ZIBELINE INTERNATIONAL™ PUBLISHING

Acta Scientifica Malaysia (ASM)

DOI: http://doi.org/10.26480/asm.02.2025.96.99

ISSN: 2521-5051 (Print)
ISSN: 2521-506X (Online)
CODEN: ASMCCQ

RESEARCH ARTICLE

THE GENOMIC-DNA RELATIONSHIP OF SOME WILD ACCESSIONS OF COWPEA (VIGNASPP) USING UN-WEIGHTED PAIR GROUP METHOD OF ARITHMETIC MEANS (UPGMA)

V.J. Adavbiele*, A.U. Omoregie and E. Akhigbe

Department of Crop Science and Agricultural Biotechnolgy, Ambrose Alli University, Ekpoma, Edo State.
****Corresponding Author Email: adavbiele_victor@yahoo.com***

ARTICLE DETAILS

Article History:

Received 10 June 2025
Revised 15 July 2025
Accepted 29 August 2025
Available online 25 September 2025

ABSTRACT

The Genomic-DNA of five wild accessions (TVnu-59, TVnu-561, TVnu-563, TVnu-1148 and TVnu-1395) of Cowpea (Vigna species) using Un-weighted Pair Group Method of Arithmetic Means (UPGMA) was carried out at African Bioscience Laboratory, Ibadan, Oyo State, Nigeria. The samples of the five wild accessions (TVnu-561, TVnu-563, TVnu1148, TVnu-1395 and TVnu-59)were collected using molecular kits and analyzed using Single Nucleotide Polymorphism (SNP) marker.SNP generated a moderate level of polymorphism and revealed existence of similar genetic diversity among four (TVnu-561, TVnu-563, TVnu1148 and TVnu-1395) out of the five wild accessions. The three level sequences of the four wild accessions showed that two (TVnu-561 andTVnu-1395) were 40% monomorphic and three (TVnu-563, TVnu-1148 and TVnu-1395) were 60% polymorphic. However, TVnu-1395 demonstrated both monomorphism and polymorphism, a distinct character that showed its slightly similar genetic diversity with others. The Dendogram revealed the three wild accessions (TVnu-561, TVnu-563 and TVnu11480) that clustered togethershowed high degree of similar genetic diversity, formed a sub-cluster with in the one accession(TVnu-1395) with slightly similar genetic diversity. Clustering ranged from 1%-20%.TVnu-1395 which had the highest yielding characters with identified slightly genetic variations should be used to improve the others as these will enhance desirable traits such as yield.

KEYWORDS

Genomic-DNA, UPGMA, SNP, Polymorphic, Monomorphic

1. INTRODUCTION

The origin of wild Vignaspecies can be traced to Africa, Asia, and Australia. Wild Vigna varieties, originating from the above regions, have unique traits that make them valuable genetic resources for improving cultivated Vigna species such as cowpea. Vigna, a genus within the Fabeceae family, includes several important species such as cowpea (Vignaunguiculata) and mung bean (Vignaradiata).These species are valuable for their nutritional content particularly protein, and adaptability to various environmental conditions (Smith et al., 2020; Olayinka et al., 2020). Wild Vigna varieties play a significant role in the genetic diversity and adaptation of cultivated Vigna species. These wild varieties have adapted to diverse environments over time, developing unique characteristics that make them valuable genetic resources for crop improvement.By studying their origins and characteristics, researchers can uncover valuable traits for crop improvement and sustainable agriculture practices (Jones and Brown, 2019).

The study of genomic relationships among wild Vigna species is crucial for understanding their evolutionary history and potential for crop improvement. The Un-weighted Pair Group Method of Arithmetic Mean (UPGMA) is a clustering method used to analyze genetic relationships based on similarity indices (Adavbiele et al., 2018). This method is particularly useful in constructing phylogenetic trees that depict the evolutionary distances between species.UPGMA is effective in revealing genetic relationships in various plant species.The UPGMA analysis group species based on genetic similarities and will unveil the evolutionary relationships among these wild Vigna species (Adavbiele et al., 2021).This understanding is crucial for identifying key genetic markers and traits that can be utilized in breeding programs to develop high-yielding Vigna cultivars (Adavbiele et al., 2023).

The study onthe Genomic-DNA Relationship of some Wild accessions of Cowpea (Vigna species) using Un-Weighted Pair Group Method of Arithmetic Means (UPGMA) was carried out to unravel the genetic diversity present in these wild Vigna species through UPGMA analysis for the selectionof parent species in breeding programs, which will facilitates the development of Vigna varieties that are not only high-yielding but also resilient to changing environmental conditions, thereby enhancing global food security.

2. MATERIALS AND METHODS

A Potted plant experiment was conducted at the Department of Crop Science and Agricultural Biotechnology, Ambrose Alli University, Ekpoma.Edo State. The Molecular characterization of some wild accessions of Cowpea (Vigna species) was carried at African Bioscience Molecular Laboratory, Ibadan, Oyo State, Nigeria.

2.1 Experimental Design and Materials

The experiment consisted of Potted plant and their analysis in the Laboratory. The Potted Plants were arranged in Completely Randomized Design (CRD) and replicated three times. The experimental layout was made up of fifteen (15) plots. Each plot contained six polybags (nursery bags) giving a total of ninety polybags. Five different accessions of wild Vigna(TVnu-59, TvNu-561, TVnu-563, TVnu- 1148 and TVnu-1395) were used. The wild Vigna accessions were collected from the Institute of

Quick Response Code	Access this article online	
	Website: www.actascientificamalaysia.com	**DOI:** 10.26480/asm.02.2025.96.99

Cite The Article: V.J. Adavbiele, A.U. Omoregie and E. Akhigbe (2025). The Genomic-Dna Relationship Of Some Wild Accessions Of Cowpea (Vignaspp) Using Un-Weighted Pair Group Method Of Arithmetic Means (UPGMA). *Acta Scientifica Malaysia, 9(2): 96-99.*

Tropical Agriculture (IITA) Ibadan, Oyo state. These were sown in standard nursery bags measuring 24.5cm length by 20.32cm width each; filled with top black well-drained loamy soil, mixed with organic manure. Each bag of soil weighed (6.3kg). The nursery bags were arranged at a spacing of 100cmx 60cm. Two seeds of each accession were sowed in soil bags containing water. Fresh leaves were harvested for analysis

2.2 Molecular Analysis

The polymerase chain reaction (PCR) amplification was carried out in African Bioscience molecular laboratory, Ibadan, Oyo State, Nigeria.

2.3 Genomic DNA extraction

DNA extraction was carried out using flower terminal genes exon from the fresh leaves collected from seedlings. The genomic DNA of the five wild accessions was isolated using the CetylTrimethyl Ammonium Bromide (CTAB) mini-prep method (Doyle and Doyle, 1990). Fresh leaves from each accession were harvested and 2cm of each leaves was then cut, ground in 600ul of extraction buffer and incubated at 65°C for 60mins. This incubation period allows the CTAB in the lysis buffer to break down the cell walls and membranes, releasing the genomic DNA from the plant tissues. The chemicals used in the extraction process, such as CTAB, EDTA, chloroform, isopropanol, and ethanol, each serve specific functions in different steps of the extraction protocol. CTAB aids in cell lysis, EDTA helps in DNA stabilization, chloroform is used for DNA extraction, and isopropanol and ethanol are involved in DNA precipitation and washing steps to purify the genomic DNA.

2.4 Gel Electrophoresis

Two percent (2%) agarose gel was used for the electrophoresis. 0.8g of agarose powder (CSL-AG100 LE multi-purpose agarose) was dissolved in 40 ml of 1X TAE (TRIS acetate EDTA) electrophoresis buffer using a microwave to ensure the powder dissolves properly. The solution was then stained with 9ulethidium bromide and was allowed to polymerise in the gel electrophoretic cast in which the comb was properly placed. TAE running buffer was poured into the electrophoresis tank to submerge the polymerized gel. The amplified PCR product of each sample was resolved in the 2% agarose gel at 80v, 250mA for 30 minutes. 4ul of the products were carefully loaded in the wells, using 100bp molecular weight marker as control for size. Resolved allelic fragments were sized using 100bp ladder and were visualized using UV trans-illuminator.

3. Results And Discussions

The number of nucleotides polymorphism varied from 48 to 86 (table 1). Forms of nucleotides polymorphism showed the same single on. All the wild accessions of cowpea revealed the same minor allele. Minor Allele Frequencies for all the genotypes showed same value (0.80). The heterozygosity is the same for all the genotypes. The same value (0.2688) was recorded for Polymorphic Information Content. The images revealed that all the genotypes lied on the same baseline when compared with the base pair ladder of 100bp (Plate 1).

Table 1: Single nucleotide polymorphisms (SNPs), major allele frequency, heterozygosity and polymorphism information content of SNPs identified in exon 3 of Terminal flower 1-like gene in four varieties of cowpea (Vignaunguiculata)

Table 1: Single nucleotide polymorphisms (SNPs), major allele frequency, heterozygosity and polymorphism information content of SNPs identified in exon 3 of Terminal flower 1-like gene in four varieties of cowpea (Vignaunguiculata)

SNPs	Genotype	Form of SNPs	MA	MAF	(He)	PIC
48A<T	TVnu-1395	Singleton	A	0.80	0.32	0.2688
52T<G	All	Singleton	G	0.80	0.32	0.2688
55C<T	All	Singleton	T	0.80	0.32	0.2688
59C<A	All	Singleton	G	0.80	0.32	0.2688
66G<T	All	Singleton	A	0.80	0.32	0.2688
69T<G	All	Singleton	T	0.80	0.32	0.2688
70G<T	All	Singleton	T	0.80	0.32	0.2688
76T<C	All	Singleton	G	0.80	0.32	0.2688
80T<C	All	Singleton	T	0.80	0.32	0.2688
81C<T	All	Singleton	C	0.80	0.32	0.2688
82A<G	All	Singleton	C	0.80	0.32	0.2688
85A<T	All	Singleton	T	0.80	0.32	0.2688
86T<C	All	Singleton	G	0.80	0.32	0.2688
52T<G	All	Singleton	T	0.80	0.32	0.2688
55C<T	All	Singleton	C	0.80	0.32	0.2688

MA: Minor Allele, MAF: Minor Allele Frequency He: Heterozygosity; PIC: Polymorphic Information Content

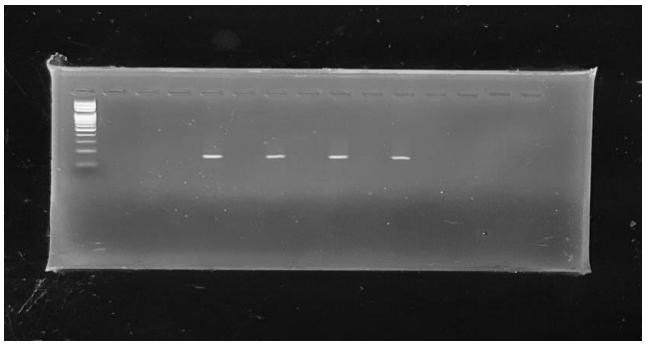


Plate 1: Gel Electrophoresis Image

SNP marker generated a moderate level of polymorphic and revealed existence of similar geneticdiversity among four (TVnu-561, TVnu-563, TVnu1148 and TVnu-1395) out of the five wild accessions. The three level sequences of the four wild accessions showed that two (TVnu-561 and TVnu-1395) were 40% monomorphic and three (TVnu-563, TVnu-1148 and TVnu-1395) were 60% polymorphic (Table 2). However, TVnu-1395 demonstrated both monomorphism and polymorphism, a distinct character that showed its slightly similar genetic diversity with others. Thus this study revealed significant similar genetic diversity among the wild accessions, with SNP markers effectively distinguishing between different accessions.

Table 2: Haplotypes present in exon 3 of Terminal flower 1-like gene in four varieties of cowpea (Vignaunguiculata)

			Genotype			
Haplotype	**Haplotype sequence**	**TVnu-561**	**TVnu-563**	**TVnu-1148**	**TVnu-1395**	**Total**
1	ATCACGCTGTTCAAT	1 (0.25 %)	0 (0.00 %)	0 (0.00 %)	0 (0.00 %)	1
2	GTGATTGTCCTGTC	0(0.00%)	1(0.25 %)	1(0.25%)	1(0.25%)	3
3	TGTGATTGTCCGTC	0(0.00%)	0(0.00 %)	0(0.00%)	1(0.25%)	1

The phylogenetic analysis of exon 3 of the Terminal Flower 1-like (TFL1-like) gene in these Vigna wild accessions (TVnu-561, TVnu-563, TVnu-1148, and TVnu-1395) provided insights into their evolutionary relationships. The forward and backward alignment of the allele sequences was shownin figure 1. Some base pairs did not align; while some aligned in their sequences. These were indications that multiples alleles co-existed at specific locus.

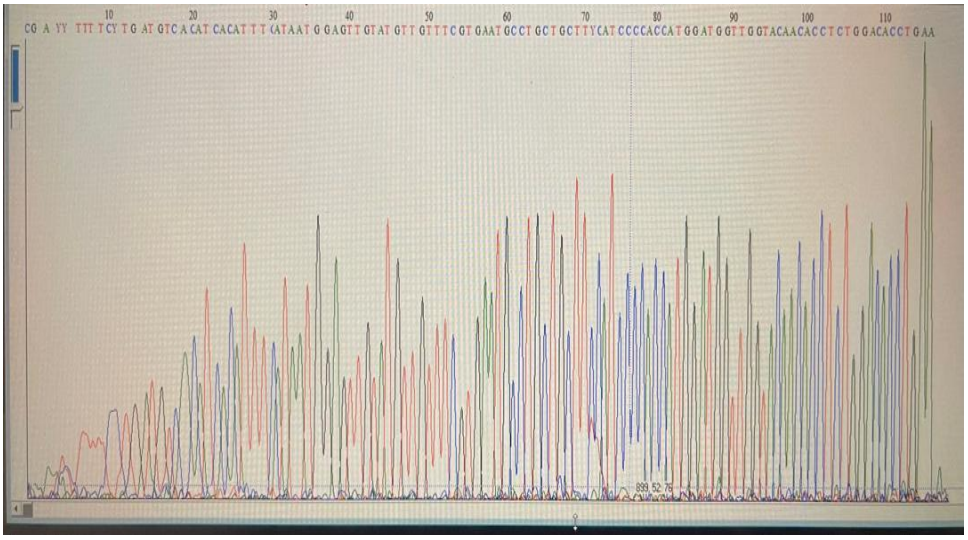
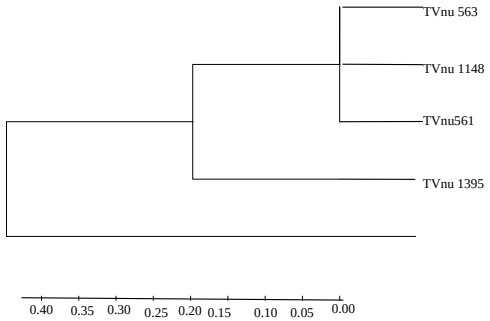


Figure 1: Alignment results of exon 3 of Terminal flower 1-like gene in four varieties of cowpea (Vigna unguiculata)



The dendogram showed similarities in their genetic diversitiesamongthe three wild accessions (TVnu-563, TVnu-1148 and TVnu-561). TVnu-1195 differed slightly in its genetic composition when compared with other three wild accessions. DNA analysis identified distinct genetic clusters, reflecting the diverse origins and genetic backgrounds of these Vigna species. Understanding these genetic differences can inform the development of improved cowpea cultivars tailored to specific environmental conditions (Xiong et al., 2016).These observations underscore the importance of slightly genetic variations, as observed in TVnu-1395, which may contribute to evolutionary adaptations and phenotypic diversity. This is in agreement with who investigated the genetic diversity and population structure of cowpea genotypes using Single Nucleotide Polymorphism (SNP) markers (Sonker et al., 2019; Sarr et al., 2021; Gumede et al., 2022; Diallo et al., 2024). UPGMA revealed a high degree of genetic similarity, with 14 consistent SNPs across three wild accessions and an additional SNP in TVnu-1395, indicating slight genetic divergence.

The clustering pattern in the UPGMA dendrogram likely showed that the three Vignawild accessions were closely related.The identified slightly genetic variations in TVnu-1395 should be explored for potential incorporation into breeding programs aimed at enhancing desirable traits such as yield.Variations in Cowpea have tremendous implications for breeding programs as they showcase the broad range of traits and genotypes that breeders have to work with in breeding for desirable traits (Nkhoma et al., 2020).

4. Conclusion

SNP marker generated a moderate level of polymorphic and revealed existence of similar genetic diversity among four (TVnu-561, TVnu-563, TVnu1148 and TVnu-1395) out of the five wild accessions. The three level sequences of the four wild accessions showed that two (TVnu-561 and TVnu-1395) were 40% monomorphic and three (TVnu-563, TVnu-1148 and TVnu-1395) were 60% polymorphic. However, TVnu-1395 demonstrated both monomorphism and polymorphism, a distinct character that showed its slightly similar genetic diversity with others. The dendogram showed a high degree of similarities in their genetic diversities among the three wild accessions (TVnu-563, TVnu-and TVnu-561), thus closely related. TVnu-1195 differed slightly in its genetic composition when compared with other three wild accessions. TVnu-1395 which had the highest yielding characters with identified slightly genetic variations should be used to improve the others.

References

Adavbiele, V.J., 2023. The Genomic-DNA Relationship of Some rice accessions Using Un-weighted Pair Group Method Analysis of Arithmetic Means (UPGMA). Proceedings of the Maiden conference of Nigerian Plant Breeders Association (NPBA) held at Abuja 25-26 September, 2023.

Adavbiele, V.J., Omoregie, A.U., and Eziashi, E.I., 2018. Qualitative characterization of some Upland rice (OryzasativaL.) in the Forest-Savannah transition zone of Edo State, Nigeria. Nigerian Journal of Agriculture and Forestry (NJAF), (6), Pp. 120-130.

Adavbiele, V.J., Omoregie, A.U., and Eziashi, E.I., 2021. Taxonomic and Genomic-DNA evaluation studies of some accessions of rice (Oryzasativa L.) in a Forest-savannatransition Zone of Edo State, Nigeria. Proceedings of the 7th National Conference of Crop Science Society of Nigeria held at Ambrose Alli University, Ekpoma, Nigeria. Pp. 214 -220.

Diallo, S., Badiane, F.A., Kabkia, B.N.P.A., Diédhiou, I., Diouf, M., and Diouf, D., 2024. Genetic diversity and population structure of cowpea mutant collection using SSR andISSR molecular markers. Scientific Reports, 14 (1), Pp. 31833.

Doyle, J.J., and Doyle, J.L., 1990. Isolation of plant DNA from fresh tissue. BRL Focus, 12, Pp. 13-15.

Gumede, M.T., Gerrano, A.S., Amelework, A.B., andModi, A.T., 2022. Analysis of Genetic Diversity and Population Structure of Cowpea (Vignaunguiculata (L.) Walp) Genotypesusing Single Nucleotide Polymorphism Markers.Plants (Basel, Switzerland), 11 (24), Pp. 3480. https://doi.org/10.3390/plants11243480.

Jone, P.R., and Brown, C.T., 2019. Assessing the adaptability and performance of Wild Vigna through field experiments and controlled growth conditions. Agricultural Science Journal, 22 (40), Pp. 153-162.

Nkhoma, N., Shimelis, H., Laing, M.D., Shayanowako, A., and Mathew, I., 2020. Assessing the genetic diversity of cowpea [Vignaunguiculata (L.) Walp.] germplasm collections using phenotypic traits and SNP markers. BMC genetics, 21, Pp. 1-16.

Olayinka, A., Kolawole, A., and Ilori C., 2020. Assessment of variation in the agronomic traits of wild cowpea (Vignaunguiculata (L) Walp.) subspecies under a rainforest agro-ecology in Nigeria GSC Biological Pharmaceutical Sciences, 11 (3), Pp. 244-253.

Sarr, A., Bodian, A., Gbedevi, K.M., Ndir, K.N., Ajewole, O.O, Gueye, B., Fonceka, D., Diop, B. M., Cisse, N., and Diouf, D., 2021. Genetic diversity and population structure analyses of wild relatives and cultrivated cowpea (Vignaunguiculata (L.) Walp) from Senegal using simple sequence repeat markers.Plant Molecular Biology Reporter, 39, Pp. 112-124

Smith, J.A., Williams, R. B., and Johnson, T. L., 2020. Morphological traits as indicators of genetic diversity and yield potential in WildVigna species.Plant genetic Resources, 18 (3), Pp. 210-219.

Sonker, S., Sharma, T., and Tandan, N., 2019. SSR based molecular characterization and diversity analysis of cowpea. Plant Archives, 19 (2), Pp. 995-1001.

Xiong, H., Shi, A., Mou, B., Qin, J., Motes, D., Lu, W., Ma, J., Weng, Y., Yang, W., and Wu, D., 2016. Genetic diversity and population structure of cowpea (Vignaunguiculata (L.) Walp.) PLoS ONE, 11 (8), Pp. e0160941. https://doi.org/10.1371/journal.pone.0160941