The Andhra Agric. J 69 (4): 513-523, 2022

Diversity analysis and fingerprinting of mung bean (*Vigna radiata* L. Wilczek) Advanced Breeding Lines using SSR markers

A Sheena Sabatina, Lal Ahamed M, J V Ramana and N Harisatyanarayana
Department of Molecular Biology and Biotechnology,
Advanced Post Graduate Centre, Lam, Guntur, A.P.

ABSTRACT

Suitability of 50 SSR markers to characterize and fingerprint thirty mung bean advanced breeding lines was studied. Among the fifty primers, forty-four primers produced clear banding pattern, of which only nine primers, CEDG 008, CEDG 015, CEDG 056, CEDG 068, CEDG-092, GLLC 108, PBALC 13, PBALC 217 and VrssR-14, produced polymorphic bands. Across the genotypes, these primers produced 236 bands, with sizes ranging from 160 to 200 bp. Primer CEDG-015 had low PIC value, primers, CEDG- 056, CEDG- 068, CEDG- 092, PBALC-013 and PBALC- 217 had moderate and primers CEDG-008, GLLC- 108 and Vr SSR-014 had high PIC values, revealing their discriminatory powers. The highest similarity coefficient value (0.33) was observed between the genotypes, LGG-705 and LGG- 688, revealing that these genotypes are highly similar in their genetic makeup compared to others. Simulated DNA fingerprinting profiles showed that each primer produced a reproducible band but there were few exceptions where two bands were observed for a primer (CEDG056 and CEDG092) and for few primers absence of bands were observed (CEDG056, CEDG068, GLLC108 and VrSSR14) indicating their usefulness in differentiating the genotypes. A larger germplasm need to be screened using more polymorphic SSR markers, ensuring a uniform coverage of the genome, to ascertain the exact genetic diversity and for precise fingerprinting.

Keywords**:** *Germplasm characterization, Green gram, Legumes, Microsatellite makers, Polymorphism and Pulses.*

Mung bean or green gram (family Fabaceae) is a herbaceous annual pulse crop suitable for dry land farming. The subgroups of *Vigna radiata* include one cultivated species (*V. r.* subsp. *radiata*), and two wild species (*V. r.* subsp. *sublobata* and *V. r.* subsp. *glabra*). It is a self-pollinated crop having diploid chromosome number of 2n = 2x = 22 with a genome size of 579 Mbp.

Characterization of genetic diversity in available cultivars is very crucial and forms the basis for creation of new variability. Morphological markers are routinely used for estimating the genetic diversity, but they are highly influenced by the environment. Molecular markers are reliable for studying the relationships and for DNA fingerprinting the cultivars. In addition, they are extensively used for phylogenetic analysis, varietal purity assessment, genome mapping, genetic linkage map construction and marker-assisted selection in crop plants (Muthamilarasan and Prasad, 2015).

Microsatellites or simple sequence repeats (SSRs) are clusters of short tandem repeated nucleotide bases distributed throughout the genome. SSR markers function based on the variability in number of such repeats in the microsatellite regions associated with each allele and are valuable tools for genetic mapping, genotyping and marker-assisted selection in breeding due to their co-dominant nature, high allelic variation and even distribution throughout the genome (Gupta and Varshney, 2000 and Hernandez *et al*., 2002). Hence, this marker system has been chosen in the present investigation to characterize advanced breeding lines of mung bean.

MATERIAL AND METHODS

Thirty advanced breeding lines of mung bean developed and maintained at Regional Agricultural Research Station, Lam (Guntur district, Andhra Pradesh state, India) (Table 1) were analyzed using

nine SSR markers. Details on primer sequences and annealing temperature are presented in Table 2. The genomic DNA of the genotypes was isolated using the modified Cetyl Trimethyl Ammonium Bromide (CTAB) method (Porebski *et al.*, 1997). DNA was assessed for its purity and intactness using both agarose gel and NanoDrop spectrophotometer. Amplification reaction mixture was prepared in 0.2 mL PCR tubes and the total volume of each reaction mixture was 10 µL (Table 3). Thermal cycles followed are presented in Table 4. The PCR products were analyzed by electrophoresis using 3% agarose gel using AAHoefer Electrophoresis unit.

Data analysis was carried out only for those primers that gave scorable patterns. The presence of an amplified product was designated as "1"and absence was marked as "0". The total numbers of bands, polymorphic bands, average number of bands per primer and polymorphism percentage were calculated. SPSS was used to perform cluster analysis of the complete data. Similarity between accessions was estimated by using Jaccard's similarity coefficient and calculated as

$$J=A/(N-D)$$

Where,

A – The number of positive matches (*i.e.*, the presence of a band in both the samples)

D – The number of negative matches (*i.e.*, absence of bands in both the samples)

N – The total sample size (*i.e.*, the number of matches and unmatched)

Similarity estimates were analyzed by unweighted pair group method with arithmetic averages (UPGMA) and the resulting clusters were prsented as a dendrogram. The genetic diversity of each SSR locus was measured in terms of the number of alleles amplified, allele frequencies, observed heterozygosity (Ho) and expected heterozygosity (He), using the "Population Genetics" (Pop Gene) software version of 1.3137. Polymorphic information content (PIC) values were calculated for each primer as per the formula.

$$PIC=1-Ó(Pij)^2$$

where, Pij-the frequency of the i^{th} pattern revealed by the j^{th} primer summed across all patterns revealed by the primers.

RESULTS AND DISCUSSION

In the present investigation, thirty advanced breeding lines of mung bean were characterized using nine SSR primers, generating detectable, distinct banding pattern. Among the fifty primers screened, forty-four produced markers. Kaur *et al.* (2018) also reported the selected amplification of SSR primers in mung bean. The primers, CEDG 008, CEDG 015, CEDG 056, CEDG 068, CEDG-092, GLLC 108, PBALC 13, PBALC 217 and VrssR-14, produced polymorphic bands among the genotypes (Table 5) (Figs. 1 and 2). A total of 236 bands were produced with nine primer sets with sizes ranging from 160 to 200 bp.

In the present study, PIC values varied from 0.06 to 0.69. PIC offers a more accurate assessment of diversity than does the number of alleles because PIC takes into account the relative frequencies of each allele. The PIC values are considered as informative when the values are more than 0.50. PIC values of less than 0.25 indicates low level of diversity while, 0.25 to 0.50 reveals moderately information of the primers. The primers, CEDG-056, CEDG-068, CEDG-092, PBALC-013 and PBALC-217, had moderate discriminatory power to distinguish the genotypes. The Primers, CEDG-008, GLLC-108 and Vr SSR-014, showed high PIC values and considered as the highly informative markers. These high PIC markers can be used for diversity studies, gene mapping and to know germplasm evolution. Similar results were also reported by Kushwaha *et al*. (2013), Andeden *et al*. (2013), Karthikeyan *et al*. (2012) and Suvan *et al.* (2020) for PIC values in the range of medium to high, while Gopal Krishna *et al*. (2010) and Kumar and Bhat (2017) reported low PIC values.

Jaccard's similarity coefficients were calculated to establish the genetic relationships among the mung bean advanced breeding lines (Table 6). The genetic similarity values range was 0.05 to 0.33. The highest similarity coefficient value (0.33) was observed between the genotypes, LGG-705 and LGG- 688, revealing that these genotypes are highly similar in their genetic makeup compared to others. The Jaccard's similarity coefficient values were used to construct the UPGMA (Unweighted Pair Group Method on Arithmetic Average) dendrogram (Fig. 3) which grouped the thirty genotypes into two clusters. Cluster I had three genotypes (LGG 696, LGG 697

and LGG 698) and Cluster II had the rest accommodated in two subclusters.

DNA fingerprinting of mung bean genotypes

DNA Fingerprinting profiles were simulated by polymorphic bands using Word Excel Sheet with the principle of presence (filled with black colour) and absence (not filled) of similar size of reproducible DNA bands (Fig. 4). Each primer produced a reproducible band but there were few exceptions where two bands for a primer were observed (CEDG056 and CEDG092) and for few primers absence of bands were observed (CEDG056, CEDG068, GLLC108 and VrSSR14) indicating their usefulness in differentiating the genotypes. Most of the genotypes were noted with nine specific bands *i.e*., one band for each primer while some had extra band *i.e.*, 10 bands (LGG 691, LGG 696, LGG 699 and LGG 700) and some were observed with only eight bands (LGG 687, LGG 688, LGG 694, LGG 698, LGG 702, LGG 703, LGG 704, LGG 708, LGG 709, LGG 710, LGG 711 and LGG 712).

DNA fingerprinting profiles were similar for the genotypes, LGG 684 and LGG 685; LGG 690 and LGG 693; LGG 709, LGG 710, LGG 711 and LGG 712, indicating they are similar to each other but they can be differentiated by employing more number of markers for the differentiation of these genotypes. DNA fingerprinting profile of the genotype, LGG 689 was similar to the genotypes LGG 684 and LGG 685 except for the marker at 160 bp for the primer PBALC-217 whereas LGG 684 and LGG 685 genotypes had marker at 180 bp, indicating the importance of this amplicon in differentiating these genotypes.

The genotype LGG 694 differed from LGG 690 and LGG 693 for the primer CEDG-056 where the marker was absent at 180 and 200 bp while 180 bp marker was observed in LGG 690 and LGG 693. The genotype LGG 695 was similar to LGG 690 and LGG 693 except for the primer PBALC-13, which generated 180 bp marker instead of 160 bp in LGG 695.

The genotypes, LGG 703, LGG 704, LGG 705, LGG 706, LGG 707, LGG 708, LGG 709, LGG 710, LGG 711, LGG 712 and LGG 460 showed very high levels of similarity in DNA profiling. The genotype, LGG 705, was similar to LGG 460 except for the primer, CEDG-068, which generated marker at 200 bp which was absent in LGG 705 but marker was generated at 180 bp. LGG 460 was similar to LGG 709, LGG 710, LGG 711 and LGG 712 except for the marker at 180 bp for the primer GLLC-108, which was present in LGG 460 and absent in others. LGG 708 was similar to LGG 709 and LGG 710 except for the primer CEDG- 015, which generated marker at 160 bp and against the marker at 180 bp in CEDG-056.

LGG 691 was similar to LGG 690 and LGG 693 except for the primer CEDG-092, which generated an extra marker at 180 bp. LGG 692 was similar to LGG 690 and LGG 693 except for the primer CEDG-092, which generated the marker at 180 bp against 160 bp. LGG 687 and LGG 688 were similar and the markers were absent for the primer CEDG-056. The genotype, LGG 689, was similar in DNA profiling to LGG 684 and LGG 685 except for the primer PBALC-217, which generated the marker at 160 bp against 180 bp. LGG 686 was similar to LGG 690 and LGG 693 except for the primer, PBALC-217, which generated 160 bp marker against 180 bp. The genotypes, LGG 696, LGG 697, LGG 699, LGG 700 and LGG 706, had markers at 200 and 180 bp for the primer CEDG-056 while these were absent in LGG 694.

The genotype, LGG 698, had the unique marker pattern for the primers, CEDG-008 (180bp) and CEDG-015 (180bp), CEDG-056 (180bp), CEDG-092 (180bp), GLLC-108 (180bp), PBALC-13 (180bp), PBALC-217 (180bp) and Vr SSR-014 (200bp) while, markers were absent for the primer CEDG- 068. This clearly indicated the importance of the DNA fingerprinting in genotype identification and protection.

The primer, Vr SSR-014, showed either 200bp or 160bp makers in the genotypes, except for the genotype, LGG 702, where these makers were absent in this genotype. The markers were absent for the primer GLLC 108 at 200bp and 180bp in the genotypes, LGG 706, LGG 707, LGG 708, LGG 710, LGG 711 and LGG 712.

The genotype, LGG 708, had the unique profile as two primers CEDG-056 at 200bp and 160bp and GLLC-108 at 200bp and 180bp, were not amplified indicating the use of these markers for this genotype identification from others.

The genotypes, LGG 684, LGG 685, LGG 686, LGG 687, LGG 688, LGG 689, LGG 690 and

LGG 691, were developed from LGG 460 as one of the parent. These genotypes showed variation in DNA profiling compared to LGG 460. These genotypes along with the parent (LGG 460) showed similar marker pattern for the primers, CEDG-015 (180bp), CEDG-068 (200bp), PBALC-13 (160bp) while, VrSSR-014 (200bp) and GLLC-108 (200bp), were absent in the parent LGG 460 and present in these genotypes.

Some of the primers like CEDG-008 (180bp) was amplified in LGG 687, LGG 688, LGG 690 and LGG 691 while, it was absent in LGG 460; CEDG-056 (180bp) was absent in LGG 687 and LGG 688; CEDG-092 (160bp) was absent in LGG 687 and LGG 688; PBALC-217 (180bp) was absent in LGG 686, LGG 687 and LGG 688.

Thus, these primers can be exploited in different combinations for the DNA profile generation to facilitate genotype identification and protection. Further, more primers have to be tested for their capability to generate more precise fingerprints.

Table 1. List of mung bean genotypes used in the study

S. No.	Name of genotype	S. No.	Name of genotype	S. No.	Name of genotype
1	LGG 684	11	LGG 694	21	LGG 704
2	LGG 685	12	LGG 695	22	LGG 705
3	LGG 686	13	LGG 696	23	LGG 706
4	LGG 687	14	LGG 697	24	LGG 707
5	LGG 688	15	LGG 698	25	LGG 708
6	LGG 689	16	LGG 699	26	LGG 709
7	LGG 690	17	LGG 700	27	LGG 710
8	LGG 691	18	LGG 701	28	LGG 711
9	LGG 692	19	LGG 702	29	LGG 712
10	LGG 693	20	LGG 703	30	LGG 460

Table 2. Details on the SSR primers used for the molecular characterization in mung bean advanced breeding lines.

Sl. No.	Primer Name	Annealing Temp. (°C)	Primer Sequence (5'-3')	Primer length (bp)
1.	CEDGAG001	56.1	F: CTCATCAGGGACATCCTCCC R: GATCGTGATCGATCCAACGGTC	20 22
2.	CEDGAG002	56.4	F:GCAGCAACGCACAGTTTCATGG R:GCAAAACTTTTCACCGGTACGACC	22 24
3.	CEDG006	51.3	F: AATTGCTCTCGAACCAGCTC R: GGTGTACAAGTGTGTGCAAG	20 20
4.	CEDG008	52.3	F: AGGCGAGGTTTCGTTTCAAG R: GCCCATATTTTTACGCCCAC	20 20
5.	CEDG015	53.5	F: CCCGATGAACGCTAATGCTG R: CGCCAAAGGAAACGCAGAAC	20 20
6.	CEDG037	52.3	F: GAAGAAGAACCCTACCACAG R: CACCAAAAACGTTCCCTCAG	20 20
7.	CEDG056	57.7	F: TTCCATCTATAGGGGAAGGGAG R: GCTATGATGGAAGAGGGCATGG	22 22

7.	CEDG056	57.7	F: TTCCATCTATAGGGGAAGGGAG R: GCTATGATGGAAGAGGGCATGG	22 22
8.	CEDG066	53.6	F: AGTAAAACAAGAACCCTCCCAAG R: GTATTAAAATTTGGGGTGGTGG	23 22
9.	CEDG068	57.7	F: TCTCCATAGGAACCCCTGAAAG R: TGGGATCAGTGAATTCGCCAG	22 21
10.	CEDG071	52.8	F:GGTCCATTGAGACGGATCGAG R: TCCCACCTCAGCGGAATCC	21 19
11.	CEDG091	58.4	F: CTGGTGGAACAAAGCAAAAGAGT R: TGCGTCTTGGTGCAAAGAAGAAA	23 23
12.	CEDG092	59.0	F: TCTTTTGGTTGTAGCAGGATGAAC R:TACAAGTGATATGCAACGGTTAGG	24 24
13.	CEDG127	56.5	F: GGTTAGCATCTGAGCTTCTTCGTC R: CTCCTCACTTGGTCTGAAACTC	24 22
14.	PBALC6	53.5	F: ATGATCCGAGTTTCCTGCA R: TACACCACCAACTTCCACCA	19 20
15.	PBALC13	50.8	F: GCAGCAGCATGAGAAAATG R: ATTACTCGACGCCCCCTAGT	20 20
16.	PBALC32	52.5	F: CTGGAGGGAAAAGATGACGA R: TTTCCCCAACTTTCCTAAGC	20 20
17.	PBALC92	52.4	F: TCAAAAGGTGGTGGAAGAGG R: AAACATCAAGTGATTAGCAATG	20 22
18.	PBALC203	51.5	F: CATAGTCAACACTTGGTCGTT R: GTCCACAATGAAACTCATCAC	21 21
19.	PBALC206	58.6	F: GATCCTGTTTTATCCCATTGT R: ACAATCACTTAGCCAAAATCA	21 21
20.	PBALC207	50.5	F: ATGGAACACAAACCAATACAC R: TGTGGTGTCCTTTGTAGAAGT	21 21
21.	PBALC217	51.6	F: TTACCAAGAAATTGAATACAGC R: AGTTTGAAAGGATCTCCAAAG	22 21
22.	PBALC221	53.9	F: AAGCGTCGTAGGTGTAAAAG R: GACTACTGTGATGATGGATGC	20 21
23.	PBALC265	49.5	F: AACATAAAGGAGAGGGTCATC R: CATCTTGTCAACAATTCCTTC	22 21
24.	PBALC353	53.8	F: CCATAACAGACAAAACCCTACT R: ATTCTCAAAGCCCATTTAGTT	22 21
25.	PBALC364	50.5	F: GACTGCTTCTATGGTTGTTTG R: GACAATGGAAGTATCCAACAC	21 21
26.	GLLC106	51.6	F: ACGACAATCCTCCACCTGAC R: AACAAGGAAGGGGAGAGGAG	20 20
27.	GLLC108	51.5	F: CGACAATCCTCCACCTGAC R: ACAAGGAAGGGGAGAGGAAG	19 20
28.	GLLC563	55.7	F: ATGGGCTCATTGAACAAAAG R: CCCCCTCTAAGAGATTTTCCTC	20 22
29.	GLLC592	48.5	F: GCGACATGGATCCTTACCC R:TCATTCAAACAAAAACAAAACAAAA	19 25
30.	BM139	54.2	F: TTAGCAATACCGCCATGAGAG R: ACTGTAGCTCAAACAGGGCAC	21 21
31.	BM154	55.6	F: TCTTGCGACCGAGCTTCTCC R: CTGAATCTGAGGAACGATGACCAG	20 24
32.	BM170	54.8	F: AGCCAGGTGCAAGACCTTAG R: AGATAGGGAGCTGGTGGTAGC	20 21

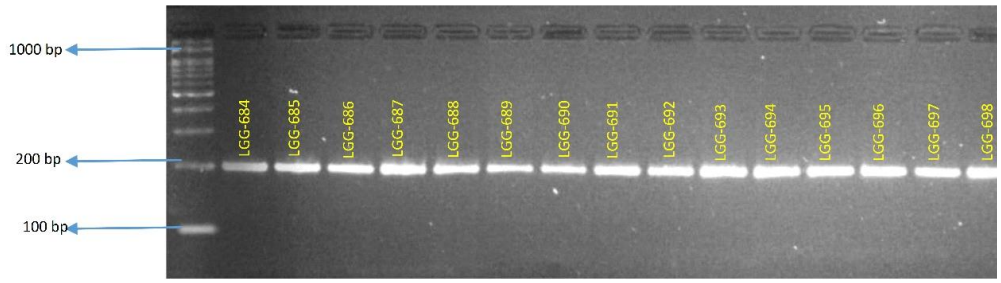
33.	BM211	52.7	F: ATACCCACATGCACAAGTTTGG R: CCACCATGTGCTCATGAAGAT	22 21
34.	SSR 3171	52.8	F: GTGGGTGTAATTATTGCTAC R: GTATCAAACTTATGGTGAAATC	20 22
35.	PLC 62	50.5	F: AAGCCAACCATTTTTGCATC R: AGTAATCCTTTGGTGCTGCG	20 20
36.	BMd9	47.6	F: TATGACACCACTGGCCATACA R: CACTGCGACATGAGAGAAAGA	21 21
37.	BMd16	53.4	F:ATGACACCACTGGCCATACA R: GCACTGCGACATGAGAGAAA	20 20
38.	BMd 33	54.4	F:TACGCTGTGATGCATGGTTT R: CCTGAAAGTGCAGAGTGGTG	20 20
39.	BMd 27	52.5	F:GGACCCACCATCACCATAAC R: TGGTGGAGGTGGAGATTTGT	20 20
40.	VrssR 01	59.6	F: ACCTCTCTTCTCGACCCCA R: GGGTTGCATGGTAAGACTGC	19 20
41.	VrssR 04	58.6	F: CTGATTCAGCCTCAGGTTCC R: CACCGCTAAGATGCTCACAA	20 20
42.	VrssR 09	56.6	F: TCCATTTTAGCCAATGAGGC R: GTGTGAATGAGCAGAAGCCA	20 20
43.	VrssR 14	57.6	F: AGCGTCGTAGGGAGAAAATG R: GCTAGAGGGATGCTTCACCA	20 20
44.	AB 128079	56.7	F: AGGCGAGGTTTCGTTTCAAG R: GCCCATATTTTTACGCCCAC	20 20
45.	AB 128113	55.8	F: TCAGCAATCACTCATGTGGG R: TGGGACAAACCTCATGGTTG	20 20
46.	X 62	56.8	F: TGGGCTACCAACTTTTCCTC R: TGAGCGACATCTTCAACACG	20 20
47.	MB 13	55.8	F: GCAGCAACAACAGCAACA R: GCAGGTTTTGTGGCTCAG	18 18
48.	MB 14	55.8	F: TGGAATTTGGAAGGAAGGA R: GATGCAGGTGTTTGGGAG	19 18
49.	PLC 17	61.8	F: AAGCTGAAGGAAATCAAAGTGG R: TCAACACACTCCATGTTTAGAGC	22 23
50.	PLC 5	58.3	F: CATTGCAGCTTATTCTCACAGC R: TGACCCATCCTCATCCTTAAT	22 21

Table 3. Composition of PCR reaction mixture

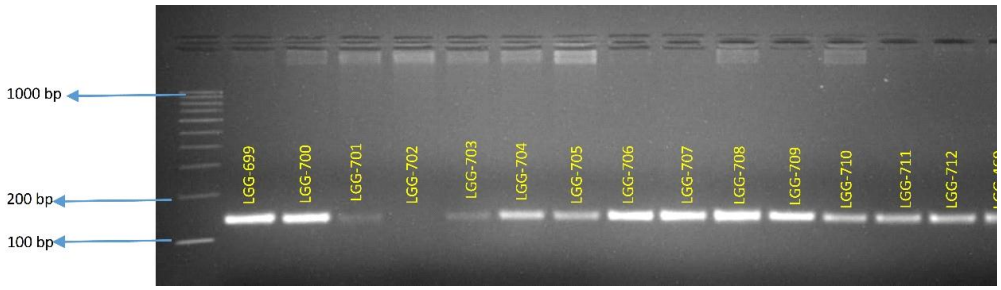
S. No.	Components	Quantity (µl)
1	Template DNA (50 ηg/µL)	2.0
2	10X buffer with 15 mM $MgCl_2$	1.0
3	‘dNTPs’ mix (2.5 mM each)	0.5
4	Primer (10 pM/µl)	0.5
5	Primer (10 pM/µl)	0.5
6	Taq polymerase (3 U/µl)	0.1
7	Sterile distilled water	5.4

Table 4. Thermal cycling conditions followed for SSR marker system

S. No.	Steps	Temperature (˚C)	Time	Cycle number
1	Initial denaturation	94.0	5	1
2	Denaturation	94.0	1	
3	Annealing	50.0-64.0	45	
4	Extension	72.0	1	35
5	Final extension	72.0	10	1
6	Final hold	4.0	infinite	

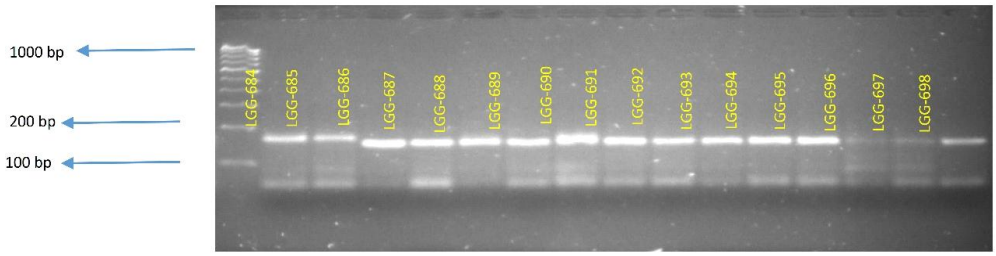


Vr SSR-014 1-1

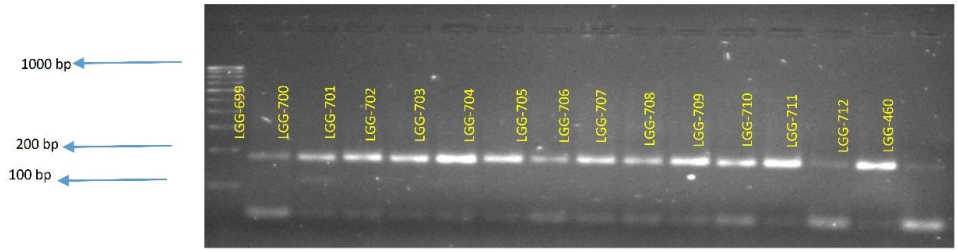


Vr SSR-014 1-2

Fig. 1. Profile of thirty mung bean genotypes with SSR marker Vr SSR-014



PBALC- 217 1-1



PBALC- 217 1-2

Fig. 2. Profile of thirty genotypes of mung bean with SSR marker PBALC- 217

Table 5. Marker pattern obtained from nine SSR primers

S. No.	Primer Name	Total no. of bands	No. of alleles	Fragment size	PIC
1.	CEDG 008	30	5	160-180	0.50
2.	CEDG 015	29	1	160-180	0.06
3.	CEDG 056	26	4	180-200	0.26
4.	CEDG 68	30	8	180-200	0.39
5.	CEDG 092	30	6	160-180	0.34
6.	GLLC 108	29	6	180-200	0.69
7.	PBALC 13	28	8	160-180	0.27
8.	PBALC 217	26	4	160-180	0.23
9.	Vr SSR 014	28	2	160-200	0.53
Total		236	44		3.27
Average			4.8		0.36

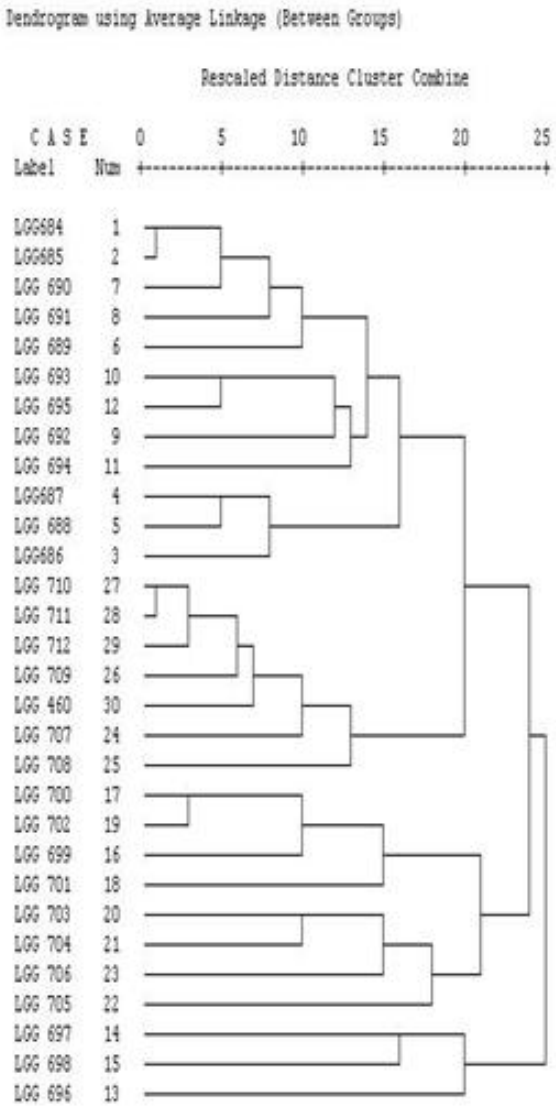


Fig. 3. UPGMA based dendrogram of thirty mung bean genotypes

Table 6. Similarity coefficient values among thirty genotypes of mung bean

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29
2	0.058																												
3	0.144	0.124																											
4	0.165	0.144	0.119																										
5	0.195	0.175	0.15	0.074																									
6	0.097	0.097	0.183	0.204	0.235																								
7	0.09	0.127	0.148	0.179	0.129	0.069																							
8	0.055	0.066	0.152	0.173	0.203	0.105	0.098																						
9	0.146	0.125	0.1	0.077	0.107	0.185	0.129	0.154																					
10	0.127	0.107	0.14	0.161	0.192	0.166	0.11	0.135	0.142																				
11	0.156	0.135	0.169	0.19	0.22	0.195	0.139	0.163	0.171	0.105																			
12	0.102	0.081	0.115	0.136	0.167	0.141	0.085	0.11	0.117	0.037	0.08																		
13	0.118	0.098	0.15	0.171	0.202	0.158	0.102	0.126	0.152	0.133	0.162	0.108																	
14	0.123	0.103	0.177	0.198	0.228	0.163	0.123	0.131	0.179	0.16	0.189	0.135	0.151																
15	0.139	0.119	0.172	0.193	0.223	0.179	0.123	0.147	0.173	0.155	0.183	0.13	0.129	0.173															
16	0.147	0.126	0.2	0.221	0.252	0.186	0.146	0.155	0.202	0.183	0.212	0.158	0.175	0.122	0.196														
17	0.16	0.14	0.214	0.235	0.265	0.199	0.159	0.168	0.216	0.197	0.225	0.172	0.188	0.135	0.209	0.084													
18	0.134	0.114	0.188	0.208	0.239	0.173	0.133	0.142	0.189	0.171	0.199	0.145	0.162	0.109	0.183	0.109	0.122												
19	0.158	0.137	0.211	0.232	0.262	0.197	0.157	0.165	0.213	0.194	0.223	0.169	0.185	0.132	0.207	0.082	0.055	0.119											
20	0.186	0.165	0.239	0.26	0.291	0.225	0.185	0.194	0.241	0.222	0.251	0.197	0.214	0.188	0.235	0.211	0.225	0.199	0.222										
21	0.19	0.169	0.243	0.264	0.295	0.229	0.189	0.198	0.245	0.226	0.255	0.201	0.218	0.192	0.239	0.215	0.229	0.203	0.226	0.111									
22	0.232	0.212	0.286	0.307	0.337	0.271	0.231	0.24	0.287	0.269	0.297	0.244	0.26	0.234	0.281	0.258	0.271	0.245	0.268	0.162	0.166								
23	0.2	0.18	0.254	0.275	0.305	0.24	0.2	0.208	0.256	0.237	0.266	0.212	0.228	0.203	0.249	0.226	0.239	0.213	0.237	0.13	0.134	0.148							
24	0.201	0.181	0.255	0.276	0.306	0.24	0.2	0.209	0.257	0.238	0.266	0.213	0.229	0.204	0.25	0.227	0.24	0.214	0.238	0.187	0.191	0.233	0.201						
25	0.174	0.154	0.228	0.249	0.279	0.214	0.174	0.182	0.23	0.211	0.24	0.186	0.202	0.177	0.224	0.2	0.213	0.187	0.211	0.2	0.204	0.247	0.215	0.216					
26	0.172	0.152	0.226	0.247	0.277	0.211	0.171	0.18	0.227	0.209	0.237	0.184	0.2	0.174	0.221	0.198	0.211	0.185	0.208	0.158	0.162	0.204	0.172	0.111	0.187				
27	0.141	0.121	0.195	0.216	0.246	0.181	0.141	0.149	0.197	0.178	0.207	0.153	0.169	0.144	0.19	0.167	0.18	0.154	0.178	0.127	0.131	0.173	0.142	0.088	0.156	0.059			
28	0.134	0.114	0.188	0.209	0.239	0.173	0.133	0.142	0.19	0.171	0.199	0.146	0.162	0.136	0.183	0.16	0.173	0.147	0.17	0.12	0.124	0.166	0.134	0.119	0.149	0.09	0.059		
29	0.163	0.143	0.217	0.238	0.268	0.202	0.162	0.171	0.219	0.2	0.228	0.175	0.191	0.166	0.212	0.189	0.202	0.176	0.2	0.149	0.153	0.195	0.163	0.148	0.178	0.119	0.089	0.037	
30	0.156	0.135	0.209	0.23	0.26	0.195	0.155	0.163	0.211	0.192	0.221	0.167	0.183	0.158	0.205	0.181	0.195	0.168	0.192	0.141	0.145	0.187	0.156	0.141	0.17	0.112	0.081	0.059	0.088

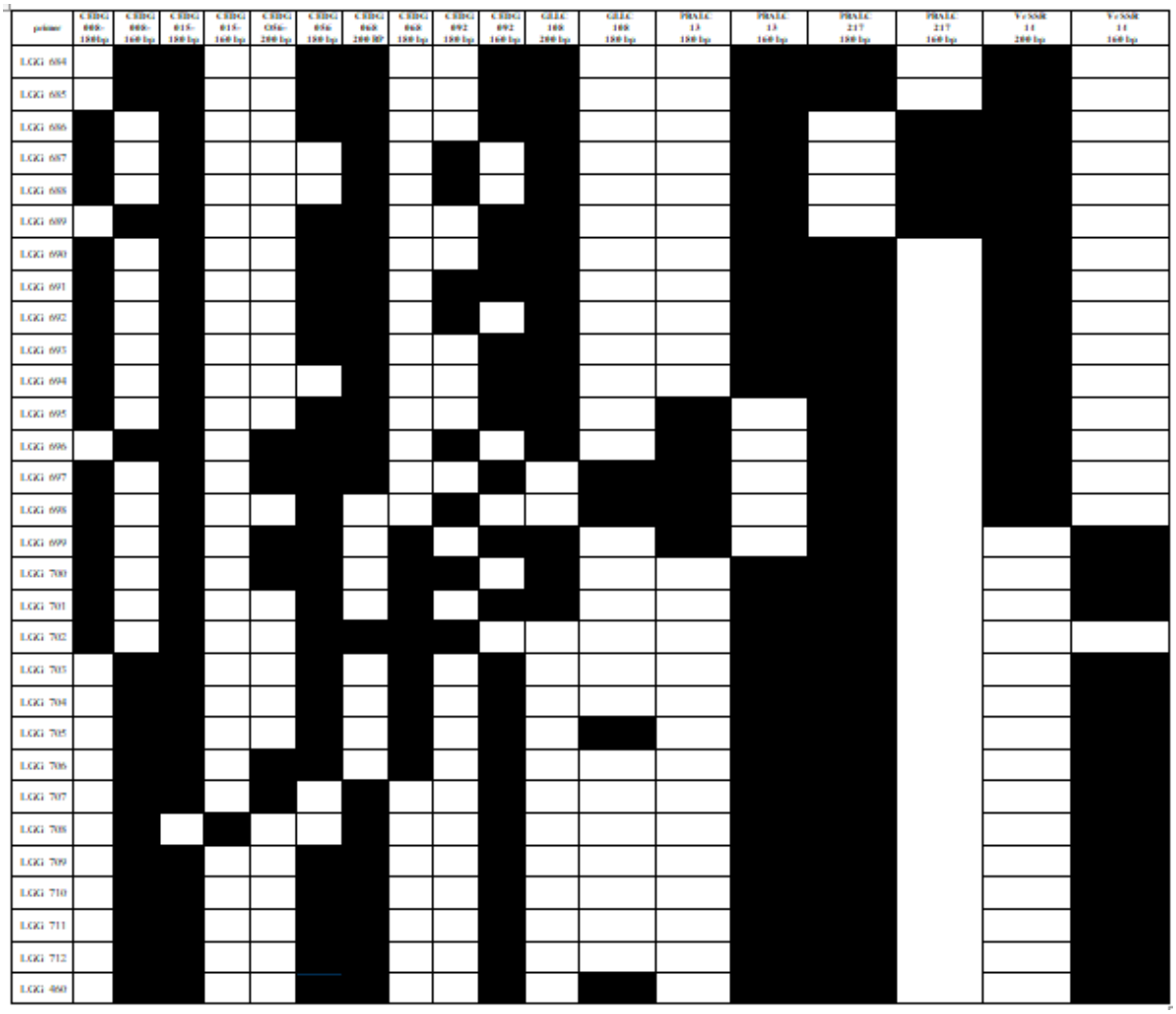


Fig. 4. DNA Fingerprint profile of mung bean genotypes using SSR markers

LITERATURE CITED

Andeden E E, Derya M, Baloch F S, Kilian B and Ozkan H 2013. Development of SSR markers in lentil. In: Proceedings of Plant and Animal Genome Conference XXI P0351, San Diego, CA.

Gopal Krishna T, Bansal R and Vaidya U G 2010. Assessment of genetic diversity at molecular level in mungbean (*Vigna radiata* (L.) Wilczek). *Journal of Food Legumes*. 26(3): 19-24.

Gupta P and Varshney R 2000. The development and use of microsatellite markers for genetic analysis and plant breeding with emphasis on bread wheat. *Euphytica*.113(3): 163–185.

Hernandez P, Lauri D A, Martin A and Snape J W 2002. Utility of barley and wheat simple sequence repeat (SSR) markers for genetic analysis of *Hordeum chilense* and titrodeum. *Theoretical and Applied Genetics*. 104: 735–739.

Karthikeyan A, Sudha M, Nagrajan P, Pandiyan M, Raveendran M, Senthil N and Angappan K 2012. Using SSR marker to identify the MYMV resistance gene in mungbean [*Vigna radiata* (L.) Wilczek]. *Romanian Journal of Biology – Plant Biology*. 57(2): 79–156.

Kaur G R, Joshi A and Jain D 2018. SSR- marker assisted evaluation of genetic diversity in mungbean [*Vigna radiata* (L.) Wilczek] genotype. *Brazilian Archives of Biology and Technology*. 61: 1-8.

Kumar M and Bhat S 2017. Molecular diversity studies in greengram genotypes differing for powdery mildew resistance. *Journal of Farm Science*. 30(4): 503 -509.

Kushwaha U K S, Ghimire S K, Yadav N K and Ojha B R 2013. Genetic relatedness of lentil (*Lens Culinaris* L.) germplasm by using SSR markers. *International Journal of Applied Sciences and Biotechnology*. 1: 16–26.

Muthamilarasan M and Prasad M 2015. Advances in *Setaria* genomics for genetic improvement of cereals and bioenergy grasses. *Theoretical and Applied Genetics*. 128: 1–14.

Porebski S, Bailey LG and Baum BR Modification of a CTAB DNA extraction protocol for plants containing high polysaccharide and polyphenol components. *Plant Mol Biol Rep* 15, 8–15 (1997).

Somta P and Srinivas P 2007. Genome research in mungbean [*Vigna radiata* (L.) Wilczek] and blackgram [*V. mungo* (L.) Hepper]. *Science Asia*. 33(1): 69-74.

Suvan P, Patel K V and Kumar S 2020. Evaluation of SSR-based genetic diversity, protein and mineral content in blackgram genotypes. *Journal of King Saud University – Science*. 32: 1029–1033.

Received on 27.05.2022 and Accepted on 30.11.2022