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SSR marker based profiling and diversity analysis of mungbean [*Vigna radiata* (L.) Wilczek] genotypes

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ABSTRACT

The evaluation and characterization of germplasm of mungbean are considered to be essential prerequisites for a rational use of its genetic resources. Accordingly in the present study, genetic diversity in 18 mungbean genotypes was assessed using a panel of 40 microsatellite based primer pairs. Out of 40 primers initially tested, only 24 showed distinct polymorphism and consequently only these primers were utilized for the purpose of genome profiling of the entries. Altogether, 183 allelic variants were detected with an average of 4.95 polymorphic fragment per primer. The number of amplified loci varied from 4 (CEDG 15) to a maximum of 12 (CEDG 92 and CEDG 172) with an average of 4.95 polymorphic fragment per primer. The polymorphic information content (PIC) ranged from 0.440 to 0.928 with an average of 0.822 per SSR primer pair. The lowest polymorphism percent was observed for primer CEDGAG001 (16.67) while the primer CEDG 154 gave the maximum polymorphism percent (63.64). The primer pairs CEDG 008, CEDG 068 and CEDG 154 among the total primer pairs were found to be highly informative. Dice's similarity coefficient ranged from 0.03 to 0.58. Dendrogram based on SSR data grouped the mungbean genotypes into seven clusters. The pattern of clustering was also reiterated by the results of principal component analysis (PCA). The SSR primer based analysis allowed unique and unambiguous genotyping of the entries. The genetic diversity observed in the present study could be useful to know the phylogenetic links among the cultivars and for the selection of suitable parents to be further used in breeding programmes and genetic mapping studies.

Key words: Genetic diversity, Molecular characterization, Mungbean, SSR, *Vigna radiata*.

INTRODUCTION

Mungbean [greengram, *Vigna radiata* (L.) Wilczek] is an Asiatic species of the pan-tropical genus *Vigna*, which is widely cultivated as pulse crop. It belongs to the subgenus *Ceratotropis* in the genus *Vigna* and is a self-pollinating diploid grain legume (2n=22) crop with a genome size of 579 Mb (Arumuganathan and Earle, 1991). Due to its protein rich edible seeds, its ability to fix nitrogen, drought tolerance, and early maturity, it is widely planted in various cropping systems (Tangphatsornruang *et al*., 2009). The seeds and sprouts of mungbean act as therapeutic agents as they possess antioxidants and have antimicrobial, anti-inflammatory, antidiabetic, antihypertensive and anti-cancerous effects (Tang *et al*., 2014). Despite its economic importance, the average yields of mungbean is low due to its susceptibility to various to biotic (mungbean yellow mosaic virus, powdery mildew and *Cercospora* leaf spot) and abiotic stresses (drought, heat and pre-harvest sprouting). Undoubtedly, the development of improved types through conventional breeding has contributed towards the genetic improvement of mungbean but in spite of all efforts made over past couple of decades, the production has not increased to a remarkably greater extent. Hybridization and recombination of available variability in mungbean and other related species have also met with limited success due to low recovery of hybrids and linkage of undesirable traits that limit the introgression of variability from related species.

The evaluation and characterization of germplasm are considered to be essential pre-requisites for a rational use of genetic resources. The estimation of genetic diversity is invaluable in selection of diverse parental combinations to generate segregating progenies with maximum genetic variability (Barrett and Kidwell, 1998) and to facilitate in introgression of desirable traits from diverse or wild germplasm into the cultivars in order to broaden the genetic base (Thompson *et al*., 1998). Earlier, analysis of genetic diversity was mostly carried out using morphological characters and isozyme markers. But, limited availability, low polymorphism and high influence of environmental factors, limited the use of morphological and biochemical markers. In the absence of distinct morphological attributes, genetic characterization of diverse genotypes and documentation of diversity among the genotypes are of utmost significance in genetic improvement of this important legume crop (Sivaprakash *et al*., 2004).

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DNA-based molecular markers have proven to be powerful tools not only for molecular characterization but also in the assessment of genetic diversity and in the elucidation of genetic relationships within and among the species. These techniques are reliable and remain unaffected across different growth stages, seasons, locations and agronomic practices. Among DNA based markers, SSRs or microsatellite markers are particularly attractive for studying genetic structure and the relationships between species (Jegadeesan *et al*., 2010) due to their hypervariability, codominant nature, locus specificity and high reproducibility. Because of several advantages offered by SSR markers, such markers have been widely used for genetic diversity analysis, marker assisted selection, genetic mapping and also in population genetics study in different crops (Zeitkiewicz *et al*., 1994). Microsatellite markers have been used for genetic diversity studies in many legumes including common bean (Khaidizar *et al*., 2012), rice bean (Tian *et al*., 2013), chickpea (Varshney *et al*., 2014), cowpea (Doumbia *et al*., 2014), urdbean (Pyngrope *et al*., 2015), pigeonpea (Njung′e *et al*., 2015), pea (Prakash *et al*., 2016) and soybean (Rani *et al*., 2016).

A carefully chosen set of SSR markers enables an unbiased assay of genetic differences and an unambiguous description of the entries, besides facilitating molecular profiling of genotypes. Therefore, in the present study, SSR markers were utilized for studying the genetic diversity of 18 genotypes of mungbean.

MATERIALS AND METHODS

Plant material: Thirty-five genotypes of mungbean procured from IIPR, Kanpur and TCA, Dholi were initially screened for MYMV disease incidence under natural field conditions (Suman *et al*., 2015-16) and forced inoculation methods (Suman *et al*., 2018). Out of the 35 genotypes, 18 genotypes were selected for their molecular profiling as they showed extreme reactions against MYMV disease incidence for resistant and susceptible responses (Table 1).

Molecular Analysis

Genomic DNA isolation and purification: Seeds of the selected 18 genotypes were germinated in small pots for 10 days and DNA was extracted from young leaves. Genomic DNA extraction was done with a modification of Doyle and Doyle (1987) method. Further purification of the DNA was done by RNase treatment. DNA purity was checked by running the sample on 0.8% agarose. Dilutions were made to maintain the genomic DNA concentrations to 25 ng/µl.

SSR amplification: A panel of forty SSR primer pairs specific to the unique sequences flanking the di-nucleotide, tri-nucleotide and complex repeat motifs derived from azukibean (Han *et al*., 2005) and mungbean were initially tested in the polymerase chain reaction (PCR) for their reproducibility and their ability to generate polymorphic amplified products across the accessions. Out of 40 primers initially tested, only 24 showed distinct polymorphism and consequently only these primers were utilized for the purpose of genome profiling of the selected entries. A breakup of primers based on their locus, chromosome number, sequence, motif and annealing temperature is depicted in Table 2.

Amplification was carried out in 15µl reaction mixture containing 2µl (25ng/µl) of DNA, 2µl (10X) of Taq buffer, 2µl (2mM) of dNTP's, 0.5 µl (25mM) $MgCl_2$, 0.5µl each of forward and reverse primer (20 pmols), 0.3 µl

Table 1: List of mungbean genotypes, their sources and developing center

Sl. no.	Name of the genotype	Source	Developing Center
1	IPM 02-14	IIPR, Kanpur	IIPR, Kanpur
2	PDM 139	IIPR, Kanpur	IIPR, Kanpur
3	Pant Mung 4	IIPR, Kanpur	Pantnagar, Uttarakhand
4	HUM 12	IIPR, Kanpur	BHU, Varanasi
5	HUM 1	IIPR, Kanpur	BHU, Varanasi
6	TMB 37	TCA, Dholi	BARC, Mumbai
7	Pusa 9531	IIPR, Kanpur	IARI, New Delhi
8	Meha	IIPR, Kanpur	IIPR, Kanpur
9	HUM 16	IIPR, Kanpur	BHU, Varanasi
10	IPM-99-1-6	TCA, Dholi	IIPR, Kanpur
11	Pusa 105	IIPR, Kanpur	IARI, New Delhi
12	AKM 8803	IIPR, Kanpur	PDKV, Akola
13	AKM 9910	IIPR, Kanpur	PDKV, Akola
14	Pusa 031	TCA, Dholi	IARI, New Delhi
15	TARM 2	IIPR, Kanpur	BARC, Akola
16	LGG 407	TCA, Dholi	APAU, Lam
17	T 44	IIPR, Kanpur	TNAU, Tamilnadu
18	LGG 450	TCA, Dholi	APAU, Lam

IIPR, Indian Institute of Pulses Research; TCA, Tirhut College of Agriculture; BHU, Banaras Hindu University; BARC, Bhabha Atomic Research Centre; IARI, Indian Agricultural Research Institute; PDKV, Panjabrao Deshmukh Krishi Vidyapeeth; APAU, Andhra Pradesh Agricultural University; TNAU, Tamil Nadu Agricultural University.

Table 2: List of microsatellite based simple sequence repeat specific primer pairs used for molecular profiling in the present study.

Sl. No.	Locus	Chr. No.	Forward and reverse sequence	Motif	Ta (ºC)
1	CEDGAG001	9	CTCATCAGGGACATCCTCCC GATCGTGATCGATCCAACGGTC	(GAG)4	56
2	CEDAAG002	2	GCAGCAACGCACAGTTTCATGG GCAAAACTTTTCACCGGTACGACC	(AAG)16	57
3	CEDG006	2	AATTGCTCTCGAACCAGCTC GGTGTACAAGTGTGTGCAAG	(AG)10 AA(AG)18	55
4	CEDG008	5	AGGCGAGGTTTCGTTTCAAG GCCCATATTTTTACGCCCAC	(AG)26	53
5	CEDG015	6	CCCGATGAACGCTAATGCTG CGCCAAAGGAAACGCAGAAC	(AG)27	61
6	CEDG037	6	GAAGAAGAACCCTACCACAG CACCAAAAACGTTCCCTCAG	(AG)16 AC(AG)8	54
7	CEDG056	9	TTCCATCTATAGGGGAAGGGAG GCTATGATGGAAGAGGGCATGG	(AG)14	58
8	CEDG066	11	AGTAAAACAAGAACCCTCCCAAG GTATTAAAATTTGGGGTGGTGG	(AG)9	54
9	CEDG068	10	TCTCCATAGGAACCCCTGAAAG TGGGATCAGTGAATTCGCCAG	(AG)16	58
10	CEDG071	8	GGTCCATTGAGACGGATCGAG TCCCACCTCAGCGGAATCC	(AG)9	58
11	CEDG091	4	CTGGTGGAACAAAGCAAAAGAGT TGCGTCTTGGTGCAAAGAAGAAA	(AG)7	61
12	CEDG092	8	TCTTTTGGTTGTAGCAGGATGAAC TACAAGTGATATGCAACGGTTAGG	(AG)17	59
13	CEDG127	4	GGTTAGCATCTGAGCTTCTTCGTC CTCCTCACTTGGTCTGAAACTC	(TG)3(AG)9	60
14	CEDG139	4	CAAACTTCCGATCGAAAGCGCTTG GTTTCTCCTCAATCTCAAGCTCCG	(AG)19	60
15	CEDG144	1	CAGTTACGAGTCTTGAACTTCAGC CAGCTCTGTACAAAGCTGTAACTG	(AG)16	56
16	CEDG149	1	GGCTGAAGGTGATGACAGAAG GGCACTGGTTTTCTAAGGTTGTTG	(AT)12(AG)16	57
17	CEDG154	4	GTCCTTGTTTTCCTCTCCATGG CATCAGCTGTTCAACACCCTGTG	(AG)14	57
18	CEDG172	9	GCTGACGTAGGTGACAACC CGGCTTGTGCTTCATTGTCTG	(AG)13	57
19	CEDG174	7	GAGGGATCTCCAAAGTTCAACGG GAAGGCTCCGAAGTTGAAGGTTG	(AG)22	60
20	CEDG181	4	CGCGAGATCTGGATCGTTGATC GCAGTACGGTAACGTCCTTGAC	(AG)13	57
21	CEDG228	9	GTCGTTTCCGGAAACTGTTC GATCCGAACCTCTTTCTGC	(AG)17	56
22	CEDG254	1	CGATGTCTCTTGCTTCAAGG GTGAAGGACTAGCCAAGTTTG	(AT)13(AG)11	54
23	CEDG264	5	GATTCCCTTCCTAGCTATGG CTGCTGGACATGAAGATTCAG	(AG)10 AT(AG)16	55
24	DMB-SSR182	2	TAGAGCCTTCTGGTTTTTCACA AGGAGGAGGATTTTGATGATGA	(TGA)3..(CT)3…(TCC)4	56

(1 units) of Taq DNA polymerase and 8.2µl of Double distilled water using a Eppendorf thermo cycler. The PCR was programmed and maintained with a temperature profile of initial DNA denaturation at 94°C for 3 mins followed by 35 cycles at 94°C for 45 seconds, annealing at 53-61°C for 1 min followed by extension at 72°C for 1 min and a final extension at 72°C for 10 mins. The amplified products were resolved by gel electrophoresis on 1.8 % agarose gel in 0.5X TBE buffer, stained with ethidium bromide, run at 100V for 2 hours and visualized and documented in Gel Documentation System (Bio-Red, Gel Doc XR+, USA).

Data analysis: To measure the informativeness of the markers, the polymorphism information content (PIC) for each marker was calculated according to the formula given by Anderson *et al.* (1993).

$$\mathbf{PIC}_i = 1 - \sum_{j=1}^{k} P^2 ij$$

where, k is the total number of alleles detected for a marker, Pij is the frequency of the jth allele for ith marker and summation extends over k alleles. The percent polymorphic loci for each marker were calculated as the number of polymorphic loci to the total number of loci compared. The genetic association among genotypes was analyzed by calculating the similarity coefficient (Dice, 1945) for pair-wise comparisons based on the proportions of shared bands produced by primers. The method used for tree building in the analysis involved sequential agglomerative hierarchical non-overlapping (SAHN) clustering based on similarity coefficients. The dendrogram based on similarity indices were obtained by Unweight Pair group Method with Arithmetic Mean (UPGMA). The nature and extent of diversity between the genotypes were assessed by identifying the clusters at appropriate phenon levels. All calculations were performed using the NTSYS-pc version 2.10 m software (Rohlf, 2000).

RESULTS AND DISCUSSION

Characterization of band profile: The microsatellite based targeted amplification using a panel of 24 primer pairs specific to the unique flanking sequences exhibited different levels of polymorphism. These primers produced comparatively the maximum number of high intensity bands with minimal smearing, good technical resolution and sufficient variation among different genotypes (Fig 1). The molecular profile generated on the basis of the evaluation of the amplified products clearly indicated that a total of 183 types of amplified products were generated during the amplification reaction (Table 3). The number of amplified loci varied from 4 (CEDG 15) to a maximum of 12 (CEDG 92 and CEDG 172) with an average of 4.95 polymorphic fragment per primer. The differences noticed in respect of the number of alleles per primer indicated that appreciably greater extent of allelic diversity existed amongst the materials included in the present investigation. The number of alleles, as it was recorded in the present study corresponded well to the reports of some of the earlier researchers (Somta *et al*., 2009). Contrarily, the average number of alleles detected in the present study was considerably higher than the average number of alleles documented in some of the previous reports (Kabir and Park, 2011; Jayamani and Sathya, 2012; Wang *et al*., 2012; Gupta *et al*., 2013) but relatively lower than the proportion of alleles per locus in some other studies (Sangiri *et al*., 2007). The inconsistency among reports might be due to the inclusion of different sets of genotypes and utilization of different sets of primers with scorable alleles during the investigation. The allelic variants revealed by the markers used in the present study showed high degree of polymorphism, suggesting that the genotypes selected for this study harboured enough genetic divergence. A total of 112 shared and 71 unique allelic variants were generated in the form of amplified products by using the 24 primer pairs. The primer pairs CEDG 008, CEDG 037, CEDG 056, CEDG 068, CEDG 139, CEDG 144, CEDG 149, CEDG 154 and CEDG 174 generated considerably greater percentage of unique alleles. In general, marker detecting greater number of alleles per locus detected more number of unique alleles in accordance with the earlier reports (Wang *et al*., 2012). The presence of unique alleles indicated that the materials used in this study are useful for plant breeders and geneticists as a rich source of genetic diversity for their effective utilization in mungbean breeding.

The PIC values revealing simple sequence length polymorphism based allele diversity and frequency among the entries varied from 0.440 in the case of CEDG 056 to 0.928 in the case of CEDG 254 with an average of 0.822 per SSR primer pair. The lowest polymorphism percent was observed for primer CEDGAG001 (16.67) while the primer CEDG 154 gave the maximum polymorphism percent (63.64). Among the primer pairs having higher PIC values, the primer pairs CEDAAG 002, CEDG 008, CEDG 066, CEDG 068, CEDG 154 and DMB-SSR182 generated considerably greater number of allelic variants due to variation in the length of SSR based amplified products as a consequence of amplification of the SSRs flanked by these primer pairs. Further, the primer pairs CEDG 008, CEDG 068 and CEDG 154 detected considerably greater percentage of unique alleles amongst these six primer pairs which had higher PIC and generated greater number of alleles (Table 3). The estimates, in general, reflected that the level of polymorphism as revealed by the magnitude of the PIC values was quite high and varied considerably among the primers. The PIC values as obtained in the present study were found to be in close agreement with earlier report in mungbean (Jayamani and Sathya, 2012) and blackgram (Gupta and Gopalakrishna, 2009). Contrarily, the polymorphism level observed in the present study was relatively higher than the that reported earlier for mungbean (Sangiri *et al*., 2007; Somta *et al*., 2009; Kabir and Park, 2011; Wang *et al*., 2012; Gupta *et al*., 2013; Lestari *et al*., 2014) and other legumes including drybeans (Cabral *et al*., 2011), cowpea (Badiana *et al*., 2012), chickpea (Choudhary *et al*., 2013), adzuki bean (Chen *et al*., 2015), pigeonpea (Njung'e *et al*., 2015) and pea (Prakash *et al*., 2016). Since markers having many alleles are considered to be useful for evaluation of genetic diversity (Ribeio-carvalho *et al*., 2004), the average PIC value of 0.822, which was obtained in this study, confirms that the primers utilized for molecular characterization and assessment of genetics divergence of genotypes under evaluation were, in general, highly informative and therefore useful for the purpose.

The product of amplification reaction directed at a particular repeat locus was not obtained in some of the entries due to failure of amplification at the target site. Similar results indicating the occurrence of null allele have also been

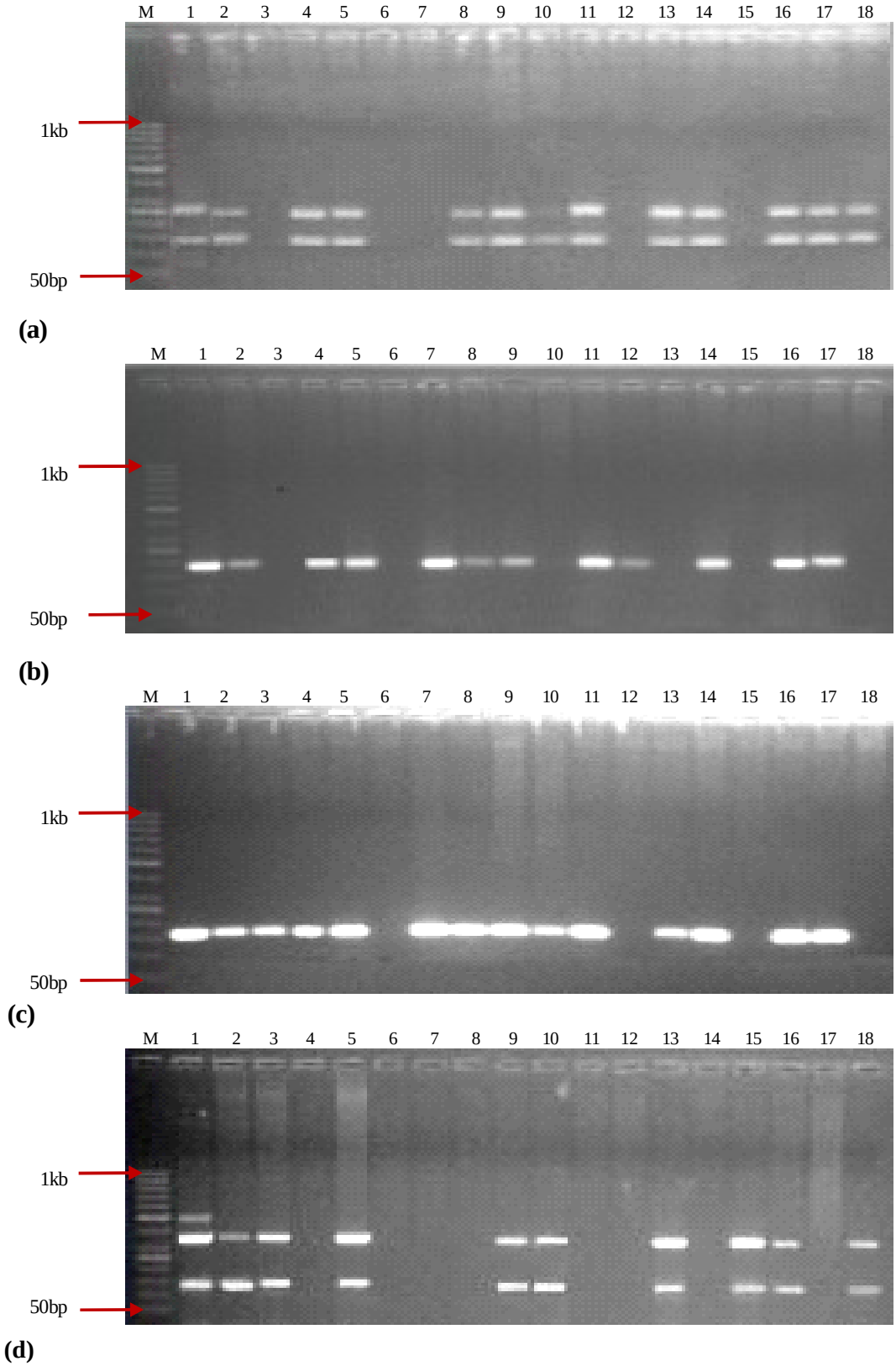

Fig 1: Amplification profile of the SSR marker (a) CEDG 056 (b) CEDG 174 (c) CEDG 264 and (d) DMG-SSR 182 in 18 genotypes of mungbean

Table 3: Analysis of primer pairs used for the amplification of genomic DNA extracted from 18 entries of mungbean.

Sl. No	Primer	No. of locus	Size of alleles (bp)	No. of alleles	No. of unique alleles	No. of shared alleles	PP	PIC
1.	CEDGAG 001	2	135-207	7	1	6	14	0.675
2.	CEDAAG 002	1	150-190	8	3	5	38	0.890
3.	CEDG 006	1	142-162	7	3	4	43	0.902
4.	CEDG 008	2	129-194	8	4	4	50	0.860
5.	CEDG 015	1	206-222	4	1	3	25	0.773
6.	CEDG 037	2	150-215	9	5	4	55	0.801
7.	CEDG 056	2	150-264	8	4	4	50	0.440
8.	CEDG 066	2	206-294	9	4	5	44	0.888
9.	CEDG 068	1	118-167	8	4	4	50	0.909
10.	CEDG 071	1	250-268	5	2	3	40	0.848
11.	CEDG 091	2	155-244	10	2	8	20	0.762
12.	CEDG 092	2	163-225	12	2	10	17	0.651
13.	CEDG 127	2	181-262	9	4	5	44	0.810
14.	CEDG 139	1	200-232	6	3	3	50	0.871
15.	CEDG 144	1	127-175	7	4	3	57	0.915
16.	CEDG 149	1	160-225	7	4	3	57	0.897
17.	CEDG 154	2	194-264	11	7	4	64	0.885
18.	CEDG 172	2	122-217	12	3	9	25	0.737
19.	CEDG 174	1	191-213	5	3	2	60	0.847
20.	CEDG 181	1	136-163	5	1	4	20	0.896
21.	CEDG 228	2	189-220	5	2	3	40	0.838
22.	CEDG 254	1	135-158	5	2	3	40	0.928
23.	CEDG 264	1	162-180	5	1	4	20	0.847
24.	DMB-SSR182	3	111-500	11	2	9	18	0.870

PP, Polymorphism per cent; PIC, Polymorphism information content.

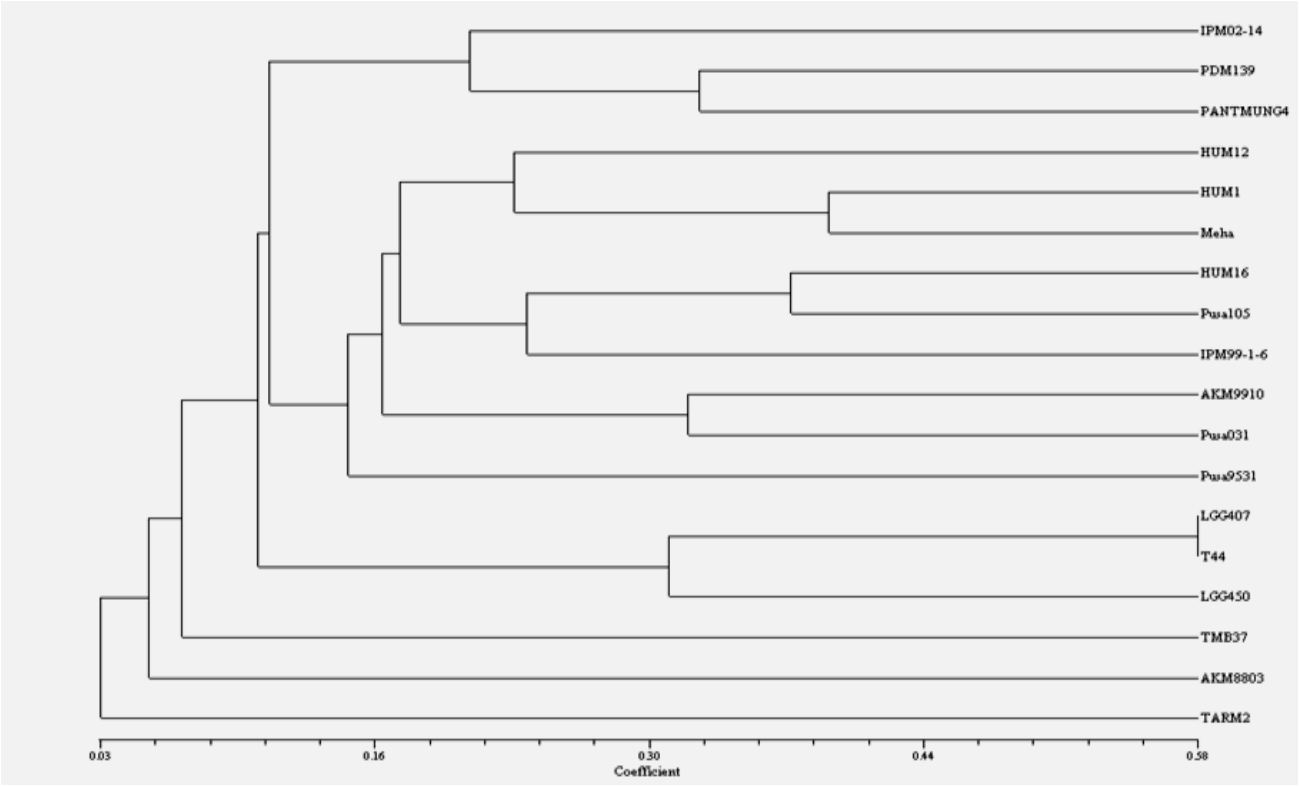

Fig 2: Dendrogram of 18 mungbean entries based on average similarity coefficients for 24 microsatellite primer pairs dependent allelic diversity.

Table 4: Estimates of 24 microsatellite repeat primer pairs based similarity coefficient among 18 mungbean entries used in the present study.

Genotype	IPM 02-14	PDM 139	Pant Mung 4	HUM 12	HUM 1	TMB 37	Pusa 9531	Meha	HUM 16	IPM 99-1-6	Pusa 105	AKM 8803	AKM 9910	Pusa 031	TARM 2	LGG 407	T44
PDM 139	0.3235																
Pant Mung4	0.1017	0.3273															
HUM 12	0.0769	0.2083	0.0000														
HUM1	0.0923	0.2295	0.2308	0.3111													
TMB 37	0.0465	0.0513	0.0667	0.0000	0.1111												
Pusa 9531	0.0000	0.0377	0.0909	0.1081	0.1600	0.1429											
Meha	0.0690	0.1481	0.0889	0.1579	0.3922	0.1379	0.2326										
HUM 16	0.0882	0.1250	0.0727	0.1250	0.1967	0.1026	0.1887	0.2593									
IPM 99-1-6	0.0377	0.0816	0.1500	0.0606	0.1739	0.0833	0.1579	0.2051	0.1633								
Pusa 105	0.0952	0.1695	0.0800	0.2326	0.1786	0.0000	0.1250	0.1633	0.3729	0.3182							
AKM 8803	0.0500	0.0556	0.0000	0.2000	0.0000	0.0000	0.0800	0.0000	0.0556	0.0000	0.1290						
AKM 9910	0.1449	0.1231	0.0714	0.1633	0.1290	0.1500	0.1481	0.1455	0.1846	0.0800	0.1667	0.1081					
Pusa 031	0.1356	0.2545	0.1304	0.2051	0.1923	0.0667	0.0909	0.1778	0.1818	0.2000	0.2000	0.1481	0.3214				
TARM 2	0.0000	0.0526	0.0690	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0870	0.0000	0.0000	0.0513	0.0690			
LGG 407	0.2154	0.0656	0.0385	0.1778	0.1034	0.0000	0.0400	0.1176	0.1639	0.0870	0.1786	0.0000	0.2581	0.1923	0.0000		
T44	0.1695	0.0727	0.0870	0.1538	0.1154	0.0000	0.0000	0.0444	0.1818	0.0500	0.2400	0.0000	0.1786	0.1304	0.0690	0.5769	
LGG 450	0.0690	0.1852	0.0000	0.1053	0.0392	0.0690	0.0465	0.0455	0.0741	0.0513	0.0000	0.0000	0.1091	0.0444	0.0714	0.3137	0.3111

obtained by earlier researchers (Sangiri *et al*., 2007; Wang *et al*., 2012). The minor product of amplification reaction was also detected in the present investigation. Presence of stutter bands indicated the presence of minor products amplified in PCR that had lower intensity than the main allele and normally lacked or had extra repeat units. Such bands were observed in the case of SSRs detected by primer pairs CEDGAG 001, CEDG 037, CEDG 066, CEDG091, CEDG 092, CEDG 127, CEDG 154 and CEDG 172.

Estimation of genetic diversity: Dice's similarity coefficient was calculated to establish the genetic relationship among 18 genotypes of mungbean using 24 polymorphic primers (Table 4). The similarity coefficient values ranged from 0.03 to 0.58, indicating the presence of wide range of genetic diversity at molecular level in these genotypes. The value of similarity coefficient was found to be the highest between the genotypes, LGG 407 and T44 (0.58). However, the value of similarity coefficient was found to be lowest in the genotypes, IPM 02-14 and IPM 99-1-6; PDM 139 and Pusa 9531 (0.03). Using similarity coefficient as a measure of similarity in numerical taxonomic approach of classification, an assessment of the nature and extent of differentiation and divergence was made amongst the entries. Based on similarity coefficients, it is evident that the genotypes LGG 407 and T44 were more similar genetically and hence the hybridization between these genotypes may not be useful in getting desirable segregates. It is also clear that the genotypes IPM 02-14, IPM 99-1-6, PDM 139 and Pusa 9531 are genetically most distant indicating that if these genotypes are used in hybridization programme by the breeder, a wide range of genetic variability will be observed in F_2 and subsequent segregating generations thereby providing scope of selecting desirable genotypes. A close genetic similarity was also observed between some of the genotypes representing that there is a close relationship in their pedigrees. The result showed the presence of narrow genetic diversity of cultivated mungbean in India. Similar observation has also been found by earlier workers using ISSR (Singh *et al*., 2012), SSR (Gupta *et al*., 2013) and RAPD (Kaur *et al*., 2017).

A dendrogram was generated following UPGMA and the clusters were identified (Fig 2). By drawing phenon line at 25 similarity units, the entries with comparatively more similar pattern for markers were allowed to be clustered together. Resultantly, the multi-genotypic group was further divided into four clusters. Therefore, seven clusters were obtained when phenon line was drawn at 25 similarity units. The multi-genotypic cluster I consisted of three entries, viz, LGG 407, T 44 and LGG 450. Cluster II contained eight entries, namely, HUM 12, HUM 1, Meha, HUM 16, Pusa 105, IPM 99-1-6, AKM 9910 and Pusa 031. The mono-genotypic cluster III consisted of only one entry Pusa 9531. Cluster IV was multi-genotypic with three entries, *viz.*, IPM

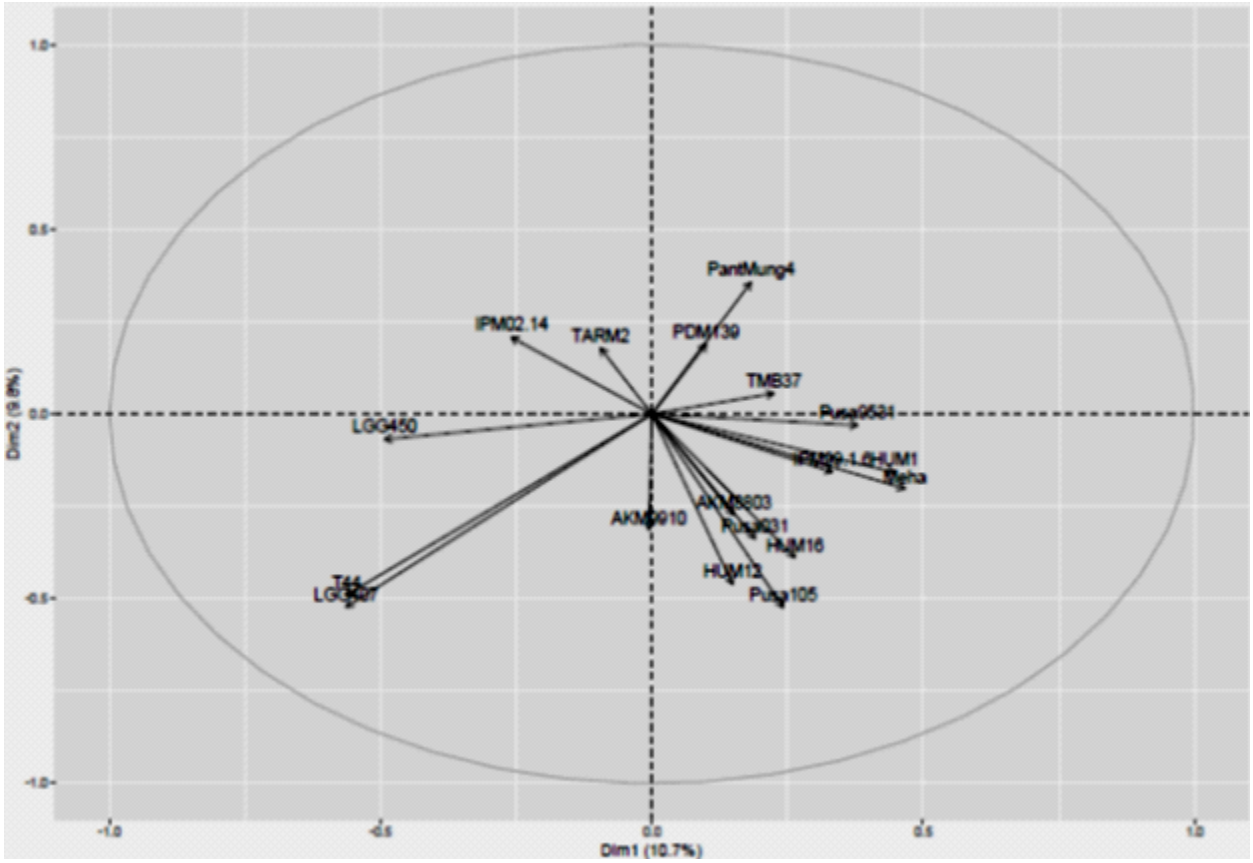

Fig 3: Pattern of relationship among the mungbean genotypes revealed by PCA (Principal component analysis) based on SSR marker data.

02-14, PDM139 and Pant Mung 4. The mono-genotypic clusters V, VI and VII included the entries TMB 37, AKM 8803 and TARM 2, respectively. A further discrimination of the entries by drawing the phenon line at 50 similarity units led to the dissociation of the clusters II and IV into sub-clusters. The cluster II was subdivided into five sub-clusters with three di-genotypic sub-clusters (IIA, IIC and IIE) and two mono-genotypic sub-clusters (IIB and IID). The sub-cluster IIA contained two entries, namely, HUM 1 and Meha. While, the sub-cluster IIC comprised HUM 16 and Pusa 105. Two entries, namely, AKM 9910 and Pusa 031 were accommodated in the sub-cluster IIE. The mono-genotypic sub-clusters IIB and IID had the entries HUM 12 and IPM 99-1-6, respectively. Keeping 75 similarity units as cut-off point, the cluster I was sub-divided into one di-genotypic and one mono-genotypic sub-cluster, while the sub-clusters IIA, IIC and IIE were divided into mono-genotypic sub-sub-clusters. The cluster I had sub-clusters IA with the entries, *viz*, LGG 407 and T 44 and the sub- cluster IB with only one entry LGG 450. The sub-cluster IIA was divided into sub-sub clusters IIAa and IIAb comprising HUM 1 and Meha, respectively. Similarly, the sub-clusters IIC was dissociated into sub-sub-clusters IICa and IICb containing HUM 16 and Pusa 105, respectively. The entry AKM 9910 was accommodated into the sub-sub-cluster IIEa of the sub-cluster IIE, while the entry Pusa 031 was a single constituent of the sub-sub-cluster IIEb of the cluster IIE. Principal component analysis (PCA) was also carried out to obtain a two-dimensional plot, in order to represent the distribution and grouping of the mungbean genotypes. The principal components 1 and 2 explained 10.7 % and 9.8 % of the total variance respectively (Fig. 3). The two-dimensional plot generated by PCA analysis also confirmed the results of cluster analysis. PCA analysis was used previously to validate and or eliminate redundancy in the dataset (Singh *et al*., 2014; Sao *et al*., 2015). These results clearly indicated that a considerably greater extent of genetic variability exists among different entries under evaluation.

CONCLUSION

The simple sequence length polymorphism based analysis in the present study which detected the polymorphism on the basis of variation in the length of SSRs using primers specific to the unique flanking sequences of the repeats, served as an efficient tool for differentiation of genotypes and diversity analysis. Since the markers were chosen from all the chromosomes of mungbean, the levels

of diversity exhibited by them seemed to be unbiased and not due to chance. So, these markers can be efficiently utilized for discrimination and unambiguous identification of different genotypes. The genetic diversity observed in the present study could be used to know the phylogenetic links among the cultivars and for the selection of suitable parents and be useful in breeding programme and genetic mapping studies.

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