analyses were constructed using MEGA 5, using the Tamura–Nei model to calculate the genetic distances between sequences (Tamura *et al.*, 2011). The inference of the phylogenetic tree was carried out with the neighbour–joining method (Saitou and Nei, 1987). The reliability of the clades formed at the species level in the tree was evaluated using the bootstrap test with 1,000 replications.

**Data Analysis**

The data obtained were analyzed using Clustal W, Bioedit, Mega 5 and NCBI website and presented in tables, figures and Plates. Major allele frequency, mean gene diversity within a population (Hs), total gene diversity (Ht), and Shannon index were computed using the R package "adegenet" (Jombart and Ahmed, 2011)and "hierfstat" (Goudet, 2005). The pairwise Nei's (Nei, 1972) D genetic distances between the populations were calculated using R software.

**Results And Discussion**

**PCR product of rpoA gene from *Vigna subterranea* cpDNA genotypes amplified using primer**

The rpoA gene from *Vigna subterranea* genotypes was amplified to test the quality of the product using electrophoresis, DNA band obtained from the amplification of the rpoA gene was ± 1000 bp (Plate 1)

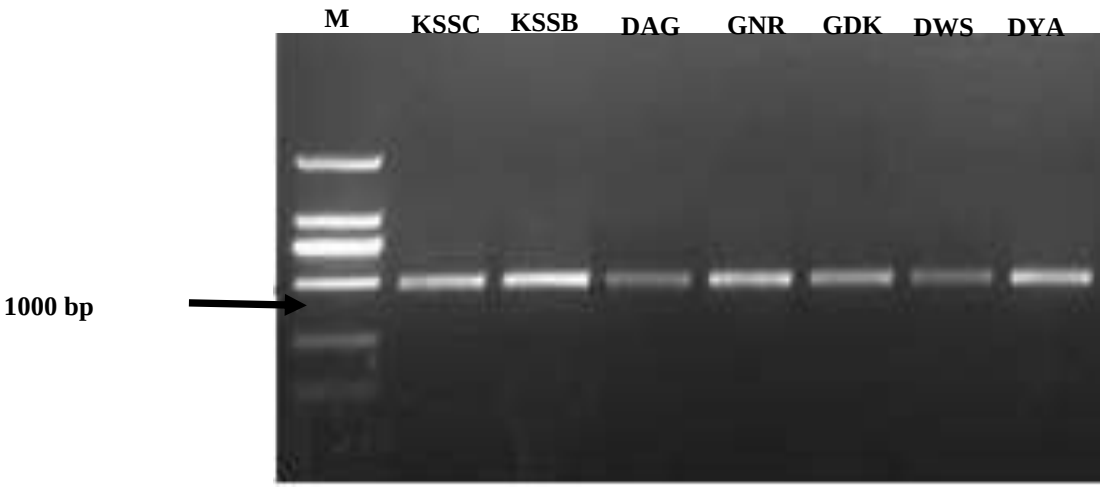


**Plate 1: Electrophoregram showing amplicon of rpoA gene from seven genotypes *Vigna subterranea* L. Verdc.** Lanes: M = DNA ladder, KSS = Kasaisa, DAG = Damagum, GNR = Gadaka, GDK = Goniri, DWS = Dawasa , DYA = Daya (Correspond to a different chloroplast DNA concentrations used for the PCR reaction).

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**Distribution of SNPs in genic [exonic, intronic, 5′- and 3′- untranslated (UTR)] and intergenic**

The SNPs in the study were annotated and classified based on their location in intergenic regions, introns, 5′-UTR, 3′-UTR or exon. Analysis of the distribution of SNPs in the rpoa gene indicated the predominance of intergenic regions (60.5%) compared to the introns (9.6%), 5′-UTR (4.8%), 3′-UTR (5.3%) and exons (19.8%) regions (Table 1).

Genetic diversity was estimated per location of the source of the studied genotype (Table 2). GNR had the highest mean of major allele frequency (0.89), followed by DWS (0.75), KSSC (0.68), KSSB (0.65), DYA (0.64), DAG (0.62) and (0.58) for GDK. DWS had the highest (0.475) gene diversity within the populations across the seven genotypes, while the least was found in DYA (0.259). The comparison of the total gene diversity analysis showed that GNR (0.480) revealed the highest diversity, followed by the DWS (0.479), DAG (0.477), GDK (0.459), KSSB (0.456), DYA (0.454) genotypes and KSSC (0.261). While in the Shannon diversity index, DWS had the highest value (0.786), while the lowest was GNR (0.455).

The KSSB and KSSC exhibited the shortest genetic distance value (0.001), according to the average Nei genetic distances among the accessions within each population (Table 3). DWS and DAG (0.002) genotypes showed a similar outcome due to a close connection. The genetic distance between GDK and DYA genotypes was determined to be (0.058), whereas GNR and DYA displayed a closer link with KSSB (0.003). The GDK genotype revealed a somewhat distant link with the other groups. The GDK genotype had a somewhat tight association with the KSSB genotype (0.011) among the groups under study, followed by the KSSC genotype (0.017).

**regions of rpoA gene from *Vigna subterranea* L. Verdc. genotypes**

**Phylogenetic Analysis of rpoA from *Vigna subterranea* L. Verdc.**

The phylogenetic relationship of rpoA gene sequence from Bambara groundnut *Vigna subterranea* L. Verdc**.** genotypes showed four clusters, the first Cluster include KSSB and KSSC, DWS and DAG in the second cluster, third cluster has only GDK and the fourth cluster has DYA and GNR (Figure 1).

**Discussion:**

Studying nucleotide quantities and patterns of nucleotide variation both between and within species is essential for gaining insights into the evolutionary mechanisms. These mechanisms encompass the transformation of genetic polymorphisms within species into genetic divergence between species, as well as the maintenance of genetic diversity. These diversities are influenced by key evolutionary processes, including selection, recombination, mutation, and population structures.

The quality assessment of rpoA gene products, amplified from seven Bambara groundnut genotypes, was conducted through electrophoresis on a 0.8% agarose gel. The gel was stained with Ethidium Bromide, and bands were visualized using a gel documentation system. The successful application of the optimized extraction method for obtaining high-quality cpDNA from all tested genotypes demonstrates its broad utility. The PCR gel exhibited bands of approximately 1000 base pairs (bp) for all seven genotypes. This 1000 bp fragment of the rpoA gene aligns with the findings of Emily et al. (2016), who cloned a 1014 bp segment of the rpoA gene. Agarose gel electrophoresis has proven to be a highly efficient method for separating nucleic acids.

**Table 1:** SNPs in the rpoA gene from *Vigna subterranea* L. Verdc. genotypes

| ıdraces | Bp | Intergene | Intron | 5'-UTR | 3'-UTR | Exon |
|---|---|---|---|---|---|---|
| **KSSC** | 996 | 613 | 91 | 41 | 54 | 197 |
| **KSSB** | 1002 | 587 | 92 | 54 | 68 | 201 |
| **DAG** | 996 | 601 | 102 | 59 | 41 | 193 |
| **GNR** | 996 | 621 | 93 | 57 | 38 | 187 |
| **GDK** | 997 | 589 | 91 | 48 | 64 | 205 |
| **DWS** | 999 | 597 | 105 | 39 | 55 | 203 |
| **DYA** | 998 | 614 | 98 | 38 | 51 | 197 |
| **Total** | 6984 | 4222 | 672 | 336 | 371 | 1383 |

**Table 2:** Estimation of major allele frequency, mean gene diversity within a population, total gene diversity and Shannon diversity among Bambara groundnut genotypes.

| on | Genotypes | allele ency | | | |
|---|---|---|---|---|---|
| **KSSC** | 1 | 0.68 | 0.410 | 0.261 | 0.725 |
| **KSSB** | 1 | 0.65 | 0.367 | 0.456 | 0.710 |
| **DAG** | 1 | 0.62 | 0.426 | 0.477 | 0.746 |
| **GNR** | 1 | 0.89 | 0.465 | 0.480 | 0.455 |
| **GDK** | 1 | 0.58 | 0.454 | 0.459 | 0.689 |
| **DWS** | 1 | 0.75 | 0.475 | 0.479 | 0.786 |
| **DYA** | 1 | 0.64 | 0.259 | 0.454 | 0.546 |

**where Hs = gene diversity within population, Ht = Total gene diversity, I = Shannon diversity**

**Table 3:** Pairwise Nei's genetic distance among Bambara groundnut populations from the geographical locations

| Locations | KSSB | KSSC | DAG | DWS | GNR | DYA |
|---|---|---|---|---|---|---|
| **KSSC** | 0.001 | | | | | |
| **DAG** | 0.022 | 0.023 | | | | |
| **DWS** | 0.012 | 0.042 | 0.002 | | | |
| **GNR** | 0.011 | 0.022 | 0.024 | 0.025 | | |
| **DYA** | 0.022 | 0.023 | 0.025 | 0.022 | 0.003 | |
| **GDK** | 0.011 | 0.017 | 0.025 | 0.046 | 0.048 | 0.050 |

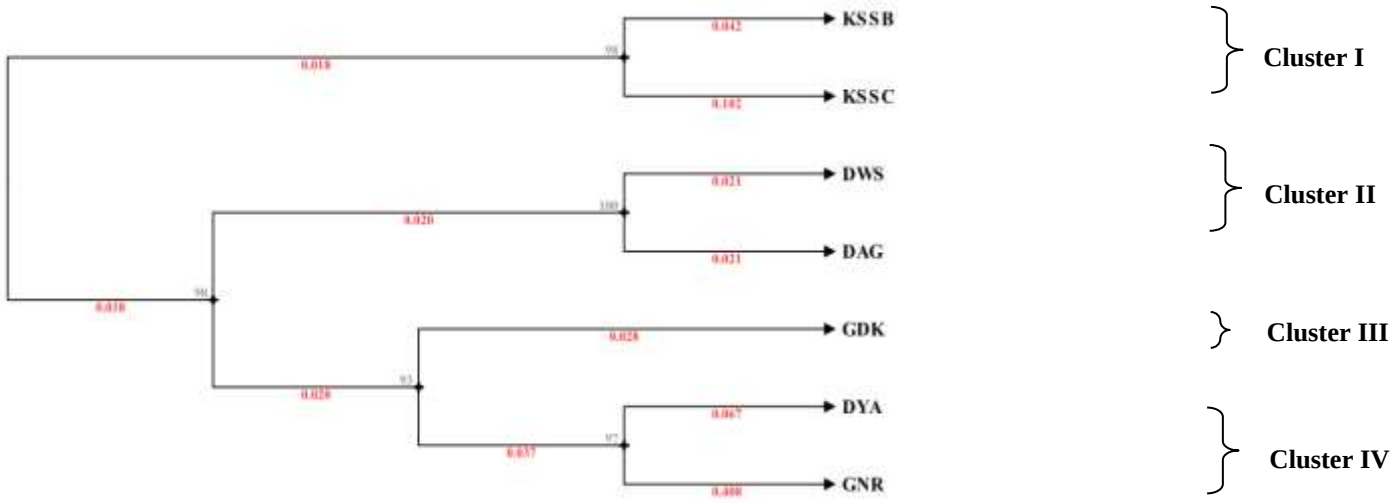


**Figure 1: Phylogenetic relationship of partial sequences of rpoA gene with 1000 bootstrap replications. Supporting values were indicated by color; bootstap value % (blue) and Branch Lenghts (red).**

The gel's robust gel strength allows for the handling of low percentage gels, particularly for the separation of large DNA fragments. The size exclusion properties are governed by the pore size created by the bundles of agarose in the gel matrix. Generally, higher agarose concentrations result in smaller pore sizes. Traditional agarose gels excel in separating DNA fragments ranging from 100 bp to 25 kb (Gao et al., 2021).

The nucleotide sequence of the plastid-encoded alpha subunit of DNA-dependent RNA polymerase gene (rpoA) of Vigna subterranea was determined. The size of the rpoA gene can vary among plant families, and differences can be observed even among species within the same family. In plants, the size of the rpoA gene typically falls within the range of 990 to 1020 nucleotides, while in bacteria, it remains below 990 nucleotides (Wincker, 2019; Wang et al., 2018).

SNPs in the rpoA gene sequences were assessed among seven *Vigna subterranea* genotypes. A total of 442 and 437 SNPs were detected in 5'-UTR and 3'-UTR regions from the amplified sequence of seven genotypes from *Vigna subterranea* rpoA gene which represents 4.2 and 4.4%, DAG genotype with 59 has the highest number of polymorphism and DYA with 38 has the lowest in the 5'-UTR region. and in the 3'-UTR region KSSB with 68 has the highest number of polymorphism while DAG with 41 has the lowest (Table 1) respectively. The results explained the heterogeneity status of the genotypes used in this study as sourced from different genotypes.

To safeguard and effectively utilize Bambara groundnut landraces from diverse African regions, it is crucial to comprehend their genetic diversity and population structure. This knowledge is vital for developing successful breeding strategies for this crop, particularly when identifying valuable alleles within landraces and other genotypes (Uba et al., 2021).

The observation of higher genetic diversity within the DWS genotype (Hs = 0.475, Ht = 0.479) in comparison to other locations, and the similarity in diversity levels with the GNR genotype (Hs = 0.465, Ht = 0.480), suggests that Bambara groundnut may be abundant in this location or has exhibited significant evolutionary potential. This may be attributed to effective crop conservation practices by local growers. Moreover, the extensive collection of Bambara germplasm from these areas offers a valuable resource for selecting improved genotypes and superior accessions for hybridization programs.

The close proximity and strong community ties among Bambara groundnut genotypes from KSSB, GNR, and DWS result in low genetic distances, mirroring the findings of Uba et al. (2021). Additionally, the reduced genetic distances observed between DWS-DAG (0.002) and GNR-DYA (0.003) indicate a shared pool of common alleles and close genetic relationships, aligning with the work of Rungnoi et al. (2012) and Somta et al. (2011). Notably, GDK exhibits a significant and substantial genetic distance from genotypes in other locations.

The phylogenetic relationship of the study evaluates the usefulness of gene sequences for molecular identification and phylogenetic study. Cluster analysis of the sequenced rpoA gene genotypes showed four clusters (Figure 1), this reflects evolutionary history and relationships among different entities, reflecting the complexity, diversity and genetic variation, This explains the existence of significant genetic diversity in the study population. The different genetic groups obtained are composed of individuals from all agro-climatic zones. The combination of genotypes from

different locations was shown by the different clusters, these results corroborate that of Ouoba *et al.,* (2019) which led to this same organisation.

Molecular genetic diversity analyses have been used to aid breeding decisions and germplasm conservation agenda in crop species (Choudhary *et al*., 2013; Huynh *et al*., 2013). Specifically, for *Vigna subterranea* , various molecular analyses of diversity have been reported to evaluate or validate the various reports based on phenotypic descriptors (Olukolu *et al*. 2012; Aliyu *et al*., 2014; Mayes *et al*., 2015; Molosiwa *et al*. 2012). The earliest report of diversity analysis at the molecular/cellular level in *Vigna subterranea* includes the report of Pasquet *et al*. (1999). In an analysis of 79 domesticated landraces of *Vigna subterranea* and 29 wild relatives at 41 isozyme loci, Pasquet *et al*. (1999) concluded that the wild relative is the true progenitor of the former based on the high level of genetic similarities.

**Conclusions:**

The amplified PCR gel products of the rpoA gene from seven Bambara groundnut (*Vigna subterranea* (L.) Verdc) cpDNA showed ± 1000 base pairs (bp), while the Single Nucleotide Polymorphism showed a detection of of 442 SNPs in the 5'-UTR and 437 SNPs in the3'-UTR from the sequenced rpoA gene of the *Vigna subterranea* L. Verdc genotypes representing 4.2 and 4.4%. The phylogenetic relationship of rpoA gene sequence from Bambara groundnut *Vigna subterranea* L. Verdc**.** genotypes showed four clusters which explains the existence of significant genetic diversity in the study population. The finding also will provide new insight into the population structure of Bambara groundnut genotype which will help in conservation strategy and management of the crop.

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**Conflict of Interest:**

Authors have declared that no conflict interests exist.

**Reference:**

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