Genetic Diversity Analysis and Molecular Diversity in Greengram (*Vigna radiata* (L.) Wilczek) Genotypes Using SSR Markers

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Original Research Article

ABSTRACT

A study on the genetic and molecular diversity of 40 genotypes of Greengram was conducted using SSR markers. The experimental material contained significant genetic diversity; nevertheless, for all yield-related and yield-attributing features, phenotypic coefficient of variation exceeded genotypic coefficient of variation. The genotypes LGG 574 (8.80), PDM 139 (8.34), and Pant Mung 6 had the highest seed yields (7.78). The highest observed cluster distances between clusters 5 and 6 (472.88) and clusters 4 and 5 (432.89). Among all the factors PC 1 to PC 10, the PC 1 (19.99) accounted maximum proportion of variability in the set of all variables and remaining

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components accounted for progressively lesser and lesser amount of variation. The first six principal components (PC-1 to PC-6) with eigen values of 2.94, 2.62, 2.18, 1.40, 1.28, and 0.86, respectively, accounted for 86.95% of the total variance for all the qualities, according to principal component analysis. The genotypes 23, 32, 33, and 21 were spread out relatively far from other genotypes in the scatter plot, suggesting that they might be different from other genotypes. PIC values of 10 SSR loci, where the VR 86 marker produced the greatest PIC Value percentage and highest heterozygosity percent. Fixation index is ranged from 1.000 to -0.076. In comparison to Cluster II, III, IV, and V, Cluster I has the most genotypes (25), and the use of SSR markers in this work to differentiate between genotypes was made possible by the high polymorphism information richness of this cluster.

1. INTRODUCTION

Mungbean, often known as Greengram, is a member of the Leguminosae family (*Vigna radiata* (L.) Wilczek, 2n=22). Crop pollination in Greengram happens naturally, although cross pollination is also present, albeit in very small amounts. India is the world's major exporter and user of Greengram. There is a lot of diversity among Greengram cultivars and within the Greengram species because it is a self-pollinated crop [1]. Greengram has a special role in the diversification of Indian agriculture and the fight against malnutrition among the nation's vegetarian population. Greengram is a short-lived legume crop produced for its high protein (25%) and lysine (504 mg/g) content. It is thought to be a cure for Beriberi and is high in vitamin B. The area of this crop in India is 5.20 million hectares, and its productivity is 2.97 million tonnes and 572 kg per ha. (Unknown 2020–2021). The biggest producers of Greengram in India are the states of Rajasthan (19.69 lakh ha; 48.65 lakh acres), Karnataka (4.14 lakh ha; 10.23 lakh acres); Maharashtra (3.68 lakh ha; 9.10 lakh acres); Madhya Pradesh (1.60 lakh ha; 3.95 lakh acres); and Telangana (0.55 lakh ha; 1.35 lakh acres) (pjtsau.edu.in 2021-22 forecast of Greengram).

Before beginning an effective breeding programme, it is essential to conduct research on genetic variation, including heritability, phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV), and genetic progress. Because there is greater variation in segregating generations that can be used to develop a cultivar, genetic divergence between the parents is necessary. Examining population variability and developing our understanding of it creates the foundation for efficient and productive breeding operations [2]. Heritability estimates alone are frequently less informative than genetic progress and heritability estimates for predicting gain under selection [3]. Breeders must study genetic variation in genetic resources in order to develop ways for incorporating valuable diversity into their breeding programmes, better comprehend the evolutionary and genetic linkages among populations, and select germplasm in a more methodical and effective manner [4]. The most crucial stage of any crop development endeavour continues to be the evaluation of the genetic variety that currently exists in the basic gene pool utilizing multivariate analytic techniques like D^2 statistics and factorial analysis. The relative significance and utility of various variables and genotypes in a data set are assessed using principal component analysis. It reduced a huge number of associated variables to a small number of independent main components, which accounted for the majority of the data variance. Molecular markers make it easier to spot labelling mistakes, track down the rightful owner of a disputed cultivar, and regularly recognize cultivars in nurseries. Use of the molecular marker simple sequence repeat (SSR) has been made to evaluate the genetic diversity in Greengram [1]. The study of molecular markers is essential to genomic research. Because of their repeatability, multiallelic nature, codominant inheritance, relative abundance, and good genomic coverage, SSRs stand out among other marker systems like restriction fragment length polymorphism (RFLP), RAPD, sequence tagged sites (STSs), and AFLP. SSRs are collections of brief tandem repeats of nucleotide bases found all over the genome. The abundance of SSRs in the genomes that have been studied so far and their hypervariability are key characteristics that contribute to their popularity [5]. Microsatellites are locus specific, highly polymorphic, co-dominant and highly reproducible [3,4]. Therefore, any breeding effort can benefit from the genetic diversity and divergence found in the

materials. In order to improve the genotypes, we would further benefit from the assessment of variance because it would provide us an accurate picture of the level of variation. In order to assess the genetic diversity and linkages among genotype lines, the primary goal of this work was to describe Greengram genotypes using molecular markers.

2. MATERIALS AND METHODS

A study on the genetic and molecular diversity of 40 genotypes of Greengram was conducted using SSR markers at the Department of Plant Breeding and Genetics, SHUATS, Prayagraj, during the kharif 2021 in a randomized block design followed by molecular work at Division of Plant biotechnology, Indian Institute of Pulses Research (IIPR), Kanpur. The recorded morphological data were subjected to statistical studies of genetic and genomic diversity. To collect data on plant height, number of primary branches, number of clusters, number of pods, pod length in cm, number of seeds pod, number of seeds per pod, number of seeds per pod, number of seeds per pod, 100 seed weight per pod, biological yield, harvest index, and seed yield plant in Greengram, five randomly chosen plants were chosen. The agronomic trait observations would aid in assessing genetic diversity based on phenotypic data, allowing one to compare genetic diversity as explained by phenotypic data with genotypic data. The distance between two populations, D^2, as defined by Mahalanobis [6], was determined using Tocher's approach, which Rao outlined (1952). The Singh and Chaudhary approach was used to evaluate the contribution of individual characters to divergence (1985). PCA was performed using XLstat ver 2021 software to assess genotype divergence in terms of spatial distance in a two-way graphical graph setting the relative location of each genotype and to determine the contribution of characters to the overall variability for all the characteristics under consideration.

2.1 Genomic DNA Extraction and Quantification

Total genomic DNA was extracted from 40 genotypes using the Cetyl Trimethyl Ammonium Bromide (CTAB) extraction technique [2] and spectrophotometrically measured using a nano spectrophotometer (Implen, Germany). The concentration of genomic DNA of each genotype was measured by comparing it to a known concentration of DNA of 200ng and adjusting it to

20ng μl $^{-1}$ by diluting with Milli-Q water for SSR marker analysis.

Accumulation of the SSR-PCR Screening of the Greengram germplasm lines shown in Table 2 was conducted using thirty SSR or microsatellite repeat primers. PCR amplification was performed in a 10 μl PCR reaction contains 0.3 μl of dNTPs, 0.2 μl of 3 units *Taq* DNA polymerase, 1 μl of 10x *Taq* buffer, 2 μl of DNA, 1 μl of primer mix and 5.5 μl of Milli-Q water. The amplification was carried out under reaction conditions of pre-denaturation at 94°C for 5 minutes, followed by 40 cycles of denaturation at 94 °C for 40 seconds, annealing at 48- 61.8 °C for 1 minute, extension at 72 °C for 60 seconds, and final extension for 10 minutes at 72 °C with a hold temperature of 4°C. Electrophoresis was utilized to separate amplification products using 3% metaphor agarose (Sigma-Aldrich, India), and gel pictures were taken.

2.2 Data Analysis

Amplicons generated using SSR primers and resolved on agarose gels for each marker allele-genotype combination were scored quantitatively for presence in a binary coding, i.e., presence was marked as (1) and absence as (0). Using the Gene Alex 6.5 programme, the binary data was analysed for the number of alleles, Shannon's information index, PIC (Polymorphism information content), and heterozygozity. The data for PCA (Principal Component Analysis) was collected using the XLstat ver 2021 application. For 40 Greengram genotypes, the NTsys programme was used to produce a molecular dendrogram cluster analysis using the ward technique and squared Euclidean distance.

3. RESULTS AND DISCUSSION

In the current study, analysis of variance revealed a significant genetic difference for each trait. This implies that there was enough room in the present gene pool to choose promising lines for yield and component characteristics. The appearance of high variability may be due to the utilization of several data sources as well as environmental factors that modify the phenotypes. In terms of biological yield, the genotypes LGG 574 (8.80) had the maximum seed yield, followed by PDM 139 (8.34), Pant Mung 6 (7.78), and IPM 99-125 (7.40). LGG 574 has the most primary branches and clusters, according to PDM-11. The estimated genotypic variance and phenotypic variance showed that,

for all yield-related and yield-attributing characters, phenotypic variation was higher than genotypic variance, indicating the influence of environmental variables on these traits. It was discovered that the phenotypic coefficient of variation was greater than the genotypic coefficient of variation, which raises the possibility that the environment may affect how the feature under study manifests. Ramakrishnan et al. reported a similar discovery [7]. The production of seeds, the number of clusters per plant, and the number of pods per plant showed the most notable differences between GCV and PCV. Nitesh et al. observed a similar finding (2017). The highest heritability was found in the following categories: days to maturity (83.254), plant height (80.163), number of primary branches (88.552), number of pods per plant (67.602), number of seeds per plant (77.891), seed index (98.782), harvest index (77.847), biological yield per plant (66.808), and seed yield per plant (76.097), respectively. Muthuswamy et al. also reported similar results [8]. The highest estimates of genetic advance as a percentage of mean were found for plant height (26.224), number of primary branches (68.006), number of pods per plant (28.558), number of seeds per plant (21.061), number of seeds per pod (21.808), number of seeds per plant (seed index (32.93), biological yield per plant (23.633), and number of seeds per plant (34.474). The data acquired on 13 yield and yield contributing variables for the 39+1(check) genotypes of Greengram were subjected to genetic divergence using the Mahalanobis D^2 statistic. The size of the D^2 values showed that there was a lot of genetic variety produced by the item under consideration. The 39+1 (check) genotypes were randomly assigned into six clusters using the Tocher technique, with Cluster I having the most genotypes (26), Cluster II having the fewest genotypes (1), and Cluster III, IV, V, VI, and VII having the fewest genotypes (1). Genetic diversity and geographic diversity do not correlate, as shown by the genotype distribution, suggesting that other factors, such as genetic drift, environmental variation, natural and artificial selection, and breeding material exchange, are more likely to be the cause of diversity than geographic isolation. According to the values of the intercluster distances between the seven clusters, cluster V and VI (472.88) had the highest divergence, followed by cluster IV and V (432.89), cluster V and VII (424.78), cluster I and V (300.20), cluster III and V (276.84), cluster II and VI (218.80), cluster II and VII (188.63), cluster II and IV (181.96), cluster III and IV

(180.58), cluster III and VII (180.07), and (173.77). Comparable outcomes have been reported by Suhel et al. [9], Manoj and Sachin [10], Sai Rekha et al. [11]. In order to increase the ability of the two parents to combine and produce greater yield traits, it is advised that two parents be chosen for a hybridization programme who differ more from one another. The current study revealed that the number of seeds produced per plant (20.30%) had the biggest contribution to total divergence, followed by harvest index (15.15%), biological yield (9.92%), seed index (8.65%), number of primary branches per plant (8.52%), and number of clusters per plant (6.55). Similar finding was observed by Katiya and Kumar [10], Aijaz et al. [12], Gaurav et al. (2017), Sen et al. [13], and Sharma et al. [14]. Thirty SSR primers were utilised for the molecular analysis, of which ten were polymorphic and the other twenty were monomorphic. Ten SSR markers were used in PCA for 40 genotypes of Greengram based on the findings of the polymorphic bands. In the current investigation, the first six principal components (PC-1 to PC-6) with eigenvalues of 2.94, 2.62, 2.18, 1.40, 1.28, and 0.86, respectively, accounted for 86.95% of the total variance for all the features. Characters' contributions to the divergence would increase with increasing absolute value in the PC [15]. The first principal component (PC-1) accounted for 22.63% of the variability, with biological yield (0.42), seed yield per plant (0.40), and plant height being the most important contributing variables (0.36). Similarly, significant loadings of days to maturity (0.37) and days to 50% flowering (0.29), which explained 20.17% of total variation, were detected in the second principal component (PC-2). Pod length (0.48), days to 50% flowering (0.40), and days to maturity (0.39), together accounted for 16.78% of total variance in the third main component (PC-3). The fourth principal component (PC-4) accounted for 10.8% of total variance and was distinguished by significant loadings for number of clusters per plant (0.50), pod length (0.47), and number of seed per pod (0.38). Days to 50% pod setting (0.70) and harvest index (0.27) contributed 9.88% of total variation to the fifth principal component (PC-5), indicating that these variables might be employed effectively for germplasm selection for yield increase in Greengram. Principal components with eigenvalues greater than one should be retained as potential contributors to diversity [16]. The sixth principal component (PC-6) contributed less to divergence, with an eigen value less than 1.0

and a variability of 6.64%. As a result, variables such as plant cluster number, plant pod number, plant height, days to maturity, days to 50% flowering, and seed yield plant-1 contribute the most to divergence. This finding corresponded to the findings of Jakhar and Kumar [17] for plant height and Mahalingam et al. [18] for seed yield plant. The fifth PC contributed less to divergence, with an eigen value less than 1.0 and a variability of 9.8%. The results agreed with those of Jakhar and Kumar [17] and Mahalingam et al. [18]. The first three main components accounted for 59.59% of the total variance with eigen values greater than one. In order to create a 2D scatter diagram, the scores of PCA for the first three principal components for 40 genotypes were calculated and plotted onto a graph, with PCA I in the X-axis and PCA II in the Y-axis (Table 5, Fig. 1). PCA was performed for 30 SSR markers for 40 Greengram genotypes Among all the factors PC1 TO PC10, the PC1 (19.995) accounted for maximum proportion of variability in the set of all variables and remaining components accounted for progressively lesser and lesser amount of variation. The PC 1 accounted for maximum variability i.e., 19.995% and PC2 (16.562%), PC3 (13.595%), PC4 (12.929 %), PC5 (10.041%), PC6 (8.824%), PC7 (6.849%), PC8 (5.615%), PC9 (4.343%), PC10 (1.247%). The highest Eigen value for PC1 (2.00) and the least is PC10 with 0.125 (Table 6). The first principal component PC1 showed high positive loading for VR 80 (0.891) followed by CEDG 271 (0.693), CEDG 048 (0.280), VR 48 (0.048) and VR 91 (0.026), where it has showed high negative loading for CEDG 21 (-0.580), CEDG 236 (-0.430), CEDG 300 (-0.310), VR (-0.120) and CEDG115 (0.100). The second principal component PC2 showed high positive loading for CEDG 300 (0.647) Followed by CEDG 048 (0.629), VR91 (0.371) and CEDG 236 (0.093). whereas it showed high negative loading for VR 48 (-0.520), CEDG 115 (-0.520), VR 86 (-0.340), CEDG 21 (-0.170) and CEDG 271 (-0.130). The third principal component PC3 showed high positive loading for VR 86 (0.708) followed by CEDG 236 (0.441), VR 48 (0.332), CEDG 048 (0.188), CEDG 300 (0.101) and VR 80 (0.076) whereas it showed high negative loading for CEDG 21 (-0.460), CEDG 115 (-0.400), VR 91 (0.320) and CEDG 271 (-0.180). The fourth principal component PC4 showed high positive loading for CEDG 236 (0.604) followed by CEDG271 (0.541), CEDG 21 (0.311), VR 80 (0.283), VR 91 (0.273), VR 86 (0.199) and CEDG 300 (0.142). whereas it showed high negative loading for CEDG 048 (-0.460), VR 48 (-0.290), CEDG 115 (-0.170). The fifth principal component PC5 showed high positive loading for CEDG 115 (0.536), VR 91 (0.406), VR 86 (0.256), CEDG 236 (0.190) and CEDG 048 (0.057), whereas it showed high negative loading for VR 48 (-0.420), CEDG 21 (-0.410), CEDG 300 (-0.240), CEDG 271 (-0.220) and VR 80 (-0.010). The sixth principal component PC6 showed high positive loading for VR 91 (0.712), VR 48 (0.501), CEDG 271 (0.075), VR 86 (0.067), CEDG 048 (0.053) and CEDG 21(0.015), whereas it showed high negative loading for VR 80 (-0.210), CEDG 115 (-0.200), CEDG 300 (-0.160) and CEDG 236 (-0.060). The seventh principal component PC7 showed high positive loading forced CEDG 300(0.480), VR 86 (0.369), CEDG 115 (0.368), CEDG 271 (0.208), CEDG 048 (0.195), CEDG 21 (0.172), VR48 (0.109), VR 91(0.046) and VR 80 (0.026), whereas it showed high negative loading for CEDG 236 (-0.240). The eighth principal component PC8 showed high positive loading for CEDG 048 (0.398), CEDG 21 (0.323), VR 86 (0.226) and CEDG 271 (0.149), whereas it showed high negative loading form CEDG 300 (-0.370), VR 48 (-0.220), VR 80 (-0.140), CEDG 115 (-0.110), VR 91 (-0.08) and CEDG 236 (-0.020).The ninth principal component PC9 showed high positive loading for CEDG 236 (0.390), CEDG 048 (0.272), CEDG 115 (0.250), VR 48 (0.224), CEDG 271 (0.137) and VR21 (0.010), whereas it showed high negative loading form VR 86 (-0.270), VR 91 (-0.080),VR 80 (0.020) and CEDG 115 (-0.010). The tenth principal component PC10 showed high positive loading for VR 80 (0.237), CEDG 21 (0.153), VR 91 (0.051), VR 48 (0.046), CEDG 048 (0.046), CEDG 236 (0.023), CEDG 115 (0.012) and VR 86 (0.007), whereas it showed high negative loading form CEDG 300 (-0.020) and CEDG 271 (-0.190). The genotypes 23, 32, 33, and 21 were located somewhat far from other genotypes, according to the 2D structure. Better recombinants would result from combining these genotypes [19]. The 40 Greengram genotypes are clustered using the ward technique and squared Euclidean distance utilising the 10 SSR markers. According to the dissimilarity coefficient, the 40 genotypes are separated into five clusters: Clusters I, II, III, IV, and V. Cluster I, which comprises 25 genotypes, is further subdivided into two more compact clusters. There are 4 genotypes in subcluster IB, compared to 21 in subcluster IA. The SSR marker CEDG 115 & VR 91 has highest number of alleles 5 followed by CEDG 300, VR 86, VR 48, VR 80 and CEDG 236 has 4 number of

alleles, CEDG 048 has 3 alleles and the lowest alleles were recorded in CEDG 271, CEDG 21 has 2 alleles each. A total of average 3.7 alleles per locus. The observed heterozygosity has range 0.117 to 1.258 with a mean of 0.779. The highest observed heterozygosity recorded for VR 86 marker with 1.258 and the lowest is in CEDG 21 with 0.117. The expected heterozygosity has range between 0.000 to 0.182 with a mean of 0.057. The highest expected heterozygosity recorded for CEDG 048 has 0.182 while in least observed for VR91, VR 80, VR 48, VR 86, CEDG 21 and CEDG 271 has 0.000 [5]. PIC is a significant measure that determines a marker's capacity to distinguish between various genotypes and evaluates the efficacy of polymorphic loci. With values ranging from 0.049 to 0.694, the average polymorphic information content value (PIC) per locus was 0.427. Singh *et al.* report a similar finding (2013). PIC values (polymorphic information content) of 10 SSR loci ranged from 4.9% to 69.4% with an average of 42.7%. Out of the 10 markers, VR 86 produced the highest percentage of PIC Value, while CEDG 21 produced the lowest. In the current study, the highest (I) diversity result was obtained for VR 86 and the lowest (I) diversity result was obtained for CEDG 21. The high (I) diversity result for VR 86 and the lowest (I) diversity result for CEDG 21 in the current study Wang et al. [20] found a similar discovery, and the average Shannon's index for the 10 SSR polymorphism markers is 0.432. The random measurement of species variety is done using Shannon's information index (I). Fixation index is ranged from 1.000 to -0.076 with mean 0.774. The highest fixation index 1.000 is present in CEDG 271, CEDG 21, VR 86, VR 48, VR 80, VR91 and the lowest present in CEDG 048. The high fixation index at most loci could be attributed to self-pollination in Greengram-positive genotypes with a small number of heterozygous loci. A high fixation index was discovered to be connected with low variance. Changyou et al. [21]; Wang et al., 2017; Kanimoli et al. [22]; Muthusamy et al. 2008; Results from this study could be used to further improve crops using advance marker systems and would be very helpful in Greengram breeding projects [23-29,18,30-31].

Table 1. Genotypes of Greengram with their pedigree and salient features

S. No.	Genotypes	Centre responsible for developing	Pedigree	Year of release	Salient features
1	PDM-11	IIPR, KANPUR	Selection from LM595	1987	Erect bushy, shining green seed, suitable for spring season
2	PDM-54	IIPR, KANPUR	Selection from Kundawa Bahraich local (UP)	1987	Erect bushy, shining green seed
3	ADT-3	TNAU, ADUTHARAI	(M 70-16 × Rajendra) × G 65	1991	Semi spreading, resistant to MYMV
4.	PUSA 9072	IARI, NEW DELHI	PUSA 106 × 10-215	1995	Moderately resistant to Powdery Mildew
5	Pant Moong 4 (UPM 92-1)	GBPAU, PANTNAGAR	T-44×UPU-2	1997	Erect, Dull green seed, resistant to MYMV
6	PUSA 9531	IARI, NEWDELHI	Selection from NM9473	2000	Resistant to MYMV
7	PUSA VISHAL IPM-99-	IARI, NEWDELHI	Selection from NM92	2002	Resistant to MYMV
8	125(Meha)	IIPR, KANPUR	PM3 ×APM36	2004	Resistant to MYMV
10	HUM-16	BHU, VARANASI	PUSA BOLD-1 ×HUM8	2006	Resistant to MYMV
11	MH-2-15	CCSHAU, HISAR	PDM116 × GUJRAT-1	2007	Resistant to MYMV and Cercospora leaf spot
12	Pant Moong 6 (UPM 02-17)	GBPAU, PANTNAGAR	Pant mung-2 × AMP 36	2007	Resistant to MYMV

S. No.	Genotypes	Centre responsible for developing	Pedigree	Year of release	Salient features
13	KM-2241	CSAU, KANPUR	SAMRAT×PDM54	2008	Resistant to MYMV
14	IPM-2-3	IIPR, KANPUR	IPM99-125 × PUSA BOLD2	2009	Resistant to MYMV
15	PUSA 0672	IARI, NEWDELHI	11/395 ×ML267	2009	Resistant to MYMV
16	IPM-2-14	IIPR, KANPUR	IPM99- 125×PUSA BOLD 2	2010	Resistant to MYMV
17	DGGV-2	UAS, DHARWAD	CHINAMUNG × TM-98-50	2014	Moderately resistant to Powdery Mildew
18	MH 421	CCSHAU, HISAR	MUSKAN × BDYR 2	2014	Resistant to MYMV
19	IPM 410-3 (Shikha)	IIPR, KANPUR	IPM 03-1 × NM 1	2016	Resistant to MYMV
20	IPM 205-7 (Virat)	IIPR, KANPUR	IPM 02-1 × EC 39889	2016	Resistant to MYMV
21	LGG 460	APAU, LAM	LAM M2 × ML 267	1997	Top bearing with more pod per cluster, Resistant to MYMV
22	Pant Mung 5	GBPAU, PANTNAGAR	Selection from VC 6368	2007	Erect, shining green large seed, tolerant to MYMV
23	TM 96-2	BARC & ANGRAU, LAM	KOPERGAON × TARM 2	2007	Resistant to Powdery Mildew
24	SML-832	PAU, LUDHIANA	SML302 × PUSA BOLD1	2010	Tolerant to MYMV and thrips
25	MH-3-18	CCSHAU, HISAR	ASHA × BDYR 1	2016	Resistant to MYMV
26	SML-668	PAU, LUDHIANA	Selected fromNM94	2002	Resistant to MYMV
27	CO -4	TNAU, COIMBATORE	MUTANT OF CO-1	1981	Erect, large seed suitable for rainfed, resistant to PM & MYMV
28	CO -5	TNAU, COIMBATORE	KM 2 × MG 50-10	1991	Tolerant to MYMV
29	CO -6	TNAU, COIMBATORE	WGG 37 × CO-5	1999	Resistant to MYMV
30	HUM - 1(Malviya Jyoti)	BHU, VARANASI	BHUM 1 × PANT U30	1999	Resistant to MYMV
31	COGG -912	TNAU, COIMBATORE	MGG 336 × COGG 902	2005	Resistant to MYMV and CLS
32	COGG -8	TNAU, COIMBATORE	COGG 923 × VC 6040	2014	Resistant to YMV
33	PDM 139 (Samrat)	IIPR, KANPUR	ML 20/19 × ML 5	2001	Resistant to YMD
34	IPM 302-2 (Kanika)	IIPR, KANPUR	PANT MUNG 4 × EC 398897	2018	Resistant to MYMV
35	IPM 2K14-9 (Varsha)	IIPR, KANPUR	EC 398885 × PDM 139	2018	Resistant to MYMV and Powdery Mildew
36	IPM 312-20 (Vasudha)	IIPR, KANPUR	IPM 3-1 × SPS 5 Interspecific Hybridization	2020	Moderately resistant to Anthracnose and powdery mildew and resistance to MYMV
37	IPM 409-4 (Heera)	IIPR, KANPUR	PDM2881× IPM 3-1	2020	Resistant to MYMV

S. No.	Genotypes	Centre responsible for developing	Pedigree	Year of release	Salient features
38	LGG 574	APAU, LAM	Mutant from Pant M2	1993	Resistant to MYMV
39	IPMD-604- 1-7	IIPR, KANPUR	GERMPLASM	------	Resistant to MYMV
40	OBGG-58	OUAT, BERHAMPUR	MUTANT OFK857	2002	Resistant to MYMV

Table 2. List of polymorphic ssr markers

S.No	Primer Name	Sequences	Tm	Annealing Temperature	Length
1	VR 91	R = 3` TGGAGATGCAGGACTAAGAAGAG 5` F = 5` ACATATGTATCTGTCTGTGTGCCTA 3`	55.3°C 54.4°C	52.4°C	23mer 25mer
2	VR 86	R = 3` ATCGGTATATGTTGCCAATCAG 5` F = 5` CTATACTGCAATGAAGTGGATCTC 3`	51.1°C 54.0°C	52.5°C	22mer 24mer
3	VR 80	R = 3` AATGGTCCCTTTACCCCTTTT 5` F = 5` TGTGAGAGTGGAAGAGCAACTT 3`	50.5°C 53.0°C	51°C	21mer 22mer
4	VR 48	R = 3` AATAGGGCCCATAACATGTCC 5` F =5` AGGTGAGTGAAAATTGGAATAGG 3`	52.4°C 51.7°C	52.5°C	21mer 23mer
5	CEDG 300	R = 3` GTGTCGGAAATGTCAGGAGG 5` F =5` CGACAAACCCAAACCCTAGC 3`	59.4°C 59.4°C	59.4°C	20mer 20mer
6	CEDG 271	R = 3` CACTCCCACTGCCAAACAAGG 5` F =5` GCACTAAAGTTAGACGTGGTTC 3`	61.9°C 58.2°C	48°C	21mer 22mer
7	CEDG 236	R = 3` CGATGATGAGTCCTTTGGAATTGGG 5` F = 5` CTTGGACGGACAGAGTTTGGATTC 3`	61.0°C 62.7°C	61.8°C	25mer 24mer
8	CEDG 115	R = 3`ATGCCTCCTTTCAGGTGATTGT 5` F =5` GGCTCATTGTACCACTGGATAT 3`	58.4°C 58.4°C	58.4°C	22mer 22mer
9	CEDG 048	R = 3` GCTCCTCTTTTTGCTGCATC 5` F = 5` TCTCTTCCTCTATGGCTTGG 3`	54.6°C 53.5°C	54°C	20mer 20mer
10	CEDG 021	R = 3` AAAGGATGCGAGAGTGTAGC 5` F = 5` GCAGAATTTTAGCCACCGAG 3`	57.8°C 57.8°C	57.8°C	20mer 20mer

Table 3. Grouping of 40 Greengram genotypes based on D^2 values

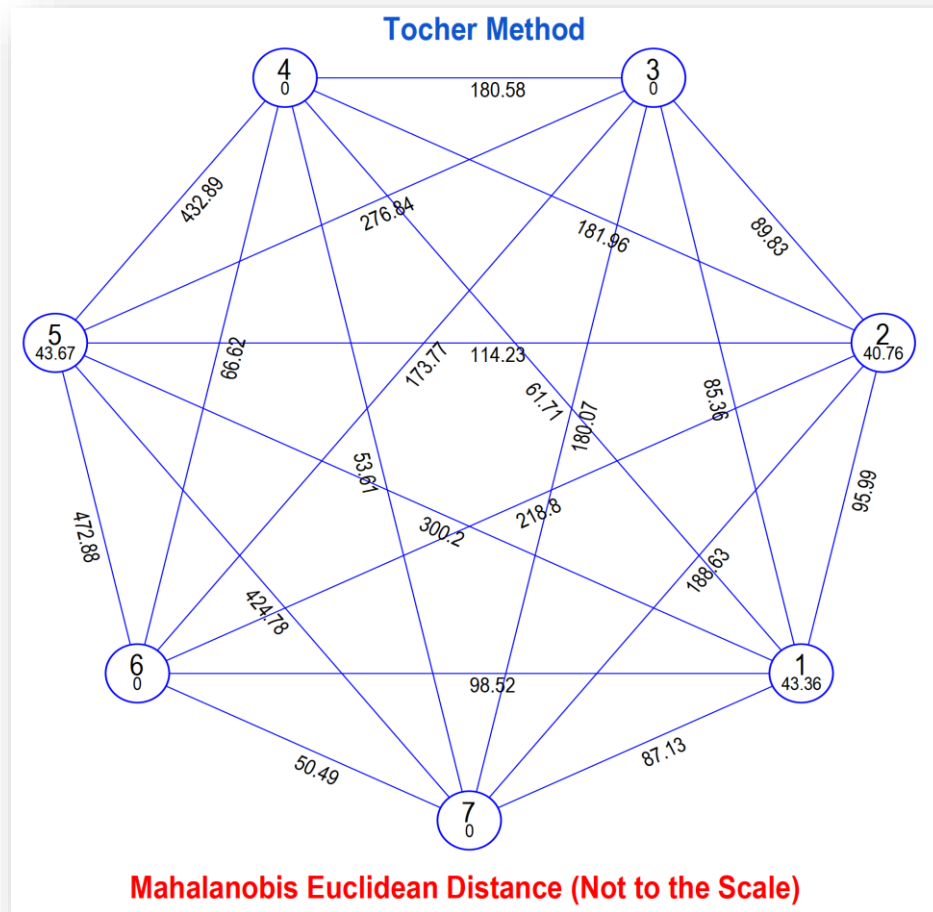| Cluster Group	No. of Genotypes	List of Genotypes
1 Cluster	26	IPM 99-125 (Check), HUM -1, IPM 410-3 (Shikha), MH 2-15, IPM 302-2 (Kanika), DGGV -2, OBGG -58, MH 3-18, PUSA 0672, SML 832, IPM 409-4 (Heera), IPM 205-7 (Virat), IPMD -604-1-7, ADT -3, IPM 2K14-9 (Varsha), PUSA 9072, IPM 2-14, KM 2241, COGG -912, IPM 2-3, LGG 574, HUM 16, MH -421, CO -4, PDM-11 & Pant Mung 5
2 Cluster	7	Pant Mung 4, CO -6, SML 668, TMB -37, PUSA 9531, LGG 460 & PUSA VISHAL
3 Cluster	1	COGG -8
4 Cluster	1	PDM 139 (Samrat)
5 Cluster	3	PDM -54, IPM 312-20 (Vasudha) & TM 96-2
6 Cluster	1	CO -5
7 Cluster	1	Pant Mung 6

Fig. 1. Diagrammatic representation of Cluster distance using Tocher method among 40 Greengram genotypes

Table 4. Average Intra and Inter cluster D^2 values in Greengram during *kharif*, 2021

	Cluster 1	Cluster 2	Cluster 3	Cluster 4	Cluster 5	Cluster 6	Cluster 7
Cluster 1	43.36	95.99	85.36	61.71	300.20	98.52	87.13
Cluster 2		40.76	89.83	181.96	114.23	218.80	188.63
Cluster 3			0.00	180.58	276.84	173.77	180.07
Cluster 4				0.00	432.89	66.62	53.61
Cluster 5					43.67	472.88	424.78
Cluster 6						0.00	50.49
Cluster 7							0.00

Table 5. Principal Components Analysis for 13 quantitative traits of 40 Greengram genotypes

S. NO		1 Vector	2 Vector	3 Vector	4 Vector	5 Vector	6 Vector
	Eigen Value (Root)	2.94239	2.62338	2.18142	1.40827	1.28463	0.86415
	% Var. Exp.	22.63380	20.17985	16.78012	10.83286	9.88175	6.64734
	Cum. Var. Exp.	22.63380	42.81365	59.59377	70.42663	80.30838	86.95571
1	Days to 50% flowering	0.25407	0.29025	0.40080	0.04205	0.11657	0.27832
2	Days to fifty percent pod	0.00278	0.04107	0.09691	0.04064	0.70550	-0.56331

| S. NO		1 Vector	2 Vector	3 Vector	4 Vector	5 Vector	6 Vector
3	Days to maturity	0.15131	0.37155	0.39661	-0.13677	0.01366	-0.00948
4	Plant height (cm)	0.36226	0.15558	0.16335	0.32086	-0.31572	-0.41856
5	Number of Primary Branches	-0.19349	-0.26814	0.20174	-0.11537	-0.50147	-0.35084
6	Number of clusters per plant	0.30859	-0.03585	-0.31699	0.50778	-0.12445	-0.19631
7	Number of pods per plant	0.28275	0.19573	-0.38377	0.27369	0.02998	0.27913
8	Pod length(cm)	-0.13520	-0.07461	0.48781	0.47262	0.09161	0.16565
9	No. of seeds/pod	-0.10037	-0.37708	0.29164	0.38106	-0.05144	0.16748
10	Seed Index (g)	-0.43522	0.19739	-0.12200	0.29609	0.09212	-0.26275
11	Harvest index (%)	-0.04655	-0.49805	-0.08604	0.11165	0.27589	0.14309
12	Biological yield per plant (g)	0.42710	-0.32967	0.05293	-0.07250	0.15840	0.03493
13	Seed yield per plant (g)	0.40786	-0.31123	0.09707	-0.23432	0.01502	-0.21017
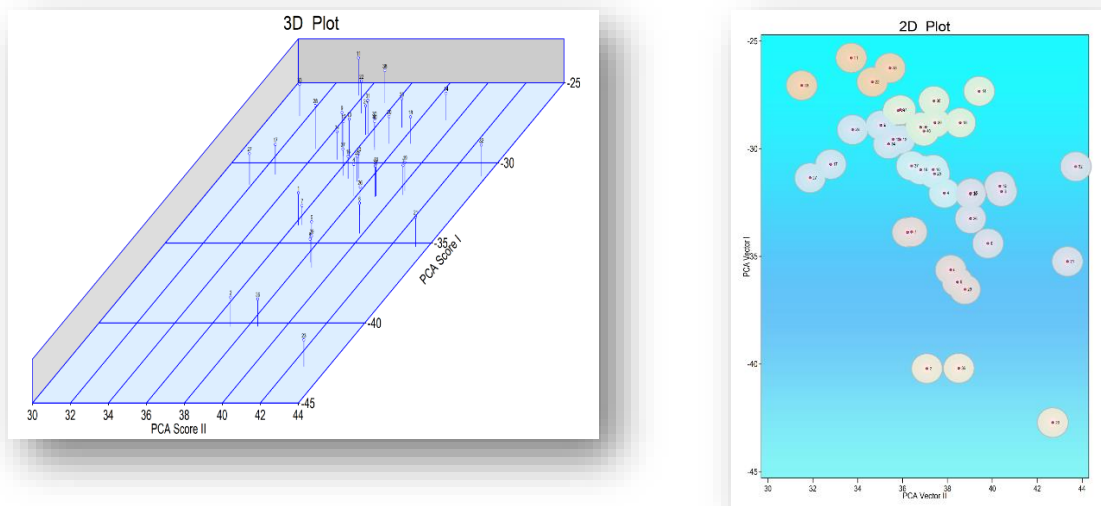
Fig. 2. 3D plot and 2D plot of Principal Components Analysis for 13 quantitative traits of 40 Greengram genotypes

Table 6. Total variance explained by different principal components in Greengram

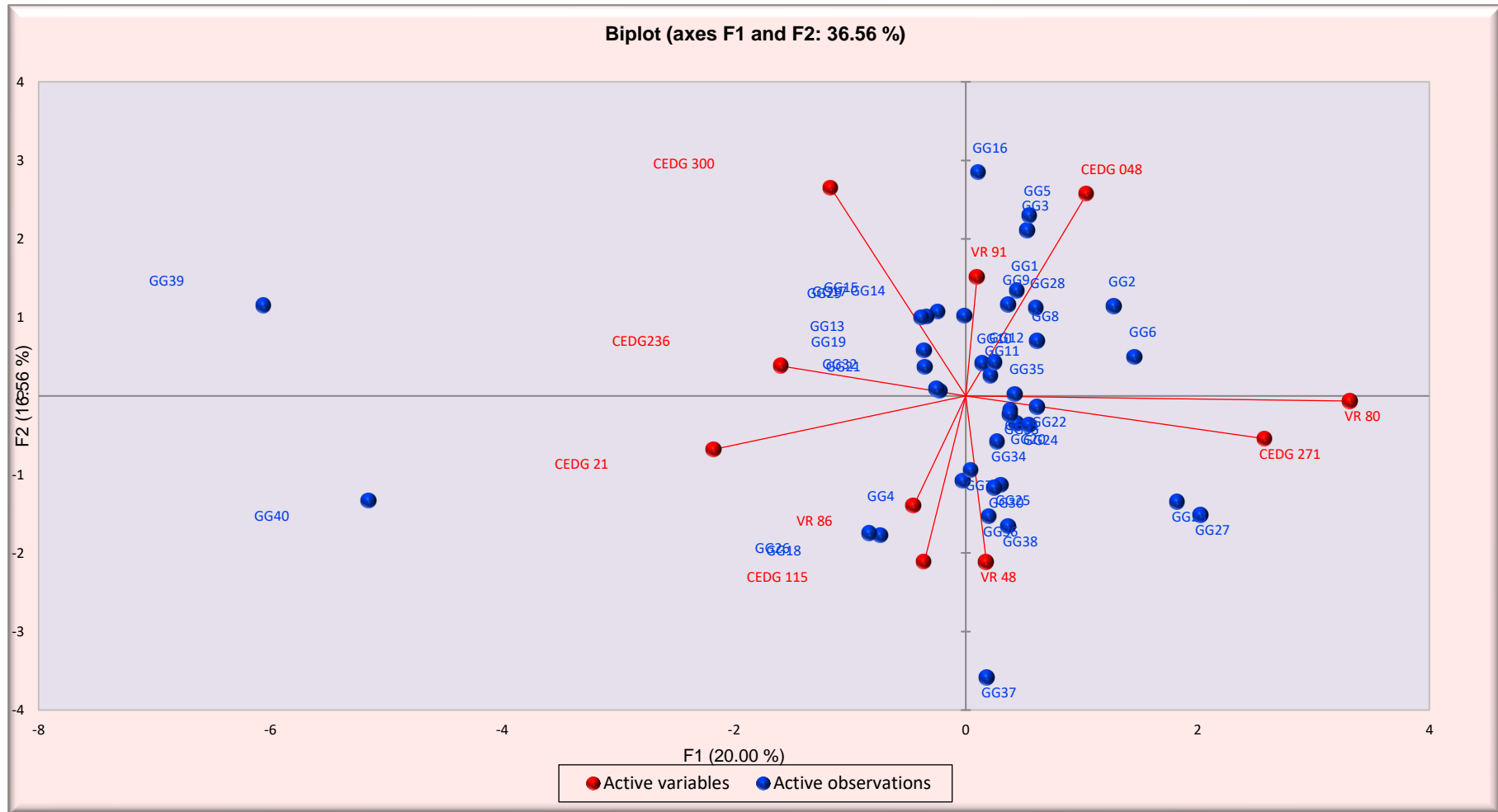| Observations	Eigenvalue	Variability (%)	Cumulative %
PC 1	2.000	19.995	19.995
PC 2	1.656	16.562	36.557
PC 3	1.359	13.595	50.152
PC 4	1.293	12.929	63.081
PC 5	1.004	10.041	73.122
PC 6	0.882	8.824	81.947
PC 7	0.685	6.849	88.795
PC 8	0.561	5.615	94.410
PC 9	0.434	4.343	98.753
PC 10	0.125	1.247	100.000

Fig. 3. Distribution of 40 genotypes on the bi-plot axes in PC 1 and PC2 of principal component analysis

Table 7. PCA analysis of 10 SSR markers for molecular diversity of Greengram

Observations	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10
CEDG 115	-0.096	-0.516	-0.400	-0.168	0.536	-0.197	0.368	-0.113	0.250	0.012
CEDG 300	-0.313	0.647	0.101	0.142	-0.237	-0.158	0.480	-0.374	-0.009	-0.021
CEDG 271	0.693	-0.133	-0.184	0.541	-0.219	0.075	0.208	0.149	0.137	-0.192
CEDG 048	0.280	0.629	0.188	-0.460	0.057	0.053	0.195	0.398	0.272	0.046
CEDG236	-0.427	0.093	0.441	0.604	0.190	-0.057	-0.239	-0.018	0.390	0.023
CEDG 21	-0.583	-0.166	-0.457	0.311	-0.411	0.015	0.172	0.323	0.010	0.153
VR 86	-0.121	-0.341	0.708	0.199	0.256	0.067	0.369	0.226	-0.266	0.007
VR 48	0.048	-0.517	0.332	-0.290	-0.417	0.501	0.109	-0.217	0.224	0.046
VR 80	0.891	-0.016	0.076	0.283	-0.006	-0.209	0.026	-0.138	-0.021	0.237
VR 91	0.026	0.371	-0.315	0.273	0.406	0.712	0.046	-0.077	-0.075	0.051

Table 8. Information generated by using 10 SSR Markers in Greengram Genotypes

| Markers	Alleles count	Number of effective alleles	Observed heterozygosity	Expected heterozygosity	PIC	I	Fixation index
CEDG 115	5	2.896	1.255	0.125	0.655	0.663	0.809
CEDG 300	4	2.276	0.972	0.125	0.561	0.569	0.777
CEDG 271	2	1.226	0.331	0.000	0.184	0.186	1.000
CEDG236	4	1.237	0.443	0.147	0.192	0.194	0.233
CEDG 048	3	1.203	0.363	0.182	0.169	0.172	-0.076
CEDG 21	2	1.051	0.117	0.000	0.049	0.049	1.000
VR 86	4	3.265	1.258	0.000	0.694	0.703	1.000
VR 48	4	2.305	0.952	0.000	0.566	0.573	1.000
VR 80	4	2.186	0.886	0.000	0.543	0.549	1.000
VR 91	5	2.920	1.215	0.000	0.658	0.666	1.000
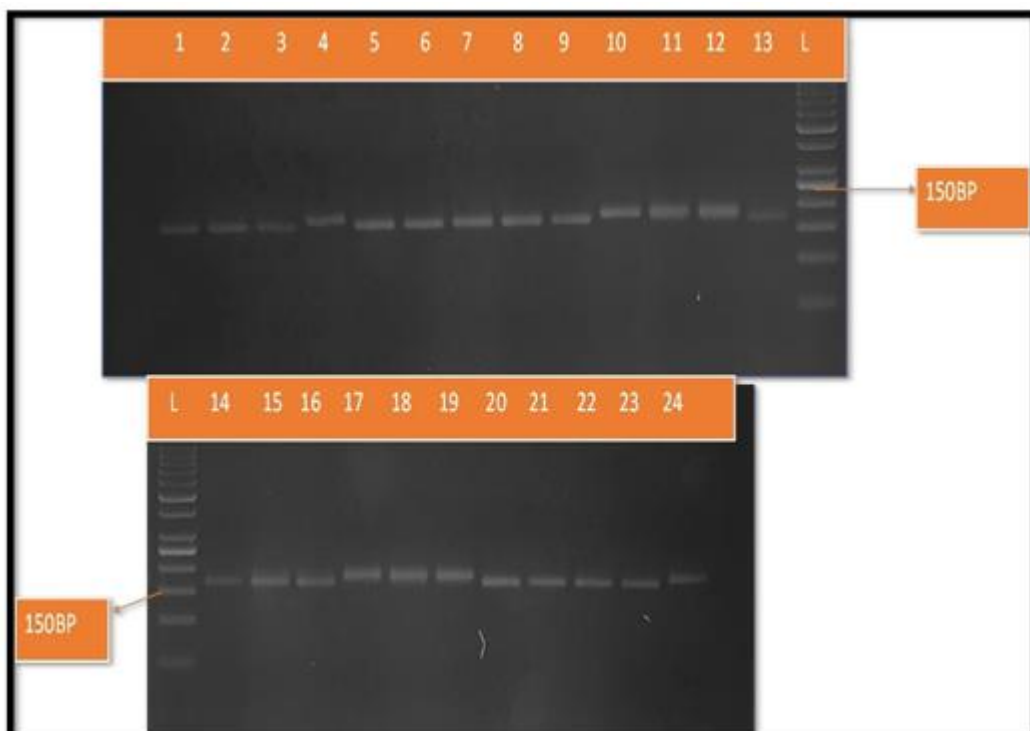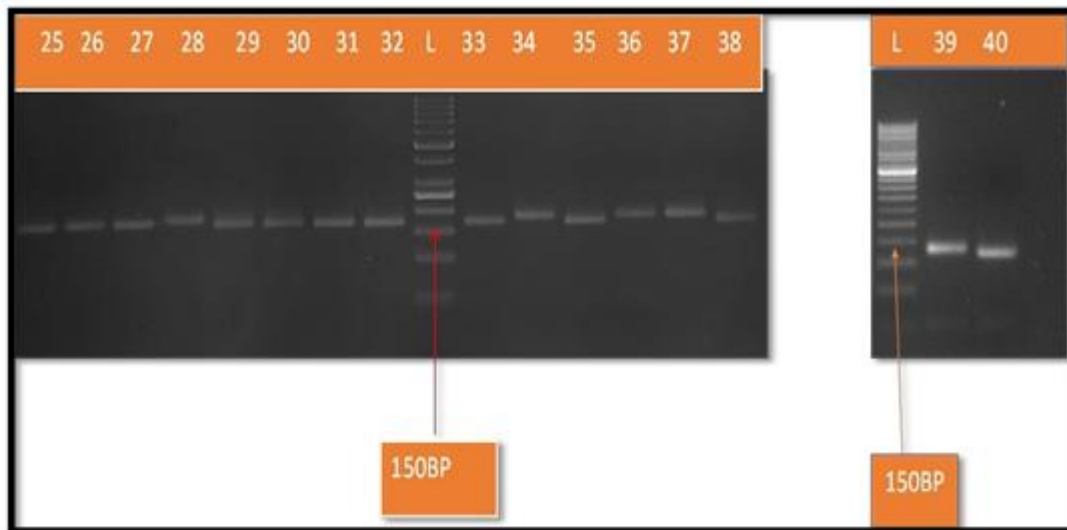
Fig. 4. Gel pictures of Agarose stained with Ethidium bromide showing genetic polymorphism among Greengram (*Vigna radiata* (L.) Wilczek) Genotypes using SSR primers
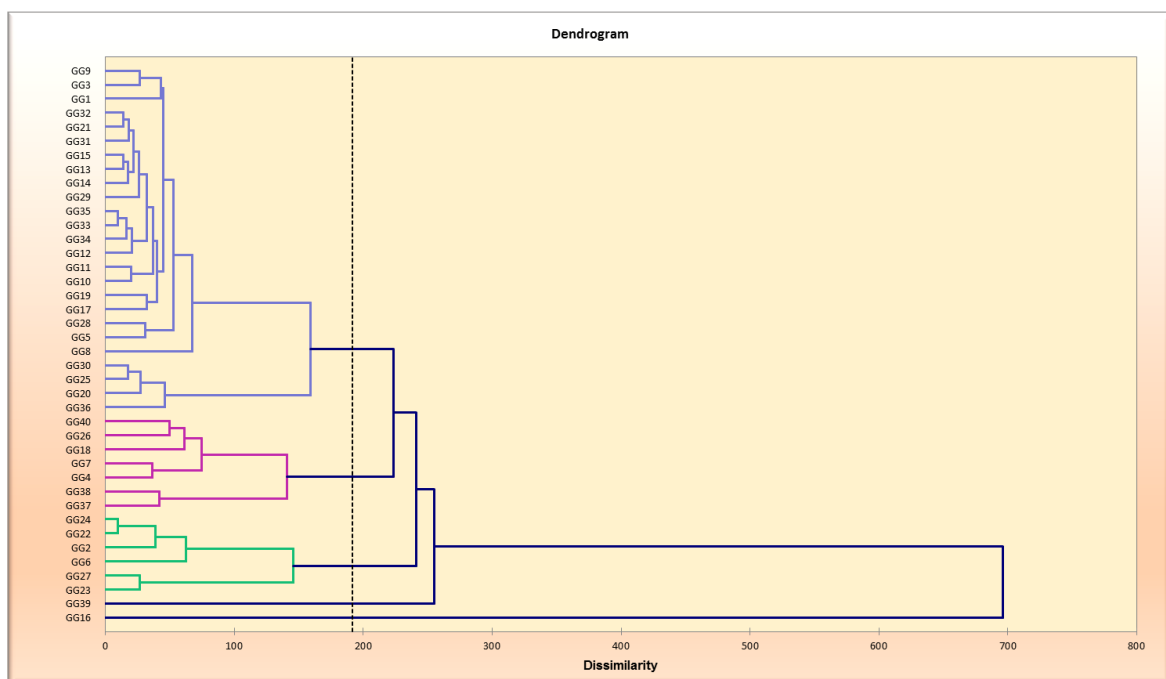

Fig. 5. Cluster analysis using ward method and squared Euclidean distance for 40 Greengram genotypes

4. CONCLUSION

Conclusions can be made based on the findings of the current study, which showed that Analysis of Variance revealed significant differences among the 40 genotypes, suggesting the possibility of selecting promising lines from the available germplasm. Based on average performance, the LGG 574 genotype produced the most seeds, followed by PDM 139 and Pant Mung 6. The separation between clusters 4 and 5 was the maximum, followed by clusters 5 and 6. The genotype compositions of these clusters show a broad range of segregational diversity. 86.95% of the variation for all the attributes was explained by the first six principal components (PC-1 to PC-6), with eigenvalues of 2.94, 2.62, 2.18, 1.40, 1.28, and 0.86, respectively. The PC

1 (19.99) factor, out of all the factors PC 1 to PC 10, accounted for the greatest proportion of variability in the set of all variables, whereas the following components accounted for progressively smaller and smaller amounts of variation. The highest PIC and heterozygosity percentages were found in VR-86. When compared to Clusters II, III, IV, and V, Cluster I has the most genotypes, at 25. In terms of Shannon's information index (I), CEDG 21 has the lowest diversity (I) result (0.049) and VR 86 has the highest diversity (0.703). From 1.000 to -0.076 is the fixation index range, Cluster I have the most genotypes when compared to Clusters II, III, IV, and V. SSRs with significant polymorphism information richness allowed for the differentiation of genotypes in our study. The results of the study show that SSR analysis can be used to evaluate the molecular diversity of various Greengram genotypes. For the purpose of marker-assisted breeding programmes, plant breeders highly value the information generated on marker data.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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