International Journal of Biotechnology Research Vol. 9(2), pp. 009-016, December, 2025
Available online at http://academeresearchjournals.org/journal/ijbr
ISSN 2328-3505 ©2025 Academe Research Journals

Full Length Research Paper

Genetic Relationship among twenty Cowpea Genotypes (*Vigna Unguiculata* (l) Walp.) Using Simple Sequence repeat Marker

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Accepted 18 November, 2025

This study assessed genetic diversity and relationships among 20 cowpea genotypes using eight Simple Sequence Repeat (SSR) markers. A total of 123 alleles were detected, with an average of 15.38 alleles per locus. The polymorphic information content (PIC) ranged from 0.9030 to 0.9258, indicating high genetic in formativeness of the markers. Genetic similarity among genotypes ranged from 0.75 to 1.00. UPGMA cluster analysis grouped the genotypes into two major clusters. These findings confirm the effectiveness of SSR markers in evaluating genetic diversity and offer valuable insights for cowpea breeding programs.

Keywords: Genetic relationship, Cowpea, Genotypes, Simple sequence repeat, Marker

INTRODUCTION

Cowpea, also known as (*Vigna unguiculata L.),* is one of the most important grain legume crops grown in tropical and subtropical regions, a member of the family Leguminosae, subfamily Fabaceae, and genus *Vigna* (Boukar *et al.*, 2019). Among legumes, cowpea is frequently used as a vegetable crop and green or dry fodder in husbandries and poultries of which is usually grown during rainy seasons (Abebe and Alemayehu, 2022). All parts of cowpea, such as the leaves, seed, and pod, serve as vegetables for human consumption, grains for various food products and feed for animals respectively (Enyiukwu *et al.*, 2018). Cowpea is also a good source of vitamins A, B, C, E particularly the B group, such as B1, B2 and B9 (Romuald *et al.*, 2017).

Information on the genetic diversity within and among closely related cowpea genotypes is essential for rational use of genetic resources (Nkhoma *et al.*, 2020). Genetic relationship among plant genotypes based on morphological characters usually varies with environment, and evaluation of characters require growing of such crops to full maturity before identification of related genotypes can be carried out (Roychowdhury *et al.,* 2014). Information on genetic diversity and relationship among cowpea genotypes is very useful for crop improvement all over the planet, promoting the efficient use of genetic variations in breeding programs through proper selection of cross combination among large sets of parental genotypes. (Nkhoma *et al.*, 2020).

Molecular markers have been proven to be powerful tools in the assessment of genetic variation and elucidation of gene relationship within and among plant species in oeder to conserve and use the plant genetic resources effectively (Salgotra and Chauhan, 2023). It is essential to utilize markers that not only distinguish individual but also reflect the inherent relationship and association among the various plant genotypes (Adhikari *et al.*, 2017). SSR markers, among others are the most frequently used markers in the analysis of genetic diversity studies (Nan *et al.,* 2020). This research is embarked upon to determine the extent of genetic diversity and relatedness among the cowpea genotypes.

MATERIALS AND METHODS

The experimental materials comprising twenty cowpea genotypes utilised for this study were obtained from the cowpea germplasm collection of the International Institute of Tropical Agriculture (IITA), Ibadan, Oyo State, Nigeria

Table 1. Names of Cowpea Genotypes and Source

S/N	Cowpea Sample ID	Source
1	TVu-8825	International Institute of Tropical Agriculture(IITA)
2	TVu-7440	International Institute of Tropical Agriculture(IITA)
3	TVu-297	International Institute of Tropical Agriculture(IITA)
4	TVu-16762	International Institute of Tropical Agriculture(IITA)
5	TVu-2300	International Institute of Tropical Agriculture(IITA)
6	TVu-16072	International Institute of Tropical Agriculture(IITA)
7	TVu-14544	International Institute of Tropical Agriculture(IITA)
8	TVu-16451	International Institute of Tropical Agriculture(IITA)
9	TVu-2269	International Institute of Tropical Agriculture(IITA)
10	TVu-4348	International Institute of Tropical Agriculture(IITA)
11	TVu-16117	International Institute of Tropical Agriculture(IITA)
12	TVu-16143	International Institute of Tropical Agriculture(IITA)
13	TVu-16123	International Institute of Tropical Agriculture(IITA)
14	TVu-9924	International Institute of Tropical Agriculture(IITA)
15	TVu-12390	International Institute of Tropical Agriculture(IITA)
16	TVu-16723	International Institute of Tropical Agriculture(IITA)
17	TVu-15203	International Institute of Tropical Agriculture(IITA)
18	TVu-12456	International Institute of Tropical Agriculture(IITA)
19	TVu-10407	International Institute of Tropical Agriculture(IITA)
20	TVu-12474	International Institute of Tropical Agriculture(IITA)

Table 2. Names and Primers Sequence of the SSR Markers used for the analysis of 20 Cowpea genotypes**.**

Marker	Forward	Tm	Reverse	Tm
CEDG156	5′CGCGTATTGGTGACTAGGTATG 3′	54.84	5′CTTAGTGTTGGGTTGGTCGTAAGG 3′	57.38
CP09781	5′CTGACGCATTCAGCATTTTACAGC 3′	55.68	\ATACGGTTGCGTCCATGTAT 3′	55.68
CEDG127	5′ GGTTAGCATCTGAGCTTCTTCGTC 3′	57.38	5′CTCCTCACTTGGTCTGAAACTC 3′	54.84
CEDG305	5′ GCAGCTTCACATGCATAGTAC 3′	52.40	5′GAACTTAACTTGGGTTGTCTGC 3′	52.97
CEDG020	5′ TATCCATACCCAGCTCAAGG 3′	51.78	5′ GCCATACCAAGAAAGAGG 3′	48.04
CEDG245	5′ GATAGAGCTTAAACCCTC 3′	45.77	5′ CTTTTGATGACAAATGCC 3′	43.49
CEDG132	5′ GGGTGTAATCCGTCAGAGC 3′	55.88	5′ CTTCCCCCTCTTCCGTTCTC 3′	55.88
CEDG093	5′ AAAACCCATGTAAAGTTCA 3′	43.58	5′ CAATCCATTCCCTTCTTAAT 3′	45.63

(Table 1).

DNA EXTRACTION

Two (2) grams of leaves from 2 weeks old of potted cowpea were ground after being surface sterilized with ethanol, and 1000µl of freshly prepared modified CTAB extraction buffer (100mMTris-HCl, pH 8.0; 20mM EDTA, pH 8.0; 1.4M NaCl; 2% CTAB; (just before use))was added in the mortar and pestle. The resultant mixture was homogenized and incubated in a 60°C water bath for 30min. Following the incubation period, the tube was transferred into centrifuge at 12,000RPM for 10 minutes. The supernatant was transferred into a new sterilized eppendorf tube, then 10µL of RNases solution was added and incubated at 37℃ for 5minutes. It was allowed to cool for 7 min, and 1000µL chloroform: isoamyl alcohol (24:1) was added in the tubes and centrifuged at 12,000 rpm for 5minutes. The supernatant recovered after centrifugation was transferred into new tubes and up to 500µL Isopropanol was added and kept in -10°C freezer for 30minutes for DNA precipitation. The pellet was collected by centrifugation at 12,000 rpm for 2 min and washed with 500µL of chilled 70% ethanol. Pellet was air-dried until no further traces of ethanol. An average of 40 µL nuclease free water T.E. buffer was added to elute the DNA and stored at -10℃.

POLYMERASE CHAIN REACTION (PCR)

SSR primers developed by Schafleitner *et al*., (2013) were used in this study, and the primer sequences are shown in Table 2. PCR reaction for SSR was performed in a 10 µl volume containing 20ng template DNA and PCR master mix (NEB). The PCR reaction conditions were an initial denaturation at 95°C for 5 min, followed by 35 cycles of 94°C for 30 s, 55°C for 30s, 72°C for 30 s, and a final extension at 72°C for 10 min. The amplified SSR products

Table 3. Alleles number, polymorphic Information Content (PIC) ,Major allele frequency and Gene diversity of the Primers

Marker	Allele No	PIC	Major Allele Frequency	Gene Diversity
CEDG156	16.0000	0.9202	0.1500	0.9250
CP09781	17.0000	0.9258	0.1500	0.9300
CEDG127	15.0000	0.9038	0.2000	0.9100
CEDG305	13.0000	0.9030	0.1500	0.9100
CEDG020	14.0000	0.9087	0.1500	0.9150
CEDG245	16.0000	0.9256	0.1000	0.9300
CEDG132	15.0000	0.9199	0.1000	0.9250
CEDG093	17.0000	0.9258	0.1500	0.9300
Mean	15.3750	0.9166	0.1438	0.9219

were separated on 6 % agarose gel electrophoresis and were visualized by silver staining with EZ-Vision. The gels were visualized under blue light using Bluebox™ (USA).

MOLECULAR DATA ANALYSIS

Statistical analysis was conducted by scoring SSR bands as co-dominant. These bands were considered polymorphic when they were present in some samples. Percent polymorphism for each marker was generated by the formula: (Number of polymorphic bands/Total number of scored bands) X 100. The PIC calculator was used to determine the degree of polymorphism, or polymorphic information content (PIC). The amplicons were scored according to band weight in order to analyze the variability, and Jaccard's coefficient was used to determine the pairwise genetic similarity between genotypes. Using the unweighted pair group method average (UPGMA) clustering, a dendrogram was created using PAST3. The robustness of the dendrogram nodes with 100 bootstrap was assessed using the computer application bootstrap.

RESULTS

Table 3 showed the SSR primers' allele number, polymorphism information content, major allele frequency, and gene diversity. Among the 20 cowpea genotypes, a total of 123 alleles were found at 8 primer loci. Each locus had anywhere from 13 to 17 of these alleles. There are 15.375 alleles on average per locus. CEDG093 and CPO9781 are the loci with the greatest number of alleles (17). With an average of 0.9166 per locus, the PIC values varied from 0.9030 (CEDG093) to 0.9258 (CEDG132 and CP09781). 62.5% of the SSR markers (CEDG156, CPO9781, CEDG245, CEDG132, and CEDG093) had PIC estimates over 0.9166 on average. The main allele frequency varied from 10% to 20%, with CEDG305 having the highest frequency (0.20) and CEDG245 and CEDG132 having the lowest. The average major allele frequency across the loci was 0.14 (14%). On the average, 75% of the markers utilized for this study recorded major allele frequency above 0.14. As regards the gene diversity of the primers, it ranged from 0.91 to 0.93 with an average of 0.92. The highest gene diversity was observed in CEDG127 and CEDG132. On the average, 67.5% of the primers recorded gene diversity above 0.92.

Table 4 displays the genetic similarity coefficients of the 20 cowpea genotypes obtained using SSR markers. The similarity coefficient's values varied from 0.75 to 1.00. While genotypes with 0.75 (75%) similarity suggested that they are from distinct origins, those with 1.00 (100%) similarity suggested that they are closely related. Figure 1 displays the dendogram derived from the UPGMA cluster analysis of the SSR data. The dendogram revealed two main groups that are not too far apart.

Table 5 shows the cowpea genotype distribution. Two distinct clusters (cluster A and cluster B, respectively) were created from the 20 cowpea genotypes. 17 cowpea genotypes from the created dendogram make up Cluster A. TVu-8825, TVu-7440, TVu-297, TVu-16762, TVu-2300,

Table 4. Genetic similarity coefficient of 20 cowpea genotypes derived from SSR markers

	TVu-7440	TVu-297	TVu-16762	TVu-2300	TVu-16072	TVu-14544	TVu-16451	TVu-2269	TVu-4348	TVu-16117	TVu-16143	TVu-16123	TVu-9924	TVu-12390	TVu-16723	TVu-15203	TVu-12456	TVu-10407	TVu-12474
TVu-8825	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0.75	1	0.75	0.75	1
TVu-7440		1	1	1	1	1	1	1	1	1	1	1	1	1	0.75	1	0.75	0.75	1
TVu-297			1	1	1	1	1	1	1	1	1	1	1	1	0.75	1	0.75	0.75	1
TVu-16762				1	1	1	1	1	1	1	1	1	1	1	0.75	1	0.75	0.75	1
TVu-2300					1	1	1	1	1	1	1	1	1	1	0.75	1	0.75	0.75	1
TVu-16072						1	1	1	1	1	1	1	1	1	0.75	1	0.75	0.75	1
TVu-14544							1	1	1	1	1	1	1	1	0.75	1	0.75	0.75	1
TVu-16451								1	1	1	1	1	1	1	0.75	1	0.75	0.75	1
TVu-2269									1	1	1	1	1	1	0.75	1	0.75	0.75	1
TVu-4348										1	1	1	1	1	0.75	1	0.75	0.75	1
TVu-16117											1	1	1	1	0.75	1	0.75	0.75	1
TVu-16143												1	1	1	0.75	1	0.75	0.75	1
TVu-16123													1	1	0.75	1	0.75	0.75	1
TVu-9924														1	0.75	1	0.75	0.75	1
TVu-12390															0.75	1	0.75	0.75	1
TVu-16723																0.75	1	1	0.75
TVu-15203																	0.75	0.75	1
TVu-12456																		1	0.75
TVu-10407																			0.75
TVu-12474																			1

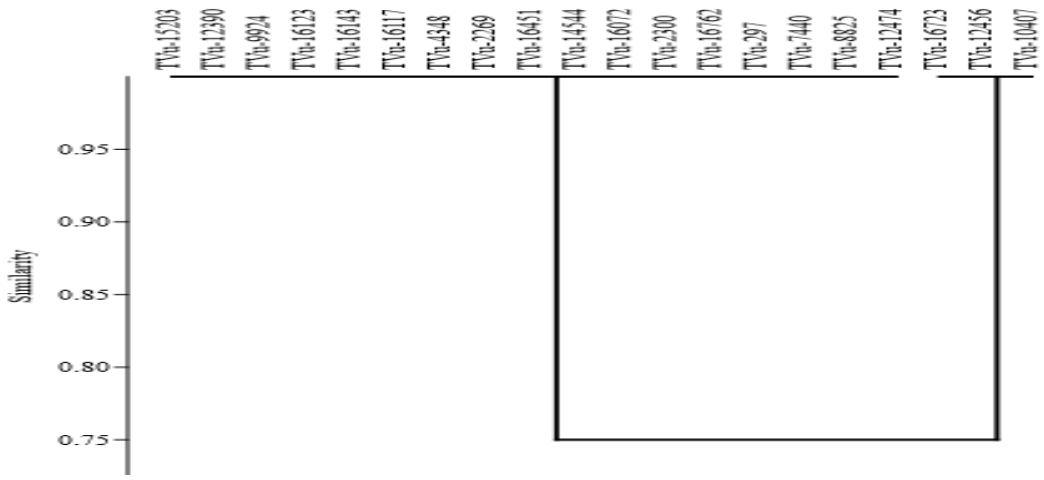

Figure 1. UPGMA based cluster analysis of 20 Cowpea genotypes using SSR Markers

Table 5. Distribution of 20 Cowpea Genotypes into different Clusters

S/no	Cluster number	No of cowpea genotypes	Genotypes
1	A	17	TVu-8825, TVu-7440, TVu-297, TVu-16762, TVu-2300, TVu-16072, TVu-14544, TVu-16451, TVu-2269, TVu-4348, TVu-16117, TVu-16143, TVu-9924, TVu-12390, TVu-16723, TVu-15203, TVu-12474
2	B	3	TVu-12456, TVu-10407, TVu-16123

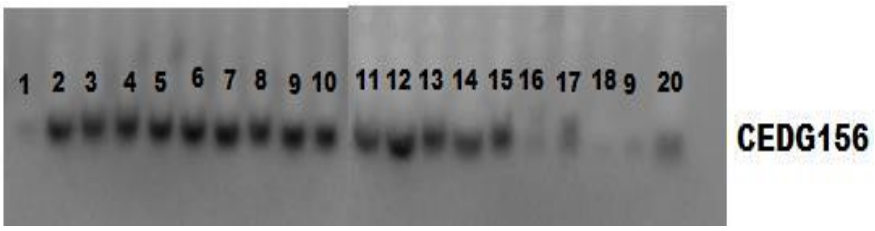

Plate 1. Gel electrophoresis. DNA bands amplified by CEDG156 Marker across 20 Cowpea genotypes

TVu-16072, TVu-14544, TVu-16451, TVu-2269, TVu-4348, TVu-16117, TVu-16143, TVu-9924, TVu-12390, TVu-16723, TVu-15203, and TVu-12474). According to Cluster A, cowpea genotypes are so similar that they most likely have a restricted genetic foundation and a common ancestor. The cowpea genotypes in Cluster B were categorized according to their reduced resemblance to the genotypes in cluster 1. This implies that these genotypes are genetically distinct and their inclusion in breeding programs can enhance genetic diversity. Plates 1 to 8 revealed the DNA bands amplified by the 8 SSR markers across the 20 cowpea genotypes respectively.

DISCUSSION

A basic requirement for plant breeding, conservation, and usage programs is an understanding of the degree of genetic variability and linkages among crop germplasm.

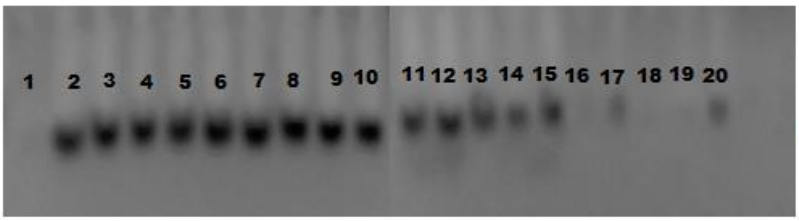

Plate 2: Gel electrophoresis. DNA bands amplified by CP09781 Marker across 20 Cowpea genotypes

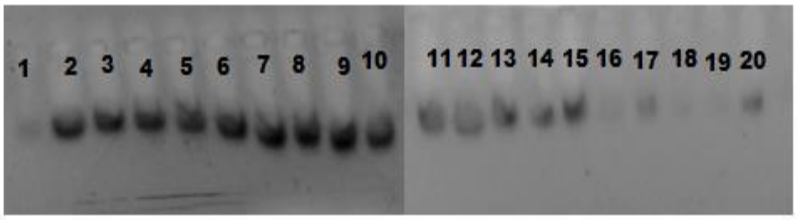

Plate 3. Gel electrophoresis. DNA bands amplified by CEDG127 Marker across 20 cowpea genotypes

Plate 4. Gel electrophoresis. DNA bands amplified by CEDG305 Marker across 20 cowpea genotypes

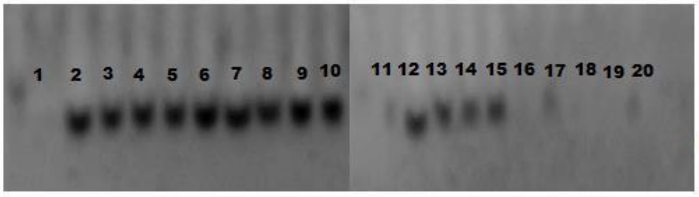

Plate 5. Gel electrophoresis. DNA bands amplified by CEDG020 Marker across 20 cowpea genotypes

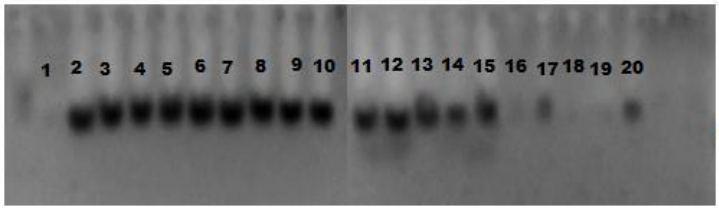

Plate 6. Gel electrophoresis. DNA bands amplified by CEDG245 Marker across 20 cowpea genotypes

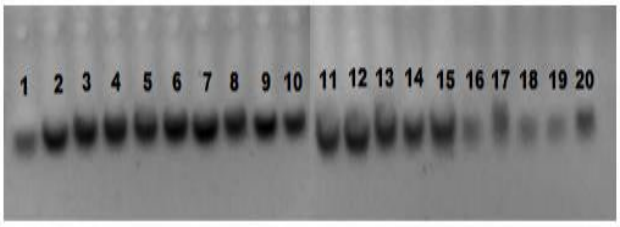

Plate 7. Gel electrophoresis. DNA bands amplified by CEDG132 Marker across 20 cowpea genotypes

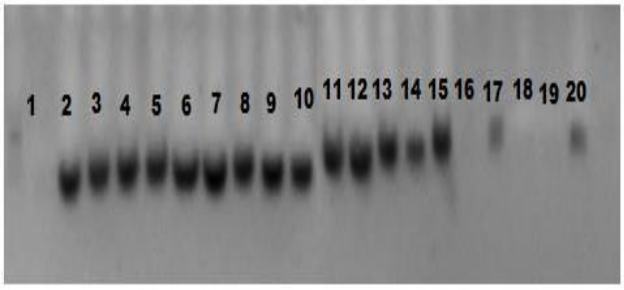

Plate 8. Gel electrophoresis. DNA bands amplified by CEDG093 Marker across 20 cowpea genotypes

Breeders can find appropriate parental lines that can produce superior segregants and novel recombinants with improved features thanks to genetic variety (Swarup et al., 2021). A high degree of polymorphism was demonstrated by the amplification of 123 alleles, with an average of 15.38 alleles per locus, throughout the genotypes. This significantly high average allele number confirms previous findings that SSR markers are multi-allelic, highly informative, and broadly distributed throughout plant genomes (Ramesh et al., 2020). Previous molecular-based cowpea investigations have revealed similar high allele numbers, which further supports the effectiveness of SSR markers in detecting genetic diversity (Zafeiriou et al., 2023). With an average PIC value of 0.9166 and a range of 0.9030 to 0.9258, the Polymorphic Information Content (PIC) showed that the markers were very informative and able to identify genetic variation within the population. Markers with PIC > 0.50 are regarded as very informative, particularly for genetic diversity investigations, according to Botstein's classification (Botstein et al., 1980). The PIC values revealed in this study are higher than those reported in comparable previous studies like Yao et al. (2023), suggesting that the selected SSR markers are quite robust and the germplasm used has a wide diversity. The majority of alleles are either rare or moderately frequent, according to the main allele frequency values, which varied from 0.10 to 0.20, indicating that no single allele dominated the genotype population. Similarly, the sampled germplasm's genetic variability is further confirmed by the mean gene diversity estimate of 0.92, increasing their potential utility in cultivar programs for genetic conservation and improvement. Different levels of relatedness between the genotypes were shown by the genetic similarity coefficient, which varied from 0.75 to 1.00. A similarity rating of 1.00 indicates that a number of genotypes are genetically identical or have very close ancestry, which may be the result of repeated use of comparable parental lines in previous breeding cycles or a shared selection history. On the other hand, a similarity value of 0.75 suggests that certain genotypes differ significantly and may have come from separate genetic backgrounds.

These results are consistent with those of Vinay et al. (2022) and Dagnon et al. (2022), who also found that cowpea lines have narrow to moderate genetic variation.The genotypes were further divided into two main clusters by cluster analysis, with Cluster A having 17 genotypes and Cluster B having just 3. The three genotypes are excellent candidates for hybridization programs meant to produce novel heterotic combinations since the big grouping in Cluster A indicates tight genetic ties, while the small grouping in Cluster B shows greater genetic distinctiveness.

CONCLUSION

The high polymorphism and informativeness of the SSR markers used in this work confirmed their use for cowpea

phylogeny and genetic diversity research. The majority of genotypes showed strong genetic similarities according to the clustering pattern, indicating a small genetic foundation. The genetically unique genotypes found may be useful resources for breeding efforts to increase agricultural improvement and diversity.

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