



Cross Genera Marker Transferability and Genetic Diversity Analysis in Elite Cultivars of Mungbean [*Vigna radiata* (L.) Wilczek]

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ABSTRACT

Backgrounds: To increase the accessibility of molecular markers for germplasm evaluation, genetic analysis and new cultivar development in mung bean, cross genera transferability of microsatellites (SSR) was studied.

Methods: Forty simple sequence repeat markers developed for adzuki bean were used to amplify genomic DNA extracted from 70 diverse elite cultivars of greengram.

Result: All SSR markers (100%) generated repeatable and clear products, signifying a high genomic homology between two species of *Vigna*. Among the 40 amplified markers, only 11 (28%) were highly polymorphic in 70 diverse elite cultivars of greengram. The polymorphism information content (PIC) ranged between 0.64 to 0.88 with an average of 0.81, while number of alleles ranged with 6 to 12 with an average of 9.27 alleles per locus. The multiple locus amplification and high PIC values indicated the effectiveness of the transferable SSRs in mungbean. Cluster analysis based on UPGMA grouped the studied genotypes into eleven clusters. The Jaccard's similarity coefficient ranged from 0.17 to 0.46 indicating the extent of transitional amount of genetic variability within the tested genotypes.

Key words: Mungbean, SSRs, Transferability.

INTRODUCTION

Mung bean [Greengram, *Vigna radiata* (L.) Wilczek] is a self-pollinating, fast growing warm-season diploid crop ($2n = 2x = 22$). It belongs to papilionoid subfamily of the Fabaceae with a small genome of approximately 579 Mbp (Arumuganathan and Earle, 1991a). Being cultivated on a large scale in South, East and Southeast Asia by small land holding farmers, it has considerable agronomic and environmental importance and is widely cultivated owing to its adaptation to wider climatic conditions, low water requirement, soil ameliorating properties and also its use in crop rotation practices. Notably, it is cultivated for its edible seeds and sprouts having high amount of dietary protein and contain higher levels of folate and iron than most other legumes (Keatinge *et al.* 2011). Moreover, mungbean, as a legume crop, fixes atmospheric nitrogen via root rhizobial symbiosis, leading to improved soil fertility and texture (Graham *et al.* 2003). India is the major producer of mungbean in the world accounting for over 65% of the global annual production, followed by China and Myanmar. In India, it is grown in about 4.5 million hectares with the total production of 2.5 million tonnes (In the year 2020) with a productivity of 548 kg/ha and contributing 10% to the total pulse production (Green gram outlook report, AMIC, ANGRAU, Lam). India is also the domestication center of mungbean as reported by different genetic diversity data and archaeological evidences (Fuller, 2007).

Over the years, a number of high yielding varieties have been developed in mungbean in India, mostly based on classical breeding approaches. Notwithstanding this, the

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productivity realized by the farmers in this crop has remained quite low as compared to that obtained in the research trials. Narrow genetic base is one among the several constraints which have direct impact on decreasing the factor productivity in mungbean owing to repeated use of parents in breeding program making the varieties vulnerable to insect and disease attack. An understanding of the genetic and genomic relationships of extant mungbean species and cultivars is critical for further utilization of available mungbean genetic diversity and genomic information in the development of superior cultivars that combine the favourable qualities conditioned by this diverse germplasm. The germplasm lines collected from different regions serve as the best natural resources in providing the required variation in traits to develop new cultivars as well as produce more heterotic effects during the crossing program besides sometimes producing desirable transgressive segregants. Thus, assessment of local varieties including collection,

evaluation and molecular characterization of germplasm lines is the most fundamental and important approach for mungbean improvement.

Despite its importance owing to stagnant attention from scientific community and lack of genetic information advancement of studies in genetic diversity, map-based cloning and molecular breeding of mungbean have lagged far behind. No doubt, a variety of molecular markers like RFLP, RAPD and AFLP have been developed and used for genetic analysis in mungbean (Lambrides *et al.*, 2000; Humphry *et al.*, 2002; Bhat *et al.*, 2005). Nevertheless, most of these markers have been transferred from different *Vigna* backgrounds and sufficient markers specifically for mungbean have not been developed for linkage saturated map construction because they were either monomorphic or not fully informative for bi-parental mapping populations. This has been the case for RFLP (Tangphatsornruang *et al.* 2010), RAPD (Young *et al.* 1992), AFLP (Kaga *et al.* 1998), CAPS (Moe *et al.* 2012) and SNP (Chen *et al.* 2007) markers. Therefore, microsatellites (also known as SSRs based on their simple sequence repeat core) are a logical choice for broadening the scope of markers available to mungbean researchers. Additionally, they also provide advantage in being locus specific, hypervariable, highly reproducible and codominant in nature. Thus, SSRs have become the molecular markers of choice. Despite all these positive attributes there are only few reports on the development of SSR markers in mungbean (Somta *et al.*, 2008; Seehalak *et al.*, 2009; Tangphatsornruang *et al.* 2009; Gupta *et al.*, 2012). Additionally, identification of SSRs requires the construction and sequencing of SSR enriched genomic libraries, which is a labor intensive and costly approach. Due to these issues transferability of DNA markers between the genomes of different species not only provides researchers an arsenal of markers, but also allows us to better understand the evolution and speciation of crops through comparative mapping by being an alternative approach for cumbersome primer design schemes. Transferability studies provide conserved and cross-species transferable markers which have many applications in this aspect. They are useful in constructing comparative genetic maps, thus facilitating the study of synteny conservation and collinearity among related genomes (Chaitieng *et al.*, 2006; Choi *et al.*, 2004; Gupta and Rustgi, 2004). Therefore, the present study was justified by keeping in view the purpose of hybridization program and less advancement in successful transferability of genomic SSRs in case of mungbean.

MATERIALS AND METHOD

The experimental material for the present study comprised seventy elite mungbean cultivars (Table 1) released for different eco-geographical regions of India over the years. These genotypes were bred at Agricultural Research Institutes across the country representing different geographical regions. The selection of such a large and

diverse set of evaluated germplasm lines for SSR diversity analysis ensured generation of informative allelic data. Total genomic DNA of these varieties was isolated from young leaves following the procedure of Doyle and Doyle (1987). The quality of extracted DNA was checked by comparing with λ DNA while quantity was determined using spectrophotometer. Stocks were maintained in TE buffer. The working DNA sample was diluted to a standard concentration of 25 ng/ μ l. DNA was amplified *in vitro* through Polymerase Chain Reaction (PCR) using SSR primers. PCR reactions were carried out in 20 μ l volume containing 1X PCR reaction buffer, 50 ng template DNA, 0.6 U of Taq DNA polymerase, 0.2 mM dNTP mix and 0.5pM of forward and reverse primers. Amplification was performed using a thermocycler (G-Storm, UK) and it was programmed for an initial denaturation of 94°C for 3 min followed by 35 cycles each of denaturation at 94°C for 1 min, annealing for 1 min, elongation at 72°C for 2 min and final extension at 72°C for 7 min. The amplified products were separated on 3% Agarose gel in 1X TAE buffer at 50-60 V. The gels were visualized and documented after staining with ethidium bromide (0.5 μ g/ml) under UV light using gel documentation system (UVITEC, Cambridge).

A total of 40 SSR markers (Table 2) belonging to CEDG, VM and BM series (Wang *et al.* 2004) which showed polymorphism in different studies in our lab were used to analyse polymorphism in all the 70 mungbean accessions. Genotyping data of each SSR marker was recorded in binary fashion *i.e.*, presence (1) and absence (0) of bands. The polymorphism information content (PIC) values were calculated following Botstein *et al.* (1980) as follows:

$$PIC = 1 - \sum_{i=1}^n (P_{ij})^2 - \left\{ \sum_{i=1}^n (P_{ij})^2 \right\}^2 + \sum_{i=1}^n \{(P_{ij})^2\}^2$$

Where,

P_{ij} is the frequency of j^{th} allele in i^{th} primer and summation extends over 'n' patterns.

The binary data on all mungbean cultivars for 11 polymorphic markers were used to calculate the correlation matrix using similarity co-efficient analysis (Jaccard, 1908) based on which a dendrogram was constructed using unweighted pair group method with arithmetic average (UPGMA) using NTSYS pc- 2.11x (Rohlf, 1998) software.

RESULTS AND DISCUSSION

Cross genera transferability and polymorphism of the SSR markers

Due to cost efficacy, transferability of markers from related species may become an unorthodox choice as compared to new SSR primer designing from genomic library. Keeping in that view many workers had reported cross-species transferability of SSR markers among legumes (Choi *et al.*, 2004; Choumane *et al.*, 2004; Guohao *et al.*, 2006). The basic principle behind the extent of transferability and use of molecular markers from one species to other related species relied on the extent of genomic similarity between

Table 1: Mungbean elite lines evaluated for diversity analysis.

Name of variety	Year of release/development	Name of variety	Year of release/development
T 44	1948	Pant Mung-4	1997
Pusa Baisakhi	1971	Pratap	1999
DGG V-2	1972	HUM 1	1999
Sona Yellow	1973	Pusa 9531	2000
ML 5	1976	HUM 2	2000
Kopergaon	1976	Pusa Vishal	2000
PS 16	1978	RMG 344	2001
KM 2	1978	HUM 6	2001
K 851	1979	PDM 139	2001
Sujata	1980	GM 4	2001
ML 131	1980	SML 668	2002
SML 32	1981	OUM 11-5	2002
Pant Mung-1	1982	BPMR 145	2002
Pusa 105	1983	HUM 12	2003
C0 4	1983	ML 818	2003
Pant Mung-2	1984	Ganga 1	2004
PDM 54	1987	MH 96-1	2004
Vamban 1	1989	Pusa Ratna	2004
Dhauli	1989	OBGG 52	2004
RMG 62	1991	Meha (IPM 99-125)	2004
NDM 1	1992	Shalimar Mung-1	2005
AKM 8803	1992	TMB 37	2005
MUM-2	1992	HUM 16	2006
BM 4	1992	AKM 9911	2007
LGG 450	1993	BM 2002-1	2007
LAM Mung-2	1993	PKVAKM 4	2009
TARM 1	1994	IPM 02-3	2009
Pusa 9072	1995	Pusa 0672	2009
LGG 407	1995	TM 2000-2(Pairy Moong)	2010
GM 3	1995	IPM 2-14(Aditya)	2010
JM 721	1995	KM 2195	2012
TARM 1	1996	MH 421	2012
SML 134	1996	BM 2003-2	2012
TARM 18	1996	IPM 410-3(new)	2016
RMG 268	1997	IPM 2005-7	2016

two species. This principle was further conceptualized by Gupta and Gopalakrishna (2010) reporting that in case of SSR markers, it depends upon the extent of conserved primer binding sites flanking the SSR loci. In the present study also, Among the 40 markers used in PCR amplification in this study all primers amplified in mungbean cultivars which shows 100% transferability rate signifying a high genomic homology between two species of *Vigna*. Tangphatsomruang *et al.* (2009) reported the success in transferability of SSR markers by indicating that majority of microsatellite markers were transferable in *Vigna* species, whereas transferability rates were only 22.90% and 24.43% in *Phaseolus vulgaris* and *Glycine max*, respectively. On the contrary, Wang *et al.* (2009) observed 75% adzuki bean SSR markers as transferable and only 14% as polymorphic among a total of 187 primers in 60 mungbean genotypes.

Likewise, Dikshit *et al.* (2012) observed transferability percentage across the genotypes ranged from 60.97 to 92.6% with 87.8% in *Vigna radiata* and *Vigna mungo*, 62.2% in *Vigna unguiculata*, 91.8% in *Vigna umbellata*, 78% in *Vigna mungo* var. *sylvestris* and 80% in *Vigna trilobata*, respectively by using 78 mapped SSRs from adzukibean cultigens. These studies exhibited the auxiliary leeway in transfer of SSR markers to mungbean from other closely related species. These transferred markers can be used to expedite the breeding efforts in the mungbean improvement program. For example, in a pioneer study, Ishemura *et al.* (2012) analyzed the genetic differences between mungbean and its presumed wild ancestor by formulating a complete linkage map of mungbean covering all the 11 linkage groups using SSR and EST-SSR markers from mungbean and its related species like adzukibean, cowpea and soybean and

Table 2: List of SSR Primers used in the study.

Name of the primer	Primer sequence		Amplified product size (bp)		Linkage Group	Reference
	Forward	Reverse	Cultivated	Wild		
CEDG204	CCTTGGTTGGAGCAGCAGC	CACAGACACCCCTGCGGATG	153	147	1	Wang <i>et al.</i> (2004)
CEDG026	TCAGCAATCACTCATGTGGG	TGGGACAAACCTCATGGTTG	-/142	60/146	10/2	Wang <i>et al.</i> (2004)
CEDG186	GGATGGGAGAGTAAGAAG	GCATGGCATGATGACTTG	155/-	161/457	3/7	Wang <i>et al.</i> (2004)
CEDG305	GCAGCTTCACATGCATAGTAC	GAACCTTAACCTGGGTTGCTGC	107	105	3	Wang <i>et al.</i> (2004)
CEDG139	CAAACTCCGATCGAAAGCGCTTG	GTTTCTCCTCAATCTCAAGCTCCG	196	200	4	Wang <i>et al.</i> (2004)
CEDG291	CCTCAAGTGGGTTACC	GGTCAACCTCATTCTCC	127	113	1	Wang <i>et al.</i> (2004)
CEDG015	CCCGATGAACGCTAATGCTG	CGCCAAAGGAAACGCAGAAG	174	176	6	Wang <i>et al.</i> (2004)
CEDG118	AACCCAAACCAACCTTGTGGTAAG	GCTGGAATCATAATACCGCCTTGT	161	163	6	Wang <i>et al.</i> (2004)
CEDG121	CTTTCAAAATAATGTTGAGGCATA	CAATACATAATAACCTTTTCTGC	83	91	6	Wang <i>et al.</i> (2004)
CEDG174	GAGGATCTCCAAAGTTCAACGG	GAAGGCTCCGAAGTTGAAGGTTG	189	185	7	Wang <i>et al.</i> (2004)
CEDG030	TGAGGGAATGGAGAGAGGC	TCCGCAGATAGAGGCTCAG	105	107	8	Wang <i>et al.</i> (2004)
CEDG073	CCCCGAAATCCCCTACAC	AACACCCGCTCTTTCTCC	207	171	8	Wang <i>et al.</i> (2004)
CEDG247	GTAGACACTGATCATCAC	GACCATCATCGATACGATTC	153	155	8	Wang <i>et al.</i> (2004)
CEDG013	CGTTCGAGTTCTTCGATCG	ACCATCCATCCATTGCGATC	92	82	11	Wang <i>et al.</i> (2004)
CEDG003	CCACTTCTCTTGACTTTGC	GACCAAGTGAAGCCCAAGAG	258	250	1	Wang <i>et al.</i> (2004)
CEDG048	TCTCTCCTCTATGGCTTGG	GCTCCTCTTTTGTGCTGC	194	218	1	Wang <i>et al.</i> (2004)
CEDG053	CGTTGCAGAGCGGTGGTGG	GCCTTGTCTCCCATCCATG	110	100	1	Wang <i>et al.</i> (2004)
CEDG141	CCAGGCATCCATGATGAC	GAAGTTGTGGTAATGGTTGCCTC	209	179	1	Wang <i>et al.</i> (2004)
CEDG275	CACACTCAAGGAACCTCAAG	GTAGGCAACCTCCATTGAAC	254	260	2	Wang <i>et al.</i> (2004)
CEDG127	GGTTAGCATCTGAGCTTCTTCGTC	CTCCTCACTGGTCTGAACTC	222	214	4	Wang <i>et al.</i> (2004)
CEDG059	AGAAAAGGGTGGCCTCGTTG	GCAGGCATTTCATCGCAG	217	215	8	Wang <i>et al.</i> (2004)
CEDG099	TGGTGAGCATGGATGTGA	GGTTCAAGGTGGAAGGCAGA	216	214	8	Wang <i>et al.</i> (2004)
CEDG271	GCATAAAGTTAGACGTGGTTC	CACTCCCACTGCCAAACAAGG	194	200	8	Wang <i>et al.</i> (2004)
CEDG147	CTCCGTCGAAGAATTGGTTGAC	GCAAAAATGTGGCGTTTGGTTGC	-/308	237/288	9/10	Wang <i>et al.</i> (2004)
CEDG150	GAAGGGAATGAAAATGAAACCC	GTTCAATCCATTCACTCTCC	162	154	10	Wang <i>et al.</i> (2004)
CEDG044	TCAGCAACCTTGCAATTGCAG	TTTCCCGTCACCTCTTAGG	133	147	11	Wang <i>et al.</i> (2004)
CEDG098	AAAGGAGTAGAAGGTGCATA	ACAAAATTGGTTGACTCACC	99	107	11	Wang <i>et al.</i> (2004)
CEDG168	CTGCTTGGTGTGAAGCTTC	CATTCTACATTCAGACCTGC	176/380	228/376	11/2	Wang <i>et al.</i> (2004)
CEDG116	TGTATCGAAACGACGACGCAGAT	AACATCAACTCCAGTCTACCCAAA	120	148	10	Wang <i>et al.</i> (2004)
CEDG295	CAAAGGTTAGATCCAAACATCG	GGTTAGTCATCAACAATCC	165	159	7	Wang <i>et al.</i> (2004)
CEDG086	GAGTTTACAACAGATGGGGCTAA	AGGCTTTGATTGACTTTCTGGGT	126	130	4	Wang <i>et al.</i> (2004)
CEDG097	GTAAGCCGCGATCCATAATTCCA	TGCGAAAGAGCCGTTAGTAGAA	100	88	10	Wang <i>et al.</i> (2004)
BM146	GAGATGAGTCTTTCCCTACCC	TGCAGACACAATTTATGAAGGC	98	95	3	Gaitan-Solis <i>et al.</i> (2002)
VM37	TGTCCGCGTCTCTAATACGC	CGAGGATGAAGTAACAGATGATC	270	266	8	Li <i>et al.</i> (2001)
VM27	GTCCAAAGCAAATGAGTCAA	TGAATGACAATGAGGGTGC	247	233	9	Li <i>et al.</i> (2001)

Table 2: Continue...

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CEDG051	AAACATACCCCTGGCAGTTCC	TTCTGACCTAAGAAAGAGCCCTGG	250	236	1	Wang <i>et al.</i> (2004)
CEDG225	GAGGAAGTGTTCAGCACC	GTAGACTCTGCAGAGGGATG	118	116	2	Wang <i>et al.</i> (2004)
CEDG176	GGTAACACGGGTTTCAGATGCC	CAAGGTGGAGGACAAAGATCGG	128/-	132/138	3/7	Wang <i>et al.</i> (2004)
CEDG257	GACTACTCTCAAGACCAAG	GATGGTTGTAGATAACACTCC	97	89	8	Wang <i>et al.</i> (2004)
CEDG156	CGCGTATTGGTGACTAGGTATG	CTTAGTGTGGGTGGTCGTAAGG	201	193	8	Wang <i>et al.</i> (2004)

observed that the amplification ratio of SSR and EST-SSR primers from the five legumes was comparatively high in mungbean, with values between 76.7% (cowpea primers) and 98.0% (mungbean primers). This cross-species utilization of SSR primers made possible due to a high level of sequence conservation among the flanking regions of microsatellites which was indicated by multiple bands found in by few microsatellites because of annealing at more than one locus or duplication of primer binding sites in the cross-species legumes (Dikshit *et al.*, 2012; Wang *et al.*, 2014). In accordance of these results, Palaniappan and Murugaiah, (2012) characterized 20 elite mungbean genotypes using 16 microsatellite markers from greengram, adzukibean, common bean and cowpea. Among these 16 makers 14 had showed polymorphism. They demonstrated that adzukibean microsatellite markers are highly polymorphic and can be successfully used in the genome analysis of mungbean.

Moreover, in the present study, out of the 40 primers showing amplification in greengram, 11 markers were highly polymorphic (for example, Fig 1) and remaining were monomorphic. In order to demonstrate the potentiality of the transferable SSR markers, PIC values were used as a parameter, which varied with a mean value of 0.81 and more than 63% SSR loci had greater than this average PIC value. PIC value of these polymorphic SSR markers ranged between 0.64-0.88 with an average PIC value of 0.81 (Fig 2) while number of alleles ranged between 6 to 12 with an average of 9.27 alleles per locus (Table 3). It has been observed that markers should have many alleles to be considered useful for evaluation of genetic diversity (Ribeiro-carvalho *et al.* 2004). The polymorphic primers which have high PIC value can be used in further molecular studies like association mapping, tagging of gene(s) of interest and the most called approach marker assisted selection (MAS). Total 102 alleles were generated by these 11 primers. Contrastingly a large representative collection of mungbean accessions was analysed using 19 SSR primers for each linkage group (on the basis of the azuki linkage map) by Sangiri *et al.* (2007) revealing detection of 309 alleles. These differences in our study pertaining to number of alleles detected are primarily due to use of 0.3% Agarose gel instead of capillary electrophoresis used in their study for genotyping as well as lines from different geographic regions and SSR markers used.

Furthermore, product size was also calculated for each of the primers by calculating the average in the present study. Some primers (CEDG141, CEDG156, VM 27and BM 146) highly deviate on their product size studied earlier by Wang *et al.* (2014). The multiple locus amplification and high PIC values indicated the effectiveness of the transferable SSRs in germplasm characterization, evolution, breeding application and phylogenetic studies in greengram. Several SSR markers also showed multiple banding patterns with very weak bands but these were not considered for analysis in present study. This poor banding pattern could be due

the non-specific annealing of the SSR primers (Williams *et al.* 1990). In accordance to these results, Chattopadhyay *et al.* (2008) identified and successfully deployed efficient molecular markers which can minimize ambiguity in varietal identification and registration in mungbean by using 15 accessions and 10 PCR-based efficient primers identified with higher polymorphic information content (PIC) values and higher marker indexes (MI).

Diversity analysis

A dendrogram (Fig 3) based on UPGMA analysis grouped 70 genotypes of greengram in eleven major clusters (Table 4). These eleven clusters had maximum similarity of 38%. The perusal of the cluster analysis revealed that the individuals within any one cluster are more closely related than are individuals in different clusters. The cluster II consisting

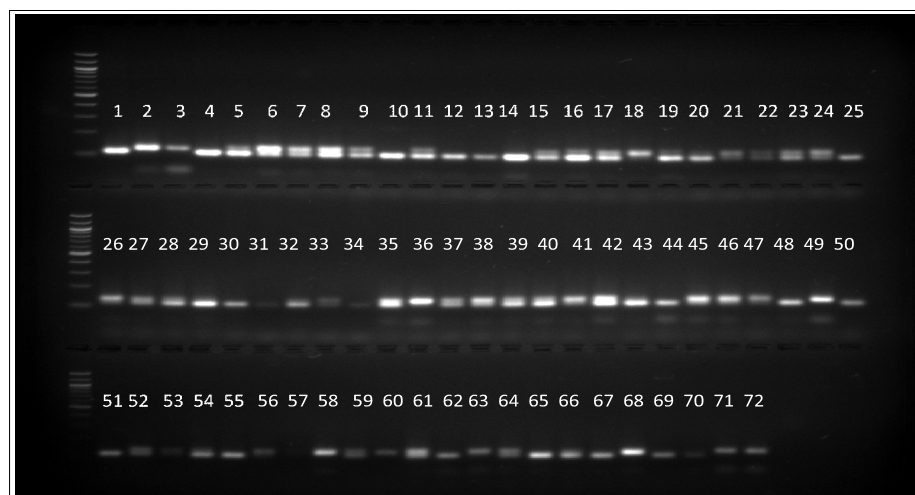


Fig 1: Pattern from amplification of genomic DNA of 72 test genotypes with SSR primer CEDG 305. Genotypes marked by order (1-72) as mentioned in table 1 with two additional genotype 71 (LPG17) and 72 (DPU8831).

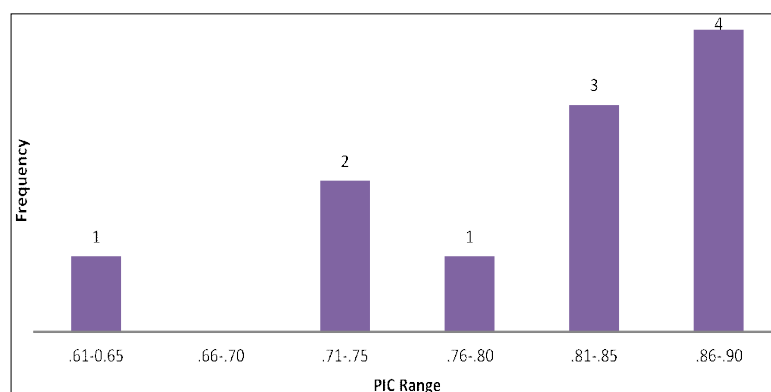


Fig 2: Frequency distribution of PIC range values of the polymorphic markers.

Table 3: Primer Sequences and characteristics of 39 transferable Adzukibean and Mungbean specific SSR primer pairs.

Primers	Amplified product size (bp)	No. of alleles	PIC value	*LG	*Distance (cM)
CEDG 204	151	8	0.75	1	32.8
CEDG 247	132	10	0.86	8	67.4
CEDG 141	137	11	0.84	1	13.8
CEDG 127	176	8	0.8	5	83.6
CEDG 044	104	9	0.84	11	18.6
BM 146	242	7	0.75	3	41
CEDG 147	275	12	0.88	9	25.7
CEDG 257	82	6	0.64	8	53.8
VM 27	192	11	0.88	9	28.3
CEDG 305	98	11	0.85	3	59.6
CEDG 156	165	9	0.86	8	2.8

of 34 genotypes was the largest and was followed by cluster XI having 9 genotypes while rest of the clusters had less than 5 genotypes. The clustering pattern thus obtained in the study confirmed the discriminating power and reliability on the SSR markers for genetic diversity studies. NTSYS also analyzed the Jaccard's similarity

coefficient which was ranging from 0.17 to 0.46. This range clearly showed that a transitional amount of diversity found among the genotypes by visualizing the extent of genetic similarities among the test genotypes. Minimum similarity of 17% was found between the genotypic pair TMB 37 and Pusa Ratna as well as TMB 37 has shown considerable

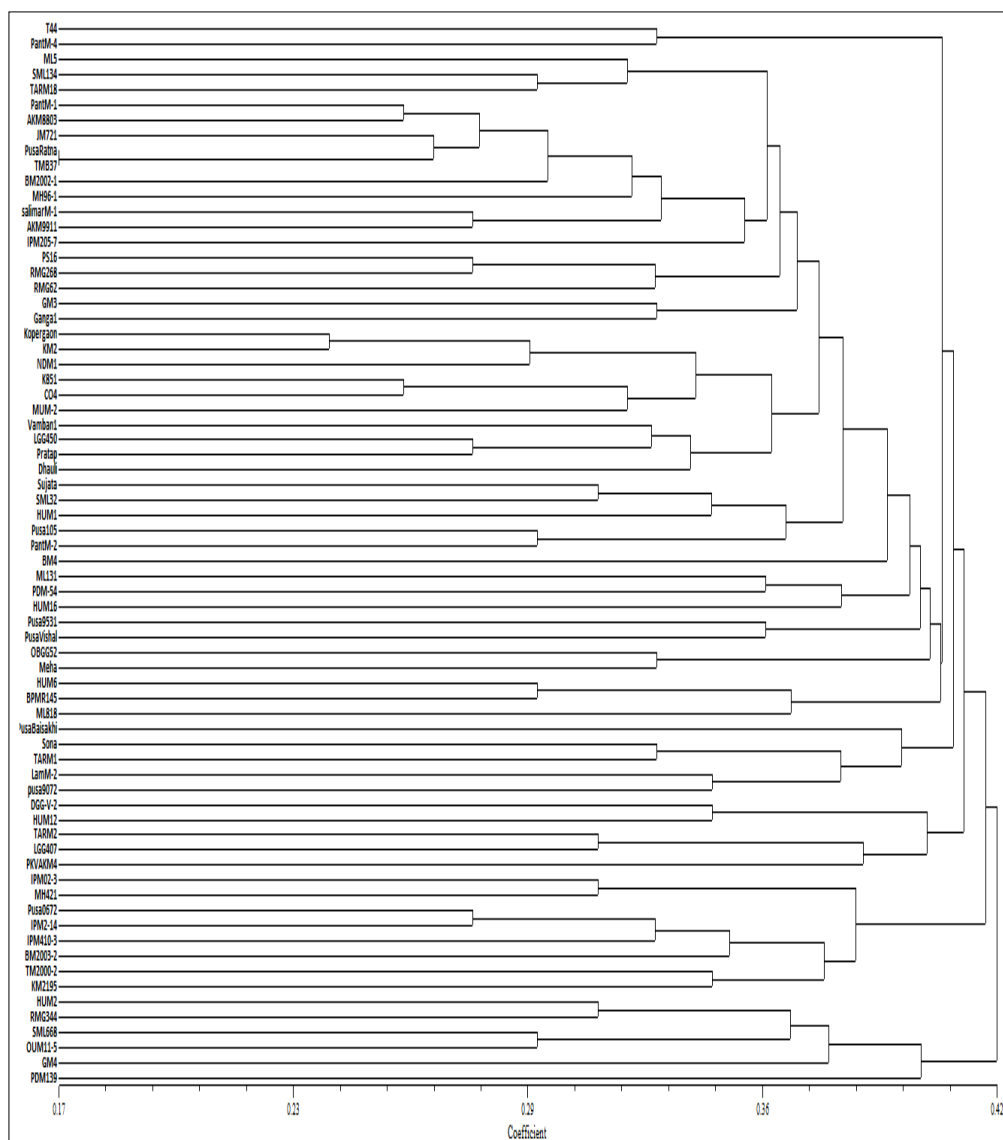


Fig 3: Dendrogram based on Jaccard's similarity coefficient using UPGMA method of clustering.

Table 4: Clustering pattern obtained by SSR analysis.

Cluster	Number of genotypes	Genotypes
I	10	TM 2000-2, KM 2195, PKVAKM 4, IPM 205-7, IPM 02-3, MH 421, BM 2003-2, IPM 410-3, PUSA 0672, IPM 2 14
II	7	SML 134, PS 16, RMG 268, Shalimar Mung-1, AKM 9911, OBGG 52, Meha
III	11	HUM 6, BPMR 145, PDM 139, ML 818, HUM 2, RMG 344, GM 4, SML 668, OUM 11-5, Pusa Ratna, TMB 37
IV	22	AKM 8803, Kopergaon, KM 12, K 851, Sujata, ML 131, SML 32, Pant Mung-1, Pusa 105, CO4, Pant M-2, Vamban 1, Dhauli, RMG 62, NDM 1, MUM 2, BM 4, LGG 450, Pratap, HUM 1, Pusa 9531, PDM 54
V	20	DGG-V-2, T 44, Pusa Baisakhi, Sona, ML 5, Lam M-2, Pusa 9072, LGG 407, GM 3, JM 721, TARM2, TARM 18, Pant Mung-4, Pusa Vishal, HUM 12, Ganga 1, MH 96-1, HUM 16, BM 2002-1, TARM 1

dissimilarity with AKM 8803 and JM 721. Furthermore, these genotypes TMB 37, Pusa Ratna, AKM 8803 and JM 721 are implicated to get minimum similarity consequently they can be used to facilitate as mapping population in various mapping studies as well as establishing the utility of microsatellite markers in identifying diverse pairs. While the maximum genetic similarity of about 46% was observed between 34 genotype pairs. This mark a possibility that the SSR markers used in the study may be linked to the genomic region in these genotypes. Furthermore, these genotypic pairs are implicated to get maximum dissimilarity consequently they can be used to facilitate as mapping population in various mapping studies as well as establishing the utility of microsatellite markers in identifying diverse pairs.

CONCLUSION

The transferability of SSRs due to homology of flanking regions between closely related species may reduce costs and avoid the laborious cloning procedures involved in their development. Recent research suggests that successful cross-species amplification in plants is largely restricted to closely related genera. This may allow for the comparative map construction, molecular characterization and evaluation of crop species such as mungbean which are lacking sufficient DNA markers. Further success rates can be achieved by using primers based on expressed sequences which may be more conserved in genomic regions. Keeping this in view, there is an ample scope to develop/transfer more SSR markers in greengram in order to saturate the present molecular linkage map in this important food legume. This can be further utilized in a systematic manner.

Conflict of interest: None.

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