The Pharma Innovation Journal 2021; 10(6): 234-238

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2021; 10(6): 234-238

www.thepharmajournal.com
Received: 09-03-2021
Accepted: 21-04-2021

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Genetic diversity studies in green gram using SSR markers

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Abstract
Mung bean (*Vigna radiata* (L.) Wilczek) is an important grain legume in Asia. Simple Sequence repeats (SSR) are the variable in short tandem repeat of DNA bases, giving codominant markers, which can distinguish between homozygotes and heterozygotes. The study was conducted during the year 2016-2018 using randomized block design of experiment with three replications at Plant Breeding Farm of Annamalai University. Twenty greengram genotypes along with 10 SSR markers were utilized. Only one marker was diallelic producing two distinct alleles among the genotypes while two markers were mono-allelic showing a dominant allele pattern. The genetic distance estimated using DICE dissimilarity co-efficient indicated highest divergence of 0.8 between GG15 and GG17. The dendogram showed five apparent clusters based on marker allele distribution. This SSR marker YMV1 can be used to screen the large germplasm for YMV resistance.

Keywords: SSR marker, genetic diversity, DICE, alleles

Introduction
Mung bean (*Vigna radiata* (L.) Wilczek) is an important grain legume in Asia. It is one of the 13 food legumes grown in India and third most important pulse crop of India after chickpea and pigeon pea. In India, the name green gram is more commonly used than mung bean (Chatterjee and Randhawa, 1952) [1]. India is the largest producer and consumer of pulses in the world accounting for 33 per cent of world's area and 22 per cent of world's production of pulses.

In any crop improvement programme, genetic diversity is an essential prerequisite for hybridization. Pulses in general being location specific, there is a need to test/evaluate the diverse germplasm in specific location or target environment. Hybridization among the diverse parents is expected to exhibit more heterotic expression in F1 generation. In addition, it generates broad spectrum of variability in segregating generations. The molecular markers are proven to be a powerful tool that can yield significant information that enhances the scope of using germplasm in crop improvement programme. Microsatellites are locus specific, highly polymorphic, co-dominant and highly reproducible (Raj *et al.,* 2020; Subramanian *et al.,* 2019) [7, 13]. These features coupled with the ease of detection and transferability between laboratories has made them an excellent marker system for genome analysis. Simple sequence repeats (SSR) are the variable in short tandem repeat of DNA bases, giving codominant markers, which can distinguish between homozygotes and heterozygotes.

Considering the potential of the DNA marker based genetic diversity analysis, the present study is aimed to analyse and assess the nature and extent of genetic diversity in mungbean using SSR markers.

Materials and Methods
The present study was conducted during the year 2016-2018 using randomized block design of experiment with three replications at Plant Breeding Farm of Annamalai University. Twenty greengram genotypes were used for the present study (Table 1). A total of ten SSR (Simple Sequence Repeats) markers were used for polymorphic studies (Table 2).

The genomic DNA was isolated from leaf tissue of 20 days old seedlings following the protocol of Doyle and Doyle (1987) [2]. The crude genomic DNA was run on 0.8% agarose gel stained with ethidium bromide following a standard method (Sambrook *et al.*, 1989) [8] for quantification. DNA templates were amplified using a set of ten SSR primer pairs. Polymorphism studies were done on the test genotypes using PCR analysis.

The band corresponding to the product amplicon were score individually for the presence and absence among the genotypes. The presence of the band was scored as 1, and absence of the band was score 0. The frequency of particular band produced by a particular marker was estimated based its presence in the entire germplasm. This was repeated all the bands produced by a particular marker and polymorphism information content was estimated. Genetic diversity of the twenty genotypes was determined from the polymorphic molecular marker pattern by estimating the genetic distance using DICE dissimilarity co-efficient. Cluster analysis was performed using the neighbor-joining (NJ) method with the DAR win v. 5.0.157 software.

Results and Discussion

The polymorphic information (PIC) value ranged from CEDG 139 (0.493) to a maximum of YMV1 (0.742). The remaining markers are intermediate PIC values (Table 3). Various banding pattern obtained are given in figures 1-3 where ten out of eight markers showed polymorphic bands among the genotypes tested. Only one marker was diallelic producing two distinct alleles among the genotypes while two markers were mono-allelic showing a dominant allele pattern. Similar observation was also made by Phansak *et al*., (2005) [6]. The genetic distance announced using DICE dissimilarity co-efficient indicated lowest dissimilarity (0.1) GG20. The highest divergence of (0.8) was between GG15 and GG17 (Table 4). Similar studies were reported by Kumar *et al*., (2002) [4] and Gwag *et al*., (2006) [3].

The dendogram constructed using the DICE dissimilarity co-efficient between genotypes showed five apparent clusters based on marker allele distribution (Fig.1). The first cluster consisted of five genotypes (GG13, GG7, GG14, GG9 and GG3) and accommodated 25% of the total population based on allelic similarity. The second cluster consisted of three genotypes having a membership density of 15.4%. It consisted of GG18, GG12 and GG17. The third cluster consisted of five genotypes accommodating 25% of total genotypes. It consisted of GG16, GG11, GG15, GG2 and GG1. The fourth cluster had remaining 25% of the population consisting of five genotypes GG10, GG8, GG5, GG6 and GG4. The fifth cluster consisting of two genotypes accommodating 5% GG20 and GG19. Although the neighbour joining procedure was bootstrapped 7000 times none of the clusters or clads showed bootstrap value more than 47%. The highest bootstrap value of 47% was shown between two genotypes GG13 and GG7 followed by their grouping with GG14 (17%). Similar reports were also made by Li *et al*., (2001) [5], Sangiri *et al*. (2007) [9] and Shukla *et al*. (2011) [10].

Table 1: List of genotypes

S. No	Genotypes	Genotypes Name	Geographical Location
1	GG1	K-17-1	Vikaravandi block, Villupuram,
2	GG2	K-17-2	Vikaravandi block, Villupuram,
3	GG3	CO6	TNAU, Coimbatore,
4	GG4	CO7	TNAU, Coimbatore
5	GG5	CO8	TNAU, Coimbatore
6	GG6	VRM-1	Virinjipadi
7	GG7	K-17-3	Vikaravandi block, Villupuram,
8	GG8	Kerala local variety	Kerala, Kollam
9	GG9	NVL-516	Thirunavalur block, Villupuram,
10	GG10	KM-2	Kollinur block, villupuram
11	GG11	K 851	Andra Pradesh
12	GG12	VBN (Gg)2	Cuddalore
13	GG13	BGS-9	Cuddalore
14	GG14	HUM-12	Kollinur block, villupuram
15	GG15	IPM 99-125	Chinnamanur, Theni
16	GG16	VBN(Gg)3	Cuddalore
17	GG17	Chidambaram local variety	Chidambaram
18	GG18	KM-1	Chidambaram
19	GG19	CO4	TNAU, Coimbatore
20	GG20	Paiyur 1	Paiyur Research Station

Table 2: SSR primers used for molecular analysis of Greengram

S. No	Marker name	Primer sequence	Repeat Motif	Product size (bp)	Annealing temperature (°C)
1	CEDG 008	F – AGGCGAGGTTTCGTTTCAAG R – GCCCATATTTTTACGCCCAC	(AG)26	110-140	55
2	CEDG 014	F – GCTTGCATCACCCATGATTC R- AAGTGATACGGTCTGGTTCC	(AT)12(AG)14	176-116	58
3	CEDG 044	F-TCAGCAACCTTGCATTGCAG R-TTTCCCGTCACTCTTCTAGG	(GT)10AT(AG)18	172-210	58
4	CEDG 092	F-TCTTTTGGTTGTAGCAGGATGAAC R- ACAAGTGATATGCAACGGTTAGG	(AG) 17	150-170	55
5	CEDG 139	F- AAACTTCCGATCGAAAGCGCTTG R-GTTTCTCCTCAATCTCAAGCTCCG	(AG) 19	190	58
6	CEDG 198	F-CAAGGAAGATGGAGAGAATC R-CCTTCTAAGAACAGTGACATG	(AG) 30	227-209	50
7	YMV1	F-GAGAGAGAGAGAGAGACAAAG R-GAGAGAGAGAGAGAGACAGGA	(GA) 17	1357	58
8	YR4	F-GGTAAGACGACACTCGCTTTA R-GACGTCCTTGTAACTTTGATCA	-	456	58
9	CYR1	F-GGGTGGTTTGGGTAAGACCAC R-TTCGCGGTGTGTGAAAAGTCT	-	1236	58
10	CEDG 180	F-GGTATGGAGCAAAACAATC R-GTGCGTGAAGTTGTCTTATC	(AG) 11	136-163	55

Table 3: Polymorphic Information Content (PIC) value for the SSR Primers

S. No	Primers	Total number of band	Frequency of each allele	PIC value
1	CEDG008	27	0.268	0.532
2	CEDG044	27	0.268	0.499
3	CEDG092	25	0.193	0.608
4	YMV1	37	0.143	0.742
5	CEDG014	48	0.125	0.733
6	CEDG198	39	0.262	0.499
7	CEDG139	27	0.308	0.493
8	CEDG180	13	0.289	0.556

Table 4: DICE Dissimilarity Co-efficient

GG1	GG2	GG3	GG4	GG5	GG6	GG7	GG8	GG9	GG10	GG11	GG12	GG13	GG14	GG15	GG16	GG17	GG18	GG19	GG20
2	0.12																		
3	0.461538	0.6																	
4	0.214286	0.185185	0.428571																
5	0.304348	0.454545	0.304348	0.28															
6	0.36	0.25	0.44	0.185185	0.363636														
7	0.416667	0.304348	0.416667	0.384615	0.52381	0.391304													
8	0.259259	0.384615	0.407407	0.241379	0.25	0.230769	0.52												
9	0.619048	0.7	0.52381	0.652174	0.666667	0.6	0.578947	0.636364											
10	0.28	0.333333	0.44	0.185185	0.272727	0.25	0.391304	0.153846	0.6										
11	0.5	0.368421	0.8	0.363636	0.647059	0.368421	0.555556	0.52381	0.733333	0.368421									
12	0.285714	0.333333	0.357143	0.266667	0.36	0.333333	0.230769	0.310345	0.478261	0.333333	0.545455								
13	0.285714	0.259259	0.428571	0.266667	0.52	0.333333	0.153846	0.37931	0.565217	0.259259	0.454545	0.266667							
14	0.310345	0.428571	0.310345	0.290323	0.384615	0.357143	0.333333	0.333333	0.583333	0.285714	0.478261	0.290323	0.16129						
15	0.391304	0.272727	0.478261	0.36	0.5	0.363636	0.333333	0.416667	0.777778	0.363636	0.529412	0.36	0.36	0.461538					
16	0.363636	0.333333	0.636364	0.333333	0.473684	0.428571	0.5	0.391304	0.882353	0.428571	0.375	0.416667	0.5	0.44	0.368421				
17	0.333333	0.478261	0.416667	0.461538	0.428571	0.478261	0.545455	0.36	0.578947	0.478261	0.555556	0.230769	0.461538	0.333333	0.428571	0.4			
18	0.185185	0.307692	0.407407	0.310345	0.416667	0.461538	0.36	0.285714	0.636364	0.307692	0.428571	0.241379	0.241379	0.2	0.333333	0.304348	0.2		
19	0.259259	0.307692	0.407407	0.37931	0.5	0.384615	0.44	0.428571	0.545455	0.384615	0.52381	0.310345	0.310345	0.333333	0.333333	0.565217	0.28	0.285714	
20	0.2	0.25	0.36	0.259259	0.363636	0.25	0.391304	0.307692	0.5	0.25	0.368421	0.259259	0.259259	0.285714	0.363636	0.52381	0.304348	0.230769	0.153846

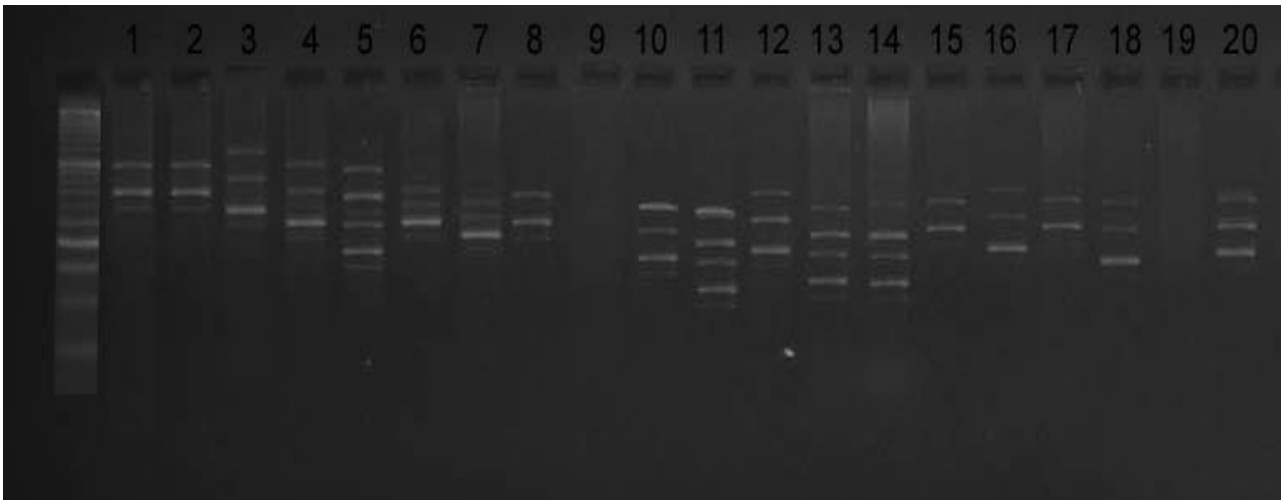

Fig 1: Gel picture of YMV1 primer

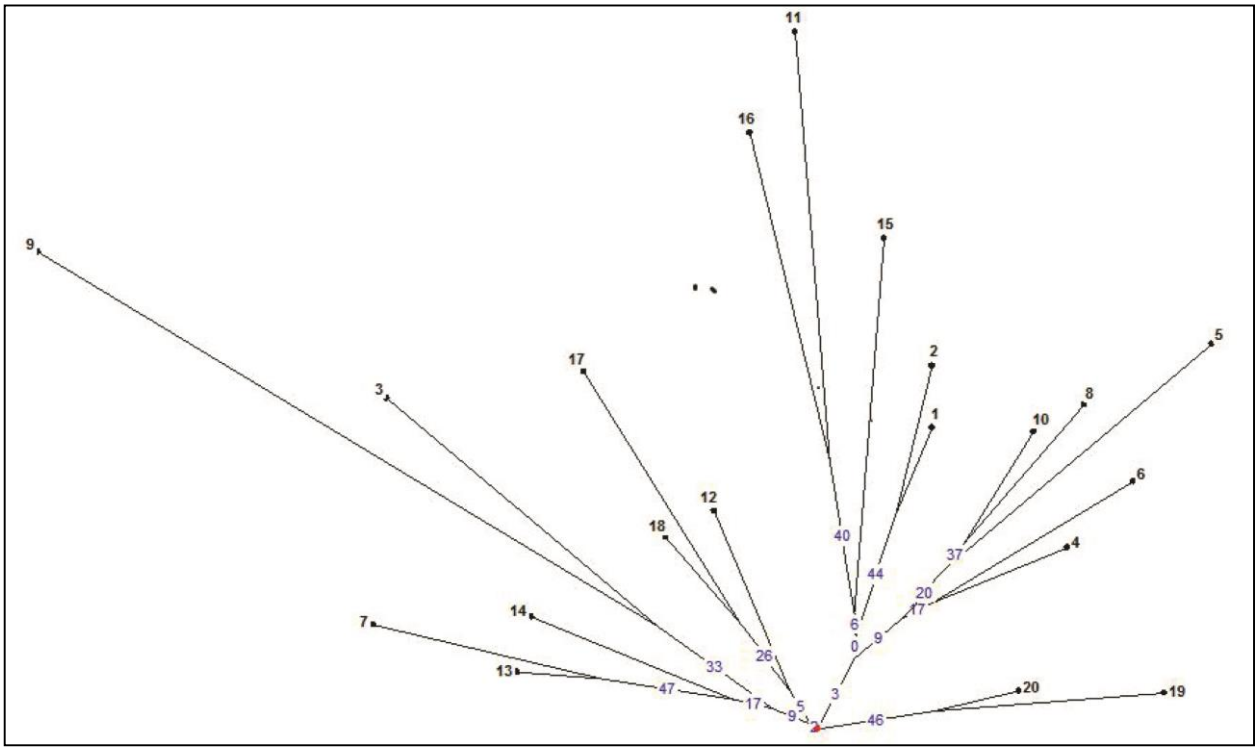

Fig 2: DICE dissimilarity dendogram

Conclusion

The genetic distance estimated using DICE dissimilarity co-efficient indicated highest divergence of 0.8 between GG15 and GG17. The dendogram showed five apparent clusters based on marker allele distribution. This SSR marker YMV1 can be used to screen the large germplasm for YMV resistance.

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