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RESEARCH ARTICLE

Molecular characterization of some wild accessions of cowpea (*Vigna spp.*) using single nucleotide polymorphisn (SNP) marker

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ABSTRACT

Molecular characterization of five wild cowpea (*Vigna* spp.) accessions was conducted at the African Bioscience Molecular Laboratory, Ibadan, Nigeria, using Single Nucleotide Polymorphism (SNP) markers. SNP analysis revealed allele counts ranging from 48 to 86, indicating nucleotide variation across accessions. All accessions exhibited singleton alleles, suggesting rare genetic variants. Four accessions (TVnu-561, TVnu-563, TVnu-1148, and TVnu-1395) showed transcriptional activity, while TVnu-59 did not. The SNP data generated moderate polymorphism, with a high Minor Allele Frequency (MAF) of 0.80 and heterozygosity of 0.32, reflecting similar genetic diversity among transcribed accessions. The Polymorphic Information Content (PIC) value of 0.26 indicated multiple alleles with comparable frequencies. Sequence alignments revealed the co-existence of multiple alleles at specific loci. Haplotype analysis showed that TVnu-561 and TVnu-1395 (haplotypes 1 and 3) were 40% monomorphic, while TVnu-563, TVnu-1148, and TVnu-1395 (haplotype 2) exhibited 60% polymorphism. Notably, TVnu-1395 displayed both monomorphic and polymorphic traits, suggesting partial genetic divergence. Variations in haplotype sequences were influenced by the number of annealing sites in the genome. These findings provide insight into intra-specific genetic variation among wild cowpea accessions and may inform future breeding strategies aimed at improving cowpea genetic resources.

Keywords: SNP; polymorphic; monomorphic; singleton; haplotype

INTRODUCTION

The origin of wild *Vigna* species spans diverse regions including Africa, Asia, and Australia, where these accessions have adapted to varied ecological niches over time, acquiring unique traits that make them valuable genetic resources for crop improvement (Onuminya et al., 2023). Wild cowpea accessions, considered ancestral forms of cultivated cowpea (*Vigna unguiculata*), harbor extensive genetic diversity with significant potential to enhance agronomic traits, nutritional quality, and resilience to biotic and abiotic stresses. Their utilization could profoundly impact global agriculture and food security. Typically found in marginal environments, wild cowpea populations face increasing threats from land-use change, deforestation, and urbanization (Gerrano et al., 2019). These accessions offer opportunities to improve consumer-relevant traits such as taste, cooking time, and nutrient density (Omoigui et al., 2021).

Despite their importance, wild cowpea accessions remain under-characterized, particularly in Nigeria, one of the world's leading cowpea producers. Limited funding, inadequate research infrastructure, and low awareness of genetic conservation have hindered progress (Ajeigbe et al., 2021). This research gap risks the loss of valuable alleles critical for climate adaptation and sustainable agriculture. Molecular characterization provides a robust framework for assessing genetic variability, identifying distinct populations, and guiding conservation strategies. Techniques such as Random Amplified Polymorphic DNA (RAPD), Simple Sequence Repeats (SSR), and Single Nucleotide Polymorphisms (SNPs) have proven effective in elucidating genetic diversity (Gerrano et al., 2019), yet their application in Nigeria remains limited (Omoigui et al., 2021).

Advanced molecular tools, including DNA sequencing and SNP analysis, offer precise insights into genome composition and trait architecture (Smith et al., 2020; Jones & Brown, 2019). Unlike morphological and biochemical methods, which are influenced by environmental conditions, DNA markers reveal inherent genetic differences among individuals (Adavbiele et al., 2018). Therefore, this study employed SNP markers to characterize selected wild *Vigna* accessions, aiming to uncover intra-specific variation and inform future breeding efforts.

MATERIALS AND METHODS

Experimental Design and Materials

A potted plant experiment was conducted at the Department of Crop Science and Agricultural Biotechnology, Ambrose Alli University, Ekpoma, Edo State, Nigeria. Molecular characterization was performed at the African Bioscience Molecular Laboratory, Ibadan, Oyo State.

The experiment comprised the cultivation of wild *Vigna* accessions and subsequent molecular analysis. A Completely Randomized Design (CRD) was adopted, with three replications. The experimental layout consisted of 15 plots, each containing six nursery polybags, totaling 90 polybags. Five wild *Vigna* accessions—TVnu-59, TVnu-561, TVnu-563, TVnu-1148, and TVnu-1395—were sourced from the International Institute of Tropical Agriculture (IITA), Ibadan.

Seeds were sown in standard nursery bags (24.5 cm × 20.32 cm) filled with 6.3 kg of well-drained loamy soil mixed with organic manure. Bags were spaced at 100 cm × 60 cm. Two seeds per accession were planted per bag, and fresh leaves were harvested from seedlings for molecular analysis.

Molecular Analysis

Genomic DNA Extraction

Genomic DNA was extracted from fresh leaf tissues using the CTAB mini-prep method (Doyle & Doyle, 1990). Approximately 2 cm of leaf tissue from each accession was ground in 600 µL of extraction buffer and incubated at 65°C for 60 minutes. CTAB facilitated cell lysis, EDTA stabilized DNA, chloroform aided phase separation, and isopropanol and ethanol were used for DNA precipitation and purification.

Gel Electrophoresis

PCR amplification was performed at the African Bioscience Molecular Laboratory. Amplified products were resolved on 2% agarose gel prepared by dissolving 0.8 g of agarose powder in 40 mL of 1X TAE buffer. The gel was stained with 9 µL ethidium bromide and allowed to polymerize. Electrophoresis was conducted at 80 V

and 250 mA for 30 minutes. PCR products (4 µL each) were loaded into wells alongside a 100 bp molecular weight marker. Allelic fragments were visualized under UV illumination and sized using a 100 bp DNA ladder.

RESULTS AND DISCUSSION

Single Nucleotide Polymorphism (SNP) generated a moderate level of polymorphism and revealed the existence of similar genetic diversity among four (TVnu-561, TVnu-563, TVnu-1148, and TVnu-1395) out of the five wild accessions. The number of single-nucleotide alleles ranged from 48 to 86, showing the position of the nucleotide in the DNA sequence of each accession, where there was a variation where either an adenine (A) or a thymine (T), Cytosine, or Guanine can be present. This SNP represents a variation at a specific location in the plant's genome, where either A or T can be found, potentially leading to genetic diversity within the species.

Singleton observed for all the accessions, indicating rare genetic structure variation. The Minor Allele Frequency (MAF) of 0.8 showed polymorphic genetic diversity (MAF≥0.01). The SNP revealed that all the accessions had moderate heterozygosity of 0.32. It indicates that 32% of the individuals in a population are expected to be heterozygous for the gene in question (≥0.1). High value of heterozygosity but the same for all the accessions indicated genetic diversity, which showed similarities. Landraces of self-pollinating crops, including cowpea, consist of heterogeneous populations due to genetic variation until complete homozygosity is reached through selection (Dairo, 2024). All the accessions had Polymorphic Information Content (PIC) of 0.26. This was agreed with Nkhoma et al. (2020), who reported the same for different cowpea varieties, which were relatively high, showing that the SNP was somewhat informative with many alleles of similar frequencies (PIC ≥ 0.25). In MAF, very rare variants ("singletons") play a surprisingly significant role in heritability, as these singletons can be strongly selected against. MAF thresholds significantly affect how population structure is inferred from genomic data (Table 1).

The gel electrophoresis image showed that only four (TVnu-561, TVnu-563, TVnu-1148, and TVnu-1395) underwent transcribed DNA. TVnu-59 (V5) did not transcribe. The base pair ladder indicated that the image was between 150 and 200bp compared to the base pair molecular weight marker. The V1-V4 is shown in the sample wells (Figure 1). The band's size varied from 150-200bp, which can be measured in 46ng-107ngDNA. In Figure 2, the chart shows the forward and backward alignment of the allele sequences. Thus, those marked with an asterisk were base pairs that did not align, while those not marked with an asterisk aligned in the sequence. An allele appeared twice in positions (48 and 66), G four times in positions (52, 59, 76, and 86), T six times (55, 69, 70, 80, 85, and 52), and C three times in positions (81, 82, and 55). These were indications that multiple alleles co-existed at a specific locus. Thymine (T) was more dominant in positions.

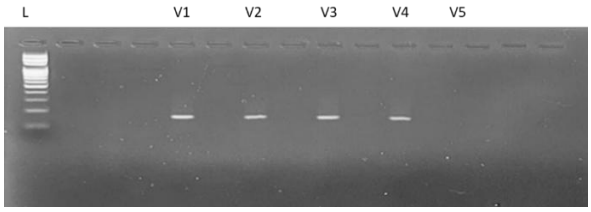

Figure 1. Agarose gel electrophoresis PCR amplification of five wild cowpea accesions using SNP markers.

Note: V1 =TVnu-561, V2 =TVnu -563, V3= TVnu-1148, V4 =TVnu-1395, V5= TVnu-59

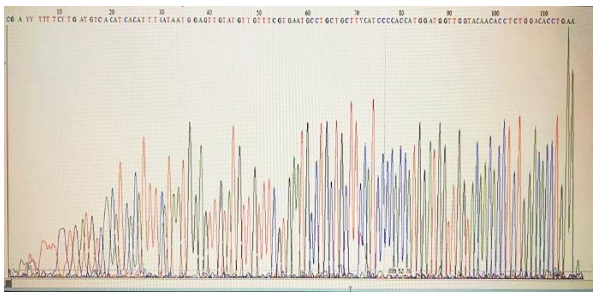

Figure 2. Alignment results of exon 3 of Terminal flower 1-like gene in four wild accessions of cowpea (*Vigna* spp.).

The four wild accessions used for haplotype DNA sequences showed several monomorphic sites to be 2, while the number of polymorphic sites was 3. A total of 4 alleles were amplified, of which 3 (75%) were polymorphic and 2 (25%) were monomorphic. The number of polymorphic fragments for each sequence varied from one (1) to three (3), averaging 1.33 alleles per sequence. The three sequences revealed polymorphism between the four wild accessions of cowpea, of which two (TVnu-561 and TVnu-1395) (haplotypes 1 and 3) were 40% monomorphic, and three (TVnu-563, TVnu-1148, and TVnu-1395) (haplotype 2), which exhibited 60% Polymorphism (Table 2). However, TVnu-1395 demonstrated both monomorphism and polymorphism, distinct characteristics that showed slightly similar genetic diversity to others. It was observed in this study that the haplotype two sequence level of polymorphism differed from haplotypes 1and 3. The haplotype sequence variation is influenced by the number of annealing sites in the genome (Adavbiele et al., 2018).

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 wild accessions of cowpea (*Vigna* spp.)

SNPs	Accession	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

Table 2. Haplotypes present in exon 3 of Terminal flower 1-like gene in four wild accessions of cowpea (*Vigna* spp.)

		Accessions				
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

Table 3. Haplotype Diversity of exon 3 of Terminal flower 1-like gene in four wild accessions of cowpea (*Vigna species*)

Indices	
Number of haplotypes	3
Haplotypes diversity (Hd)	0.7000
Nucleotide diversity (Pi)	0.13953
Average number of nucleotide difference (K)	6.000

The proportion of polymorphism variations was higher (40%-60%). Thus, information on intraspecific variation from this study might help make decisions for cowpea improvement. In this study, the number of haplotypes observed was 3, while the number of haplotype diversity was 0.7000, and the average nucleotide difference was 6.0. The nucleotide diversity was 0.13953. These results also revealed the variation of nucleotide polymorphism between the wild accessions of cowpea. The results suggest that the SNP used in this study showed a moderate level of polymorphism and revealed the existence of genetic diversity among the tested accessions. This result agrees with Mbali et al. (2022), who recorded similar observations.

CONCLUSION

Single Nucleotide Polymorphism (SNP) analysis revealed a moderate level of polymorphism and indicated the presence of similar genetic diversity among four transcribed wild cowpea accessions (TVnu-561, TVnu-563, TVnu-1148, and TVnu-1395). All accessions exhibited a high Minor Allele Frequency (MAF) of 0.80 and moderate heterozygosity (0.32), suggesting a polymorphic and genetically comparable population. The Polymorphic Information Content (PIC) value of 0.26 (PIC ≥ 0.25) further confirmed the presence of multiple alleles with relatively balanced frequencies. Haplotype analysis showed that TVnu-561 and TVnu-1395 (haplotypes 1 and 3) were 40% monomorphic, while TVnu-563, TVnu-1148, and TVnu-1395 (haplotype 2) exhibited 60% polymorphism. Notably, TVnu-1395 displayed both monomorphic and polymorphic traits, indicating partial divergence and shared genetic similarity with other accessions. The observed variation in haplotype sequences was influenced by the number of annealing sites within the genome. These findings underscore the value of molecular characterization in uncovering intra-specific variation and provide a foundation for the strategic utilization of wild cowpea accessions in breeding programs aimed at enhancing genetic diversity, resilience, and productivity.

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AUTHORS CONTRIBUTIONS

All the authors contributed equally to this work. All authors read and approved the final manuscript.

CONFLICT OF INTERESTS

The authors declare no conflict of interest.

ETHICAL APPROVAL

Not applicable.

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AVAILABILITY OF DATA AND MATERIALS

All datasets analyzed and described during the present study are available from the corresponding author upon reasonable request.

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