Website : www.asthafoundation.in
Frontiers in Crop Improvement
Vol 12 (1) : 56-60 (January-2024) Print ISSN : 2393-8234 Online ISSN : 2454-6011
Astha Foundation, Meerut (U.P.) India

Exploring Genetic Diversity in Promising Genotypes of Mung Bean (*Vigna radiata* (L.) Wilczek) through RAPD Markers

Taviyad K.L., Timbadiya P.N., Chovatiya N.C.*, Kandoliya U.K., Vala A.G. and Parakhia M.V.
Department of Biotechnology, Junagadh Agricultural University, Junagadh-362001, Gujarat
**Corresponding Author Email : chovatiyanirbhay936@gmail.com*

Abstract

The present research delves into the investigation of molecular diversity within a set of 20 mung bean genotypes, utilizing PCR-based molecular markers. The analysis employs 20 RAPD primers to assess both the phylogenetic relationships and polymorphism exhibited among the genotypes. Among these primers, 10 generated a total of 63 distinct bands, with band counts per primer ranging from 4 to 9. Within these bands, 18 displayed monomorphic characteristics across all genotypes, while 45 exhibited polymorphic variations. The calculated percentage of polymorphism stood at 75.19%. Evaluation of RAPD primer index (RPI) values indicated a range of 3.72 to 8.82, with an average of 6.08. Additionally, the polymorphism information content (PIC) values of RAPD markers ranged from 0.93 to 0.98, averaging at 0.96 per primer. The genetic comparison of the genotypes highlighted significant similarities. Notably, GJM-2025 and GJM-2027, GJM-2024 and GJM-2023, GJM-2026 and Meha emerged as the most akin, boasting a Jaccard's similarity coefficient of 0.95. Conversely, the least resemblance was observed between GJM-1701 and GJM-2017, reflecting a Jaccard's similarity coefficient of 0.50. Employing UPGMA in conjunction with the Jaccard similarity coefficient, an insightful dendrogram was constructed through the amalgamation of data from all 20 mung bean genotypes. The outcome of this hierarchical clustering categorized the genotypes into two principal clusters, namely cluster-A and cluster-B, interconnected by a 68% similarity.

Key words *: Mung bean, RAPD, PCR, molecular marker.*

Introduction

Mung bean (*Vigna radiata* (L.) Wilczek), commonly known as green gram, belongs to the *Fabaceae* family and the *Vigna* genus. Although the pulses and legume crops are self-pollinated crops, It also showed very wide variety of response to altered environmental condition like abiotic stresses, biotic stress as well as application of various chemicals and hormones (1,2,3). The genetic diversity of mung bean has drawn scientific attention, particularly due to insights from prior evaluations of morphological traits, which unveiled limited diversity within the working gene pool of this crop. Enhancing genetic improvement through strategic crosses involving parents with substantial genetic divergence has been an established principle. To this end, genotype characterization plays a pivotal role, offering plant breeders the tools to harness genetic variability and create advantageous combinations for the development of new varieties (4). Molecular markers, like Random Amplified Polymorphic DNA (RAPD) markers have emerged as a popular primary choice for analysing genetic diversity. RAPD markers are renowned for their quickness, simplicity, lack of environmental biases, extensive genome coverage, and relatively high polymorphism levels. This technique generates a multitude of molecular markers for comparative analysis, which are not only straightforward and versatile but also exhibit random yet broad genome coverage, ultimately showcasing a notable level of polymorphism (5,6).

In the context of mung bean research, previous endeavours have explored its genetic landscape through RAPD profiles, both individually and in conjunction with ISSR and SSR profiles (7). These studies have paved the way for the current investigation, which focuses on unravelling the intricate genetic diversity within 20 distinct mung bean genotypes through RAPD molecular markers.

Materials and Methods

The experiment was carried out in Department of Biotechnology, Junagadh Agricultural University, Junagadh during 2021-22. Genomic DNA isolated from young leaves germinated mung bean seedlings by following modified CTAB (Cetyl Trimethyl Ammonium Bromide) method as described by (8).

RAPD Analysis : Amplification of RAPD fragments was performed according to (9) with some modification using arbitrary primers The PCR reaction mixture (15µl) contained 10x PCR buffer (10 X Tris – HCL, pH 8.3), 2.5 mM each dNTPs, 25 pmoles primer, 50 ng of genomic DNA and 3 unit of Taq DNA polymerase (Promega, Madison, WI). The samples were subjected to 40 repeats of the following cycle: 94° C 1 min, 35° C for 1 min, 72°C for 2 min following a final extension of 10 minutes

Received : December-2023; Revised : December-2023; Accepted : January-2024

All the above PCR amplification was performed in 0.2 ml thin-walled tubes placed in a thermal cycler (Light Cycler, Eppendroff). The amplified products were analyzed by electrophoresis in 1.2% (RAPD), agarose gel stained in ethidium bromide (10 mg/ ml) and run 1x Tris borate EDTA buffer at 80 V for 45 minutes, the separated bands were visualized under UV transilluminator and photographed using a gel documentation system (BioRad).

Statistical Analysis : Polymorphic information content (PIC) for RAPD was calculated on the basis of allele frequency (10).

$$PICi = 1 \quad {}^{n}_{j\ 1} Pij^2$$

Where,

Pij is the frequency of jth allele for marker i and summation extends over n alleles.

PIC values were used to calculate a RAPD primer index (RPI), ISSR primer index (IPI) and SSR primer index (SPI) which were generated by multiplying the PIC values of all the markers amplified by the same primer.

Dendrogram Analysis : Clear and distinct bands amplified by RAPD, primers were scored for the presence (10) and absence (0) for the corresponding band among the genotypes. The data were entered in to MS-Excel data sheet and subsequently analyzed using NTSYS-pc version 2.10 e (11). The data matrix was read by NTSYS-pc version 2.2 (Numerical Taxonomy and Multivariate Analysis System for Personal Computers, Exeter Software) and analyzed by the SIMQUAL (similarity for qualitative data) program with Jaccard's similarity coefficient. SIMQUAL is a program for computing a variety of similarity and dissimilarity coefficients for qualitative data. The qualitative nature of the absence (0) or presence (1) state of a RAPD marker was used as the basis for similarity analysis among various sesame genotypes. A matrix of 0 and 1 act as the input, and the output is a matrix of similarity or dissimilarity coefficients. The resultant similarity matrix was entered into SAHN (sequential, agglomerative, hierarchical, and nested clustering method) clustering program, a tree matrix was produced and a dendrogram constructed using UPGMA (Unweighted Pair-Group Method with Arithmetic Averages). The assumption underlying the use of UPGMA clustering is the equal rate of evolution along all dendrogram branches. Dendrogram of publication quality were produced from the output tree file of SAHN by TREE (tree display) program in graphics mode.

Results and Discussion

Out of the 20 RAPD primers subjected to screening, 10 of them exhibited amplification, resulting in a cumulative count of 63 bands. The RAPD markers OPL-03 displayed the highest band yield of 9, while OPK-05 presented the lowest count of 4 bands. Among these 63 bands, all were found to be polymorphic, averaging at 4.5 bands per primer. Among the 68 polymorphic bands, all were shared within at least one variety, while 18 bands remained monomorphic. Notably, within the 46 polymorphic bands, 43 were shared among various genotypes, while 2 stood as unique polymorphic bands (as shown in Table-1). The computed percent polymorphism for the RAPD primers reached 100%, with an average of 75.09% per primer. The Polymorphism Information Content (PIC) values for the RAPD markers spanned from 0.93 to 0.98, holding an average of 0.96 per primer. Furthermore, the RAPD

Table-1 : Size, number of amplified bands, percent polymorphism and PIC obtained by RAPD primers.

Sr. No.	Primer	Allele/ Band Size (bp)	Total number of bands	Number of Polymorphic band			Number of Mono-morphic bands	% Polymor-phism	PIC value	RPI
				S	U	T				
1.	OPD-18	313-1378	6	1	0	1	5	16.7	0.96	5.76
2.	OPD-20	241-1435	8	3	2	5	3	62.5	0.98	7.84
3.	OPK-02	157-836	7	7	0	7	0	100.0	0.97	6.79
4.	OPK-05	254-3162	4	3	0	3	1	75.0	0.93	3.72
5.	OPL-01	164-1550	6	6	0	6	0	100.0	0.97	5.82
6.	OPL-02	349-2683	6	6	0	6	0	100.0	0.97	5.82
7.	OPL-03	201-1585	9	1	0	1	8	11.1	0.98	8.82
8.	OPN-14	160-658	5	5	0	5	0	100.0	0.95	4.75
9.	OPN-16	495-1349	5	5	0	5	0	100.0	0.95	4.75
10.	OPN-18	214-1402	7	6	0	6	1	85.7	0.97	6.79
		Total	63	43	2	45	18	-	-	-
		Average	-	-	-	4.5	1.8	75.09	0.96	6.08

S= Shared; U= Unique; T= Total Polymorphic bands; PIC= Polymorphism Information Content; RPI= RAPD primer index= number of bands x PIC.

Table-2 : Jaccard's similarity coefficient of 20 mung bean genotypes based on RAPD data analysis.

	GJM-2017	GJM-2018	GJM-2019	GJM-2020	GJM-2021	GJM-2022	GJM-2023	GJM-2024	GJM-2025	GJM-2026	GJM-2027	GJM-2028	Meha	GM-4	GM-6	GM-7	GAM-5	Virat	K-851	GJM-1701
GJM-2017	1.00																			
GJM-2018	0.72	1.00																		
GJM-2019	0.66	0.84	1.00																	
GJM-2020	0.64	0.89	0.84	1.00																
GJM-2021	0.64	0.84	0.93	0.81	1.00															
GJM-2022	0.61	0.82	0.85	0.86	0.91	1.00														
GJM-2023	0.64	0.84	0.90	0.88	0.93	0.91	1.00													
GJM-2024	0.59	0.80	0.88	0.89	0.88	0.93	0.95	1.00												
GJM-2025	0.59	0.80	0.85	0.86	0.88	0.90	0.89	0.90	1.00											
GJM-2026	0.57	0.80	0.88	0.83	0.88	0.90	0.92	0.93	0.87	1.00										
GJM-2027	0.62	0.82	0.90	0.82	0.90	0.85	0.90	0.89	0.95	0.89	1.00									
GJM-2028	0.55	0.79	0.82	0.82	0.88	0.93	0.88	0.90	0.90	0.90	0.85	1.00								
Meha	0.57	0.81	0.86	0.81	0.86	0.88	0.87	0.88	0.88	0.95	0.90	0.91	1.00							
GM-4	0.77	0.70	0.64	0.64	0.64	0.62	0.68	0.66	0.58	0.66	0.63	0.62	0.64	1.00						
GM-6	0.82	0.74	0.71	0.74	0.71	0.66	0.74	0.69	0.67	0.67	0.69	0.63	0.64	0.83	1.00					
GM-7	0.61	0.82	0.85	0.80	0.91	0.86	0.92	0.87	0.87	0.84	0.89	0.83	0.82	0.63	0.72	1.00				
GAM-5	0.59	0.69	0.75	0.69	0.78	0.79	0.75	0.80	0.80	0.83	0.79	0.79	0.81	0.67	0.70	0.79	1.00			
Virat	0.61	0.73	0.79	0.79	0.85	0.86	0.85	0.86	0.90	0.83	0.85	0.86	0.81	0.60	0.71	0.86	0.79	1.00		
K-851	0.53	0.76	0.82	0.79	0.85	0.86	0.85	0.86	0.86	0.86	0.82	0.86	0.81	0.59	0.66	0.86	0.82	0.86	1.00	
GJM-1701	0.50	0.63	0.69	0.67	0.69	0.76	0.70	0.74	0.71	0.71	0.70	0.76	0.72	0.52	0.53	0.71	0.66	0.76	0.76	1.00

primer index (RPI) spanned from 3.72 to 8.82, exhibiting an average of 6.08, as presented in Table-2.

A comparable outcome was previously reported by (9) who explored genetic diversity and relationships among 7 exotic and 3 advanced green gram germplasms using 3 RAPD primers (OPA01, OPB06, and OPB07). Their study yielded an average of 6 amplified products per primer and a percent polymorphism of 78.33. Similarly, It was previously identified RAPD markers for mung bean resistance against the yellow mosaic virus under south Gujarat's agro-climatic conditions (12). Among 200 RAPD markers, OPG-5, OPJ18, and OPM-20 emerged as optimal markers, producing 28, 35, and 28 amplicons, respectively, with an overall polymorphism of 70.17%.

Assessment of genetic similarity utilizing Jaccard's similarity coefficient revealed intriguing insights (as shown in Table-2). Notably, genotypes GJM-2025 with GJM-2027, GJM-2024 with GJM-2023, and GJM-2028 with Meha demonstrated the highest similarity (Jaccard's similarity coefficient: 0.95). Following closely were GJM-2024 and GJM-2026 (Jaccard's similarity coefficient: 0.93). On the contrary, genotypes GJM-1701 and GJM-2017 displayed the lowest similarity (Jaccard's similarity coefficient: 0.50), followed by K-851 and GJM-2017 (Jaccard's similarity coefficient: 0.53), indicating a greater diversity among these genotypes.

Corroborating findings were obtained by (13), who assessed genetic diversity among 26 French bean genotypes, showcasing an overall polymorphism of 74.62%. The range of Jaccard similarity indices among these genotypes extended from 0.48 to 0.98, affirming substantial genetic variability. Similarly, it was also investigated by the scientists that RAPD primers to differentiate a set of 39 green gram genotypes, reporting percent polymorphism values ranging from 42.85% to 100% (7). Their study demonstrated Jaccard's similarity coefficients spanning from 40.8% to 90.3%.

Utilizing UPGMA and Jaccard's similarity coefficient, a dendrogram was constructed to illustrate the genetic relationships among the 20 mung bean genotypes (as depicted in Table-2 and Figure-1). This dendrogram facilitated the classification of genotypes into two primary clusters: cluster-A and cluster-B, sharing a 64% similarity.

Cluster-A underwent further subdivision into subclusters I and II, boasting approximately 79% similarity. Subcluster I exclusively contained genotype GJM-2017, while subcluster II encompassed genotypes GM-4 and GM-6 (as illustrated in Figure 1). These three genotypes exhibited greater diversity in comparison to the remaining 17. Cluster-B, on the other hand, bifurcated into subclusters III and IV, displaying a 71% likeness.

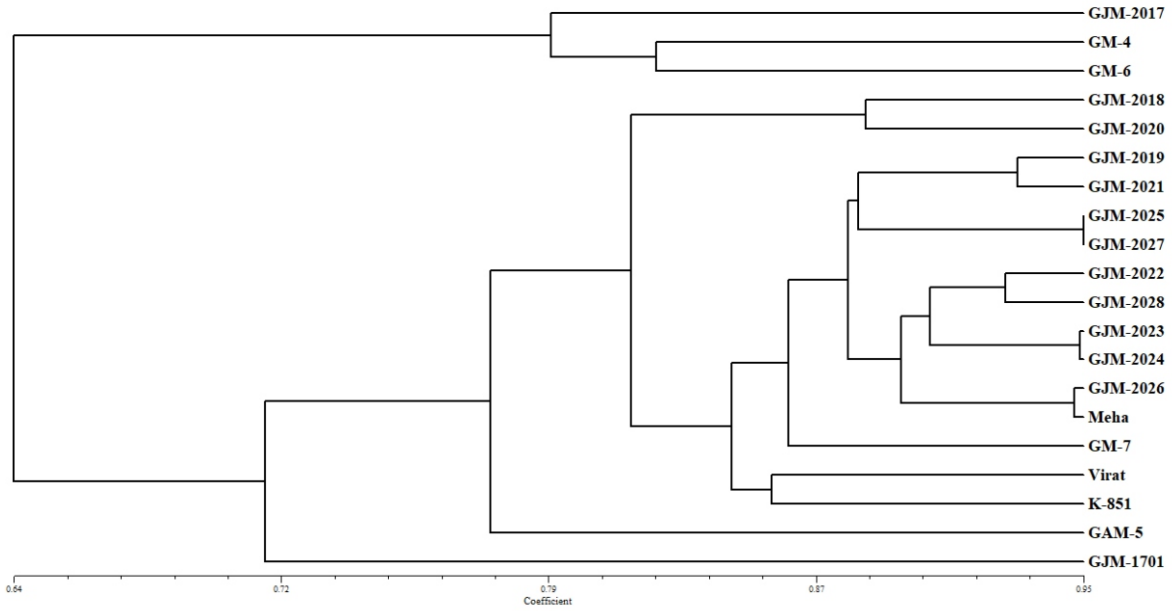
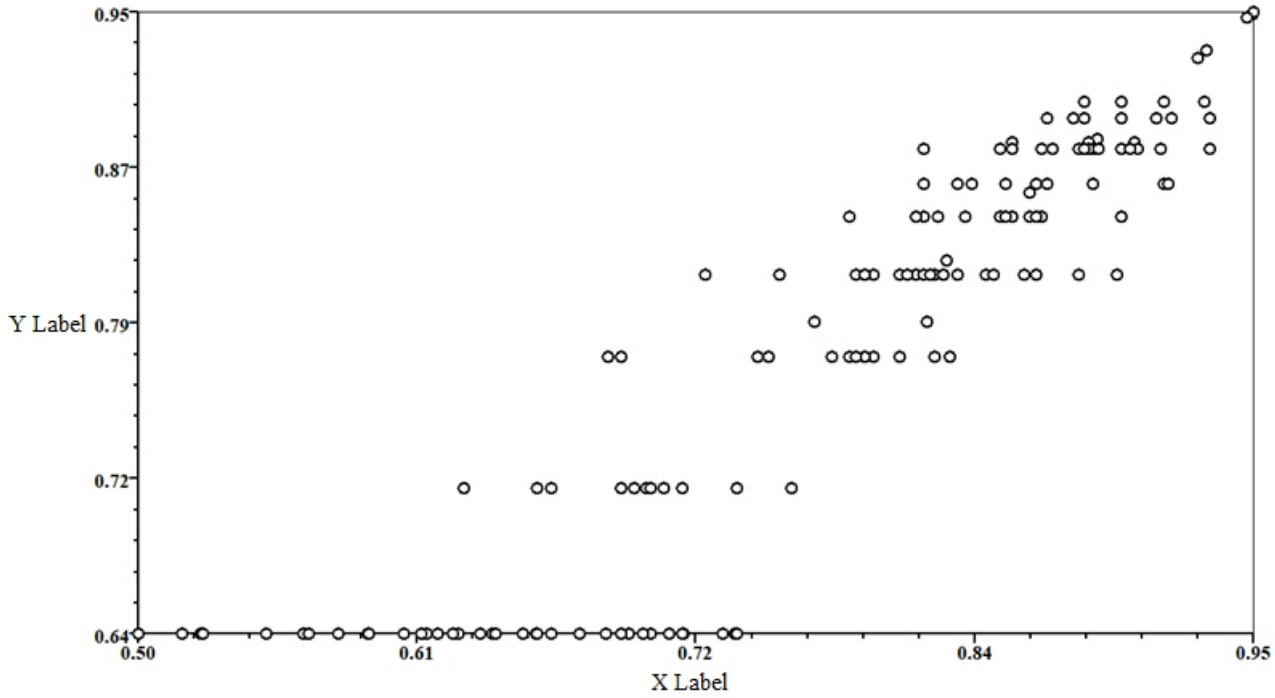


Figure-1 : Dendrogram analysis depicting genetic relationship among 20mung bean genotype sbasedon RAPD data.



Matrix correlation :r=0.93(=Normalized Mantel statistics Z)

Figure-2 : Cophenetic values against Jaccard's similarity coefficients from RAPD data of 20 mung bean genotypes.
(1: GJM-2017, 2: GJM-2018,3:GJM-2019,4:GJM-2020,5:GJM-2021,6:GJM-2022,7:GJM-2023,8:GJM-2024,9:GJM-2025,10: GJM-2026,11:GJM-2027,12:GJM-2028,13:Meha, 14:GM-4,15:GM-6,16:GM-7,17:GAM-5,18:Virat,19:K-851,20: GJM-1701

Subcluster III further branched into two subclusters, a and b. Subcluster a encompassed 15 genotypes, including GJM-2018, GJM-2022, and more. Subcluster b featured a single variety, GAM-5. Lastly, subcluster IV exclusively comprised genotype GJM-1701.

The evaluation of the clustering's goodness of fit was gauged using matrix cophenetic values, comparing the matrix produced by SIMQUAL to the cophenetic matrix. The normalization of the mental test statistic Z provided a measure of goodness of fit for cluster analysis, resulting in a matrix correlation of 0.93, aligning with the "very good fit" category as categorized by (11).

Conclusions

The utilization of Random Amplified Polymorphic DNA markers has illuminated the genetic diversity of mung bean genotypes, revealing pronounced variability influenced by diverse factors. This comprehension bears significance for breeding and conservation, fostering robust cultivars and sustainable productivity. Integrating a range of molecular markers augments our comprehension of mung bean genetics, collectively advancing breeding, conservation, and the enduring advancement of this pivotal crop. Additionally, our research delved into molecular diversity within 20 mung bean genotypes, employing PCR-based molecular markers. The genetic similarities between genotypes were notable, with GJM-2025 and GJM-2027, GJM-2024, GJM-2023, GJM-2026 and Meha showing the highest resemblance. Conversely, the least similarity was found between GJM-1701 and GJM-2017.

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