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Exploring the Molecular Variability of Diverse Black gram (*Vigna mungo* L. Hepper) Genotypes Using SSR Markers

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Abstract— *This investigation aimed to explore the molecular diversity of 117 black gram genotypes employing Simple Sequence Repeats (SSR) markers. Out of 43 SSR markers studied, 15 markers showed polymorphism. Polymorphic Information Content (PIC) values ranged from 0.4 to 0.7 with a mean of 0.6. A total of 52 alleles, ranging from three to four with a mean of 3.4 alleles per locus, were detected. Population structure analysis grouped the 117 genotypes into four sub-populations. UPGMA cluster analysis grouped the genotypes into three main clusters: Cluster I (51 genotypes), Cluster II (19 genotypes), and Cluster III (47 genotypes). Principal coordinate analysis indicated that the genotypes were distinctly separated from one another. The results of the unweighted neighbor-joining clustering tree and PCoA analysis were in close correspondence with the results of model-based STRUCTURE analysis. The findings provide valuable insights into the genetic diversity of black gram, which can assist plant breeders in developing improved cultivars.*

Keywords— Simple Sequence Repeats (SSR) Markers, Polymorphic Information Content, Genetic Diversity, Population Structure, Black gram.

I. INTRODUCTION

Black gram (Vigna mungo L. Hepper) is a short-duration, cleistogamous, self-pollinating, diploid species with a chromosome number (2n-2x-22) and a genomic size of 574 mega base pairs (Mbp). It is a member of the Leguminaceae family (Gupta et al., 2008). India is the world's largest user and producer of black gram. Black gram in India is cultivated extensively over an area of about 4.6 million hectares (Mha), with annual production and productivity of 24.5 lakh tonnes and 533 kg per hectare, respectively. In Andhra Pradesh, black gram is grown over an area of 3.93 lakh ha with 3.65 lakh tonnes production and 929 kg ha^{-1} productivity (2020-21) (www.agricoop.nic.in). According to El-Karamany (2006), most nutrients, including vitamins, minerals, and amino acids, are abundant in black gram, with proteins making up 25–26%, carbohydrates 60%, and fats 1.5%. It is high in vitamins A, B1, and B3, and contains trace amounts of riboflavin, niacin, and vitamin C.

As one of the most valued pulses in India and Pakistan, black gram is native to India and has been grown there since ancient times. Grown practically throughout India, this leguminous pulse has become the most renowned and is appropriately called the "king of the pulses." Asia, Pakistan, Myanmar, and other southern Asian nations are the main growing regions. Black gram is a member of the genus Vigna and the family Leguminaceae. There are only seven Vigna species that have been raised for their edible seeds: two African species of the subgenus Vigna, Vigna unguiculata (cowpea) and V. subterranea (the Bambara groundnut), and five Asian species of the subgenus Ceratotropis: Vigna mungo (black gram), Vigna radiata

(mungbean), Vigna aconitifolia (mothbean), Vigna angularis (adzuki bean), and Vigna umbellata (rice bean). Black gram belongs to the Asian Vigna group.

Black gram has been the subject of less investigation than cowpea and mung bean, particularly regarding molecular genetic diversity. It is essential to research and utilize the genetic variability of black gram in order to maximize its potential as a food and feed crop. Microsatellite or SSR markers are the preferred markers for molecular genetics research in crops due to their multi-allelic, co-dominant, and reliable characteristics. They are PCR-based, easy to score, and require only a tiny quantity of DNA for analysis. It is possible to employ SSR markers from related species. Although no SSR marker has been developed specifically from black gram, thousands of SSR markers have been developed in other Vigna crops, such as adzuki bean and cowpea.

SSR markers derived from cowpea and adzuki bean were utilized in the present study to investigate the genetic diversity and population structure of 117 diverse black gram accessions. The findings will provide valuable insights into domestication patterns and genetic diversity of black gram, which can assist plant breeders in developing enhanced cultivars.

II. MATERIALS AND METHODS

2.1 Genomic DNA Isolation

DNA was extracted from seedling leaves that were 20–25 days old using the Cetyltrimethylammonium bromide (CTAB) method (Murray and Thompson, 1980).

Stock solutions prepared for DNA extraction:

- **1M Tris Hydrochloric acid (HCl) (pH 8.0):** 30.285 g of Tris base was dissolved in distilled water, adjusted pH to 8.0 with HCl, and the final volume was brought to 250 mL.
- **0.5M Ethylenediaminetetraacetic acid (EDTA) (pH 8.0):** 46.53 g of EDTA was dissolved in distilled water, pH was adjusted to 8.0 with 4 g NaOH pellets, and made up to 250 mL.
- **5M Sodium chloride (NaCl):** 73.05 g of NaCl was dissolved in distilled water and made up to 250 mL.
- **1% Polyvinylpyrrolidone (PVP):** 1 g of PVP was dissolved in 100 mL of sterile distilled water.
- **2% CTAB:** 2 g of CTAB was dispersed in 100 mL of sterile distilled water.

The DNA extraction buffer (100 mL) was composed of 10 mL Tris HCl, 4 mL EDTA, 28 mL NaCl, 1 g PVP, and 2 g CTAB, supplemented with 50 µL β-Mercaptoethanol. Chloroform-isoamyl alcohol (24:1) and 70% ethanol were prepared for utilization in DNA extraction. RNase solution (25 mg) was prepared in 5 mL buffer (T10E1) and stored at -20°C for use.

Procedure for DNA isolation: 500 µl of extraction buffer was used to homogenize 2 g of fresh leaf samples. After adding another 300 µl of CTAB, the mixture was incubated for 45 minutes at 65°C. The supernatant was centrifuged again after being treated with chloroform-isoamyl alcohol (24:1). Ice-cold isopropanol was used to precipitate the DNA, which was then washed three times with 70% ethanol, allowed to air dry, and then dissolved in 100 µL of molecular biology-grade water.

DNA Quality and Quantity Estimation: The DNA purity and concentration were measured using a NanoDrop spectrophotometer (Jenway Genova Nano). A 260/280 ratio of 1.8-2.0 indicated DNA purity, while ratios closer to 2.0 suggested RNA contamination. DNA was diluted to 100 ng/µl for PCR reactions.

Agarose Gel Electrophoresis for DNA Quantification: For quality assessment, DNA samples were run in a 0.8% agarose gel in 1X Tris Acetate EDTA (TAE) buffer. The gels were stained with ethidium bromide, and bands were visible when exposed to ultraviolet (UV) light. By comparing the band intensity to a lambda DNA standard, the concentration of DNA was determined.

2.2 Genomic DNA Amplification by PCR Employing SSR Markers

Two µL of template DNA, 1 µL of each forward and reverse primer (10 pmol), 0.5 µl of dNTPs, 2 µL 10X buffer, 0.5 µL of $MgCl_2$, 0.1 µL of Taq polymerase, and 3.9 µL of molecular-grade water were used to set up PCR reactions (10 µL). Using an Eppendorf thermocycler, PCR amplification was performed as follows: 94°C for 5 minutes, followed by 35 cycles of denaturation at 94°C for 45 seconds, annealing at 55-60°C for 45 seconds, extension at 72°C for 1 minute, and final extension at 72°C for 7 minutes.

2.3 Agarose Gel Electrophoresis for SSR Marker Resolution

PCR products were electrophoresed at 100 V for one hour and 120 V for 30 minutes on 3% agarose gels prepared in 1X TAE buffer for SSR marker resolution. To confirm marker amplification, gels were stained with ethidium bromide and examined under a UV lamp.

2.4 Molecular Data Scoring

For every genotype and marker, marker allele data were created based on the presence or absence of bands and the size of the bands. These data were then sorted in various input formats (matrix form, HapMap format) for each genotype-marker pair.

2.5 Marker Polymorphism

The Polymorphic Information Content (PIC) for each marker was determined using the formula provided by Anderson et al. (1993) to estimate the markers' informativeness:

$$\mathbf{PIC = 1 - \Sigma(p_{ij})^2} \quad (1)$$

Where p_{ij} is the frequency of the jth allele for the ith marker, summed over n alleles. The calculation was based on the count of alleles per locus.

Using POPGENE (version 1.32) software, estimates of each polymorphic marker's utility information, number of alleles (Na*), number of effective alleles (Ne*), major allelic frequency (MAF), Shannon's information index (I*), Polymorphic Information Content (PIC), and genetic diversity index (Nei*) were made (Yeh et al., 1999).

2.6 Population Structure

As previously mentioned, 117 black gram genotypes were screened using SSR markers. The Q (population structure) model was utilized to report population structure and individual relatedness among 117 black gram genotypes to prevent any potential spurious relationships. The program STRUCTURE version 2.3.4 was employed to assess the genetic structure (Q) and cluster the population (Pritchard et al., 2000). The most likely K value for the examined data was determined using Structure Harvester version 0.6 and the Evanno et al. (2005) ΔK approach (Earl and Vonholdt, 2012). Following ten separate runs with a burn-in duration of 10,000 steps and 100,000 Monte Carlo Markov chain (MCMC) repeats, the optimal number of populations (K) was chosen. K = 1 to 10 was the range of genetic clusters. The posterior probability values (LnP(D) and ΔK) were employed to estimate the number of subpopulations (K).

2.7 Cluster Analysis

For dissimilarity analysis, the allelic data Excel file was fed into the DARwin (Dissimilarity Analysis and Representation) program version 6.0.12 (Perrier and Jacquemoud-Collet, 2006). Dissimilarity was computed using the allelic data as a simple matching dissimilarity index with a bootstrap value of 1000. To infer genetic links, the produced dissimilarity matrix was utilized to create a dendrogram using the Unweighted Pair Group Method with Arithmetic Means (UPGMA), which is based on the neighbor-joining approach.

2.8 Principal Coordinate Analysis (PCoA)

The first three components were utilized to represent the genotypes, and principal coordinate analysis was executed to highlight the resolving power. The dissimilarity matrix created with DARwin version 6.0 was used for PCoA.

III. RESULTS AND DISCUSSION

3.1 Genotyping of Black Gram Genotypes with Microsatellite Markers

A total of 117 black gram genotypes were screened using 43 SSR markers that were arbitrarily dispersed throughout the 11 chromosomes. Of these, 15 SSR markers (35.7%) were discovered to be polymorphic.

3.1.1 Allele Count at Each Locus

From 15 polymorphic microsatellite markers, 52 alleles were detected, ranging from three to four, with a mean of 3.4 alleles per locus. The markers CEDG 154, CEDG 116, CEDG 91, TWSSR 66, CEDG 180, CEDG 53, and CEDG 105 generated a maximum of four alleles, while CEDG 6, CEDG 97, CEDG 92, CEDG 44, CEDG 176, TWSSR 87, CEDG 128, and TWSSR 167 produced three alleles. In their analysis of genetic diversity in black gram using yellow mosaic virus-resistant SSR markers, Korattukudy et al. (2022) reported 60 alleles, with a mean of 4.2 alleles per locus and a range of two to seven alleles

per locus. Similarly, Mwangi et al. (2021) analysis of black gram genotypes revealed a total of 23 alleles from eight polymorphic SSR markers. The estimated genetic diversity within the genotypes being studied is depicted by this value. The average unique allele number for each marker was 2.8, with a range of 2 to 5. The number of alleles per locus for 15 polymorphic markers is represented in Table 1.

3.1.2 Effective Number of Alleles

The effective number of alleles is the count of equally frequent alleles needed to produce the same expected heterozygosity. The effective number of alleles should never exceed the observed number of alleles. The number of effective alleles in the current study ranged from 1.8 (CEDG 105) to 3.3 (CEDG 180), with a mean value of 2.6. CEDG 91 (3.2) and TWSSR 66 (3.3) displayed the greatest number of effective alleles, whilst CEDG 44 (2.1) and TWSSR 87 (2.1) displayed the fewest. The outcomes of the investigation regarding the count of effective alleles were higher than those of Korattukudy et al. (2022), who conducted an assessment of black gram genetic diversity analysis and reported a mean value of 2.4 with a range of 1.5 to 3.5. The effective number of alleles for 15 polymorphic markers is represented in Table 1.

3.1.3 Polymorphic Information Content (PIC)

PIC is a measure of diversity for SSR markers. PIC values assess a locus's discriminatory power by accounting for the extent of expressed alleles and their relative frequencies. With an average of 0.6, the PIC values of the 15 polymorphic markers varied from 0.4 to 0.7. The highest PIC value was noted for SSR markers CEDG 128 (0.7), followed by CEDG 44 (0.7), TWSSR 87 (0.7), CEDG 154 (0.7), CEDG 53 (0.7), CEDG 92 (0.7), and CEDG 91 (0.6). Markers with a PIC value ≥ 0.60 are more informative and can distinguish the genotypes effectively. The lowest PIC value was exhibited by CEDG 105 (0.4), followed by TWSSR 167 (0.5), depicting the less discriminatory power of these markers in differentiating the genotypes. Similarly, Santhees et al. (2014) reported that out of 42 SSR markers used, 15 SSR markers expressed polymorphism with PIC values varying from 0.3 for SSR marker (CEDG 024) to 0.7 (CEDG 154), with a mean of 0.6 in their study on screening of black gram varieties for yellow mosaic virus resistance employing SSR markers. According to Mwangi et al. (2021), with a mean of 0.3, the PIC values varied from 0.1 (CEDG 056) to 0.5 (CEDG 092). Of all the markers, CEDG 092 was the most informative, with a high PIC value of 0.8 reported by Prajapathi et al. (2022) in their study on molecular variability in MYMV-resistant and susceptible black gram using SSR markers. PIC values for 15 polymorphic markers are represented in Table 1.

3.1.4 Nei's Genetic Diversity Index

Nei's genetic diversity index is an estimate of the average genetic variation or diversity found at each locus. Based on Nei's genetic diversity index, the highest level of genetic diversity was recorded in the case of CEDG 180 (0.7) and TWSSR 66 (0.7), and the lowest was recorded by CEDG 105 (0.4), with a standard value of 0.6. Nei's genetic diversity was found to range from 0.2 to 0.7 with a mean value of 0.4 in Pyngrope et al. (2015) genetic diversity study of 30 black gram genotypes utilizing 12 SSR markers. Nei's genetic diversity index for 15 polymorphic markers is represented in Table 1.

3.1.5 Shannon's Information Index

Variation at various levels of genetic structure can be described using Shannon's information index, which is also used to quantify genetic diversity. Shannon's information index in the present investigation had a mean value of 1.0 and varied from 0.8 (CEDG 105) to 1.2 (TWSSR 66). The highest Shannon's information index was displayed by TWSSR 66 (1.2), CEDG 180 (1.2), and CEDG 91 (1.2), whereas the lowest was exhibited by CEDG 105 (0.8), TWSSR 87 (0.8), and CEDG 128 (0.9). Shannon's information index ranged from 0.6 to 1.2 with an average of 1.0 in the previous reports of Gangadhar et al. (2023). Shannon's information index for 15 polymorphic markers is represented in Table 1.

3.1.6 Major Allele Frequency

The major allele frequency in this study averaged 0.4 and varied from 0.3 (TWSSR 167) to 0.7 (CEDG 105). TWSSR 66 (0.3), CEDG 180 (0.3), and CEDG 91 (0.3) had the lowest major allele frequencies, whereas CEDG 105 (0.7) had the highest, followed by CEDG 44 (0.5), CEDG 176 (0.5), and TWSSR 87 (0.5). Among the 15 polymorphic markers studied, CEDG 180 followed by CEDG 128, CEDG 44, TWSSR 66, CEDG 91, and CEDG 154 recorded higher PIC values along with a high number of effective alleles, Nei's genetic diversity index, and Shannon's information index. Hence, these markers can be considered more effective for discriminating genotypes and are useful for genetic diversity studies. Major allele frequency for 15 polymorphic markers is represented in Table 1.

TABLE 1
SUMMARY OF STATISTICS OF GENETIC DIVERSITY ASPECTS AMONG 15 POLYMORPHIC MICROSATELLITE MARKERS EMPLOYED IN THE PRESENT INVESTIGATION

S. No.	SSR Marker	Linkage Group	Pos. (cM)	Na*	Ne*	MAF	I*	PIC	Nei*
1	CEDG 6	2	14.5	3	2.5	0.5	1	0.6	0.6
2	CEDG 97	10	37.1	3	2.6	0.4	1	0.5	0.6
3	CEDG 92	8	54.2	3	2.5	0.5	1	0.7	0.6
4	CEDG 44	11	18	3	2.1	0.5	0.8	0.7	0.5
5	CEDG 176	7	60.9	3	2.4	0.5	0.9	0.6	0.5
6	CEDG 154	4	23.5	4	2.8	0.4	1.1	0.7	0.6
7	CEDG 116	10	28	4	2.9	0.5	1.2	0.6	0.6
8	CEDG 128	15	5.3	3	2.3	0.5	0.9	0.7	0.5
9	CEDG 180	10	9.9	4	3.3	0.3	1.2	0.6	0.7
10	CEDG 91	4	25.5	4	3.2	0.3	1.2	0.6	0.6
11	TWSSR 87	—	—	3	2.1	0.5	0.8	0.7	0.5
12	CEDG 53	12	4.8	4	2.9	0.3	1.1	0.7	0.7
13	TWSSR 66	1	—	4	3.3	0.3	1.2	0.7	0.7
14	TWSSR 167	10	—	3	2.9	0.3	1	0.5	0.6
15	CEDG 105	—	—	4	1.8	0.7	0.8	0.4	0.4
Maximum				**4**	**3.3**	**0.7**	**1.2**	**0.7**	**0.7**
Minimum				**3**	**1.8**	**0.3**	**0.8**	**0.4**	**0.4**
Mean				**3.4**	**2.6**	**0.4**	**1**	**0.6**	**0.6**

Na = Number of alleles; Ne* = Number of effective alleles; MAF = Major allelic frequency; I* = Shannon's information index; PIC = Polymorphic information content; Nei* = Genetic diversity index*

3.2 Population Structure and Genetic Diversity Analysis of Black Gram Genotypes

3.2.1 Population Structure Analysis

Only after considering population structure is the true association revealed (Yu et al., 2006). The LnP(D) graph delineated with number of subpopulations (K) on the x-axis and logarithmic probability distribution on the y-axis did not give a vivid picture of the true value of K, so ΔK value was charted against number of subpopulations, displaying the highest peak at K = 4, which revealed that there are four subpopulations within the panel. Structure Harvester (Earl, 2012) was a web-based program used for the visualization of STRUCTURE output and implementation of the Evanno method for measuring maximum ΔK value. The summary plot of Q matrix estimates and clustering of 117 genotypes is represented in Fig. 1 and Table 2.

Based on the Q values that were obtained from the structure software, the genotypes were assigned to each subpopulation with consideration for the highest membership likelihood criterion. Based on the membership fractions, the genotypes with a Q value ≥ 75% were assigned to corresponding subgroups and those with ≤ 75% were defined as admixtures. The genotypes with a score > 0.75 were considered pure, while those with < 0.75 were considered admixtures.

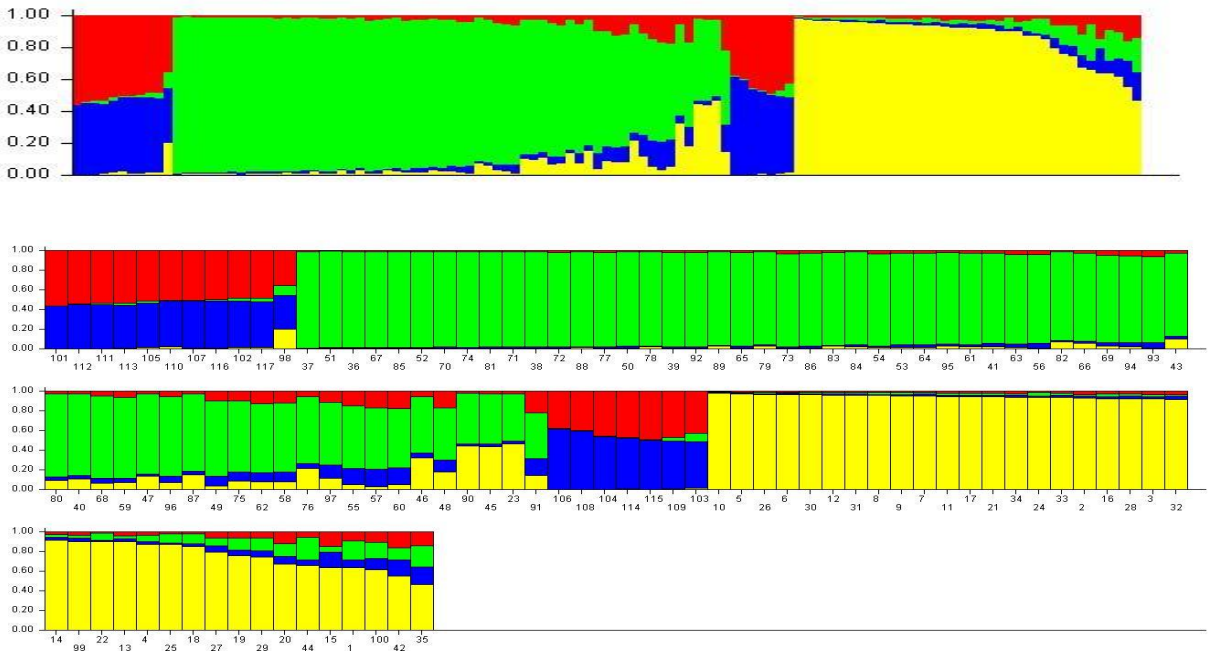

FIGURE 1: The summary plot of Q matrix estimates showing (a) cluster 1 (red), cluster 2 (green), cluster 3 (blue), and cluster 4 (yellow); (b) clustering of 117 genotypes representing membership fractions

TABLE 2
GROUPING OF 117 BLACK GRAM GENOTYPES INTO 4 SUB-POPULATIONS BASED ON POPULATION STRUCTURE

S. No.	Sub-Population	Number of Genotypes	Names of Genotypes
1	Sub-Population 1	9	VBN-10, GBG 92, PBG 272, GBG 5, PU 1537, LBG 685, LBG 1012, GBG 30, LBG 806
2	Sub-Population 2	58	KUG 898, 0BG 38, KUG 883, TBG 152, TJU 262, OBG 39, TBG 155, TBG 138, TBG 149, TBG 156, LBG 623, TBG 129, TU 94, TBG 145, OBG 102, TBG 146, LBG 645, VBG-13-003, UTTARA, TBG 142, TBG 147, TBG 141, TU 40, TJU 111, TJU 258, PANTU 19, OBG 43, TBG 104, VBG-17-026, SB-42-8, LBG 8282, SUE17-52, PBG 32-2, TBG 150, TBG 151, TBG 154, VBG-17-012, LBG 890, TBG 148, LBG 787, TU 94, PUSA 851, LBG 969, VBN-7, TU 67, OBG 101, TBG 143, SEKHAR-2, PU-31, TBG 144, VBN-6, PBG-1, PU 1527, RUB-15-04, LBG 965, MBG 1069, VBG-12-034
3	Sub-Population 3	9	ADT 5, KPU-12-1735, LBG 1064, LBG 1006, IPU-13-6, GBG 23, GBG 86, LBG 1013, PU 1805
4	Sub-Population 4	41	VAMBAN 7, LBG 938, KPU 405, GBG 1, COBG 653, KU-19-299, COBG-13-04, KU-21-162, GBG 111, KU-21-165, DKU-90, DKU-99, DKU-87, GBG 108, IPU-12-15, KPU-514-75, KU-21-170, ABFBG 4, IPU-11-16, KU-21-158, ABFBG 12, KU-21-167, IPU-12-22, KU-21-163, KPU-13-241, GBG 99, BDU-12, KU-21-160, IPU-16-09, KU-21-157, IPU-2-43, KU-21-159, KPU-520-69, LBG 907, IPU-11-16, ABFBG 3, LBG 826, LBG 852, KUG 479

Sub-population 1 (POP1), represented with red colour in the bar graph, comprises a total of 9 genotypes. Sub-population 2 (POP2), represented with green colour in the bar graph, consists of 58 genotypes. Sub-population 3 (POP3), represented with

blue colour in the bar graph, consists of 9 genotypes. Sub-population 4 (POP4), represented with yellow colour in the bar graph, consists of 41 genotypes. Singh et al. (2022) also reported four subpopulations in 117 diverse black gram germplasm in their study of genome-wide association studies for yield and yield-related traits in black gram germplasm.

3.3 Cluster Analysis Based on UPGMA

Genetic similarity ratings between the black gram genotypes under investigation were used to generate the dendrogram displayed in Figure 2. The UPGMA approach was employed to compute a similarity matrix utilizing SSR markers based on Jaccard's coefficient using DARwin 6.0 software. Cluster I, Cluster II, and Cluster III are the three crucial clusters into which the 117 genotypes used in this investigation were separated. With 51 genotypes, Cluster I was the largest, followed by Cluster II (19 genotypes) and Cluster III (47 genotypes). Further subdividing Cluster I resulted in two sub-clusters: IA (28 genotypes) and IB (23 genotypes). Cluster II was further sectioned into two sub-clusters: IIA (18 genotypes) and IIB (1 genotype). Cluster III was further categorized into two sub-clusters: IIIA (40 genotypes) and IIIB (7 genotypes). Additionally, as the similarity coefficient rose, the sub-clusters were further segmented into distinct sub-clusters. Table 3 shows the specifics of the DARwin software's grouping of 117 black gram genotypes. Similar to the present study, Kaewwongwal et al. (2015) constructed a phylogenetic tree using 534 black gram genotypes and 22 SSR markers. The phylogenetic tree developed consisted of three major clusters: C-I, C-II, and C-III. Furthermore, the results of the neighbor-joining tree coincide with the results of the model-based population structure analysis.

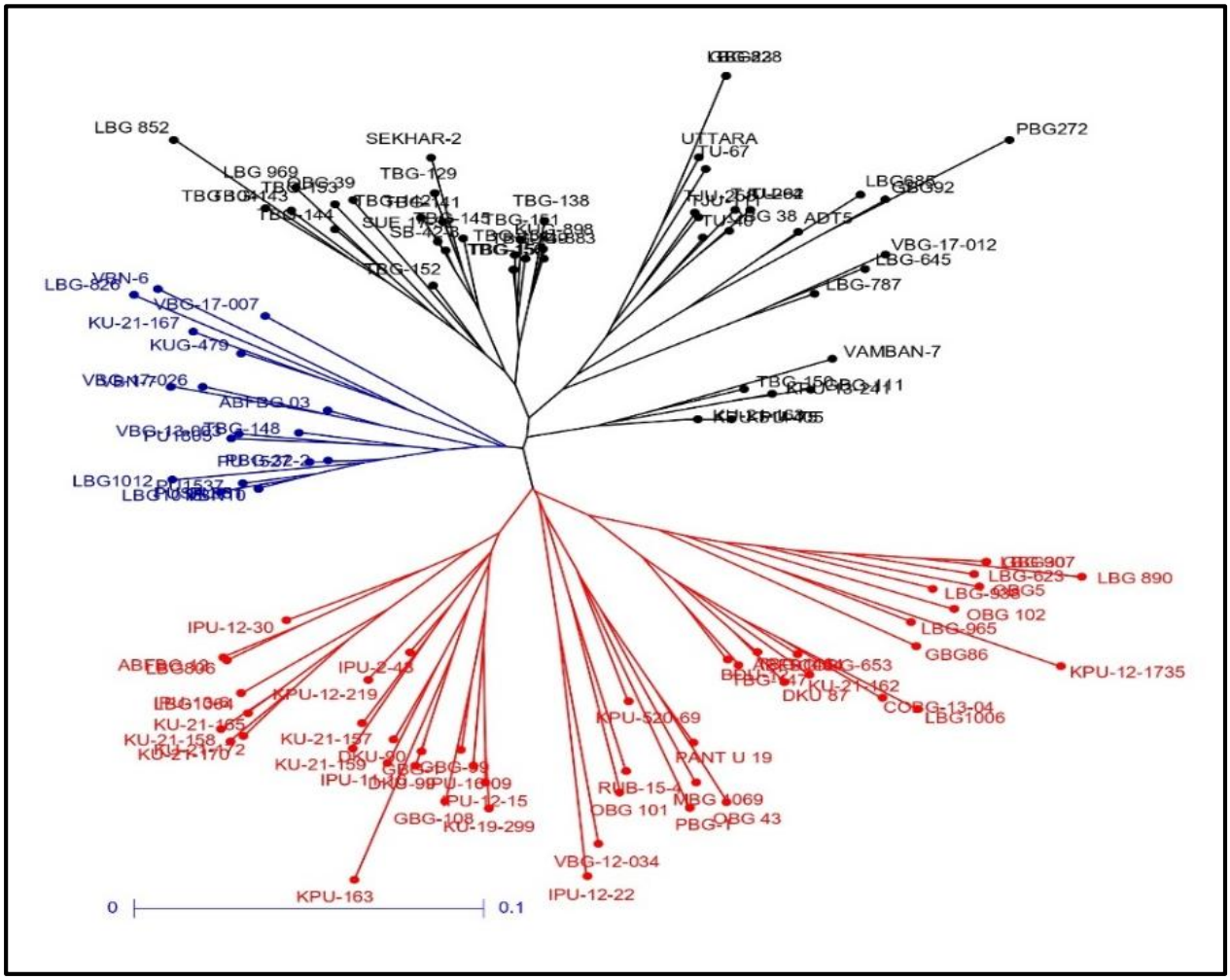

FIGURE 2: Neighbor-joining dendrogram depicting the genetic relationships among black gram genotypes based on SSR marker data. Colored branches indicate distinct genetic clusters: blue, black, and red. Scale bar represents genetic distance.

TABLE 3
GROUPING OF 117 BLACK GRAM GENOTYPES INTO DISTINCT CLUSTERS BASED ON JACCARD'S SIMILARITY COEFFICIENT USING UPGMA METHOD

S. No.	Cluster	Sub-Cluster	Number of Genotypes	Names of Genotypes
1	I	IA	28	GBG30, LBG907, LBG890, LBG623, GBG5, LBG938, OBG102, KPU-12-1735, LBG965, GBG86, LBG1006, COBG-13-04, COBG-653, KU-21-162, TBG146, ABFBG04, DKU87, TBG147, BDU12, PANTU19, OBG43, MBG1069, PBG1, KPU-520-69, RUB-15-4, OBG101, VBG-12-034, IPU-12-22
		IB	23	KU-19-299, IPU-12-15, IPU-16-09, GBG99, GBG108, GBG1, DKU99, KPU163, IPU-11-16, DKU90, KU-21-159, KU-21-157, IPU-2-43, KPU-12-219, KU-21-172, KU-21-170, KU-21-165, KU-21-158, LBG1064, IPU-13-6, LBG806, ABFBG12, IPU-12-30
2	II	IIA	18	VBN10, PU31, PUSA851, LBG1013, PU1537, LBG1012, PU1527, PBG32-2, PU-1805, VBG-13-003, TBG148, VBN7, VBG-17-026, ABFBG3, KUG479, KU-21-167, LBG826, VBN6
		IIB	1	VBG-17-007
3	III	IIIA	40	TBG104, LBG852, TBG143, TBG144, LBG969, TBG153, OBG39, TBG152, TBG142, SUE 17-52, SB-42-8, TBG141, TBG129, SEKHAR2, TBG145, TBG156, TBG155, TBG154, TBG151, TBG149, TBG138, KUG898, KUG883, GBG23, LBG828, UTTARA, TU67, TJU258, TJU111, TU40, TJU262, TU94, OBG38, ADT5, LBG685, GBG92, PBG272, VBG-17-012, LBG645, LBG787
		IIIB	7	VAMBAN7, TBG150, KPU-13-241, GBG111, KPU-21-163, KPU-514-75, KPU-405

3.4 Principal Coordinate Analysis (PCoA)

The principal coordinate analysis delineated that the 117 black gram genotypes were clearly separated from one another. The initial three principal coordinates explained 11.1%, 10.0%, and 8.2% of the total variation of 41.6%, as depicted in Table 4 and Figure 3.

The genotypes that are adjacent to the axis and clustered in one place are more strongly related to each other. Certain genotypes are extremely far from the axis, indicating a lack of a close relationship between them. The genotypes were sorted according to model-based analysis using PCoA analysis as well. The population STRUCTURE results were further validated by the outcomes of the model-based STRUCTURE analysis, which were almost correlated with the results of the PCoA and unweighted neighbor-joining clustering tree analyses. Principal Coordinate Analysis (PCoA) was used by Kaewwongwal et al. (2015) to determine genetic diversity among 534 genotypes of black gram. Of the total variation of 37.3%, the first coordinate explained 14.8% and the second coordinate explained 13.0% of the variation.

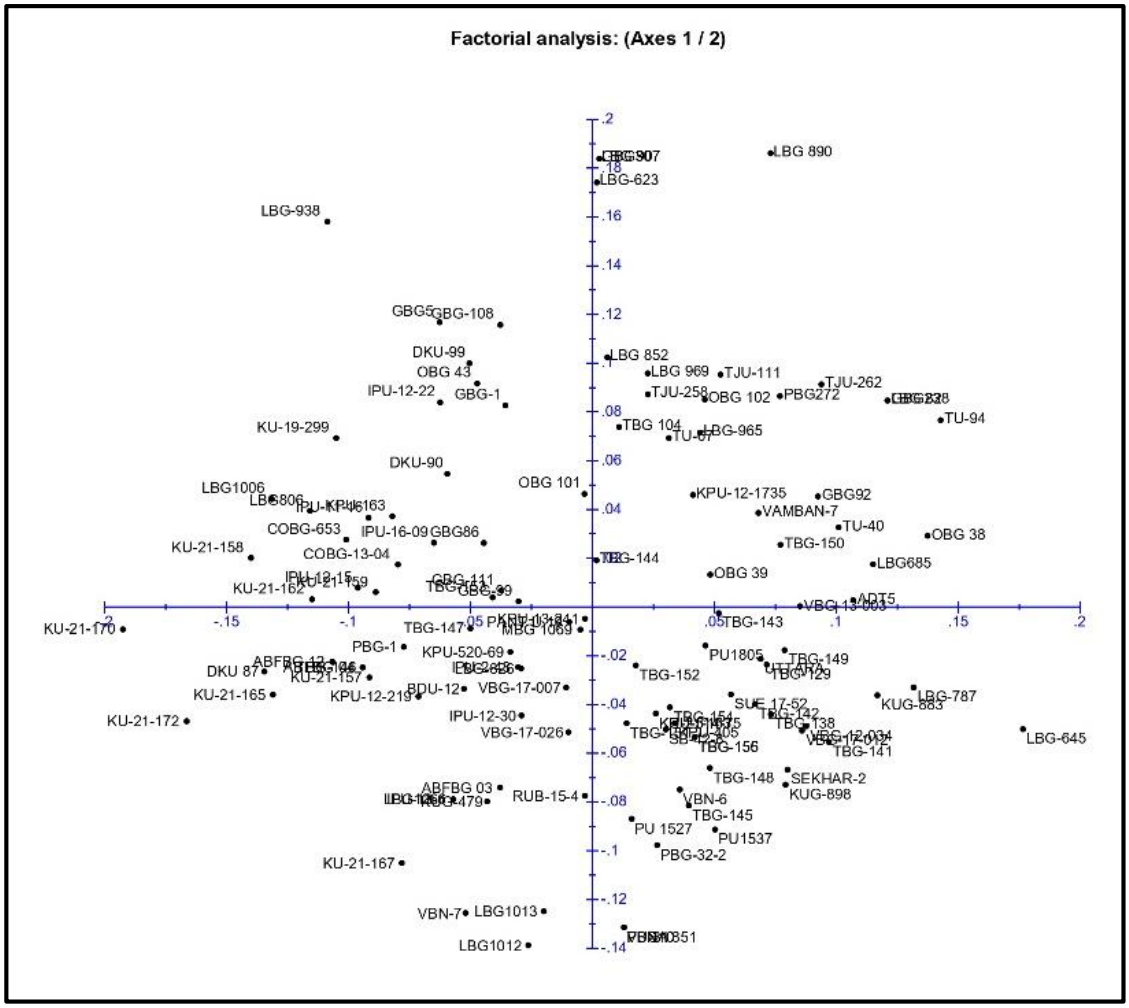

FIGURE 3: Principal Coordinate Analysis (PCoA) plot showing the distribution of black gram genotypes based on SSR marker data across the first two axes. The axes represent the major components of genetic variation among the accessions.

TABLE 4
FACTORIAL ANALYSIS BASED ON DISSIMILARITY

Axis	Eigenvalue	Inertia (%)
1	0.0056	11.1
2	0.005	10
3	0.0041	8.2
4	0.0032	6.5
5	0.0028	5.7

aCluster I generated through neighbor-joining analysis showed 13.4% similarity of genotypes with sub-population 1 and 2 generated through model-based analysis, whereas cluster I showed 32.6% correspondence with sub-population 4 and cluster II showed 18.7% correspondence with sub-population 4.

IV. CONCLUSION

From the results of population structure analysis, the maximum value for Evanno's measure of ΔK showed a clear peak at an optimal K value of four (K = 4), which indicated that the entire population can be grouped into four subgroups. Diversity analysis using DARwin based on the unweighted neighbor-joining method segregated black gram germplasm into three main clusters: Cluster I, Cluster II, and Cluster III. The grouping of genotypes based on UPGMA clustering and PCoA analysis was in close correspondence with the results of model-based STRUCTURE analysis, which further confirmed the population structure results. The identified polymorphic SSR markers, particularly those with high PIC values (CEDG 128, CEDG 44,

TWSSR 87, CEDG 154, CEDG 53, CEDG 92, and CEDG 91), can be effectively utilized for future genetic diversity studies and marker-assisted selection programs in black gram.

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CONFLICT OF INTEREST

The authors declare that they have no financial or non-financial competing interests related to the submitted work.

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