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Detection and characterization of polymorphic simple sequence repeats markers for the analysis of genetic diversity in Indian mungbean [*Vigna radiata* (L.) Wilczek]

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Abstract

Mungbean is a widely cultivated grain legume in Asia, Africa and South America. In this study, 52 varieties of mungbean were profiled with 39 polymorphic SSR primers after screening a total 315 SSR primers. A total of 96 alleles were scored from the 39 primers with an average of 2.46 alleles per locus indicating the low diversity among varieties. The Nei's genetic diversity index and the Shannon information index of SSR primers varied from 0 to 0.649 and 0 to 1.169, respectively. The results showed that the potential transferability of adzuki bean primers (83.3%) was greater than the cowpea primers (25%). The varieties profiled were grouped into four major clusters. But the clustering pattern did not reflect on their geographical origin. Further, the AMOVA indicated presence of moderate genetic differentiation among groups compared to higher differentiation among varieties within populations. The SSR markers identified here will add valuable genomic resources for germplasm characterization, cultivar identification and assessment of genetic diversity of mungbean varieties.

Key words: AMOVA, genetic diversity, mungbean, marker transferability, SSR markers

Introduction

Mungbean (*Vigna radiata* (L.) Wilczek) is a diploid (2n=22) fast growing warm-season legume belonging to the Papilionoid subfamily under Fabaceae. Mungbean is a ready source of protein, amino acids, carbohydrates, antioxidant and fibers, suggesting it to be an excellent and balanced source of diet. Despite being a nutritious crop, overall production of mungbean is low due to abiotic and biotic stresses, low level of crop management by farmers and the shortage of suitable varieties for varying geographical conditions (Singh et al. 2015).

Assessment of genetic diversity in the varieties is of immense importance for planning an effective and scientific breeding program for enhancement of crop yield as well as its stability (Chandra et al. 2013; Kaur et al. 2016). For assessing genetic diversity, molecular markers in addition to the conventional morphological and biochemical markers are powerful tools since they remain unaffected by environmental factors and are independent of tissue or developmental stage of crops (Zhang et al. 2015). The small genome size of mungbean (579 Mbp) makes it a model plant system among pulses for diversity studies. Earlier, in mungbean, RAPD (Lakhanpaul et al. 2000), AFLP (Bhat et al. 2005) and ISSR (Singh et al. 2012) markers were used for diversity study. However, Simple Sequence Repeat (SSR) markers are more popular among markers systems because of their attributes like high level of polymorphism, co-dominant nature, locus specificity, reproducibility and random distribution throughout the genome. In comparison to other legumes, limited polymorphic SSRs have been reported in mungbean, reflecting the scarcity of genomic resources (Gwag et al. 2010; Shrivastava et al. 2014; Chen et al. 2015). Moreover, genetic diversity was found to be less within the mungbean germplasm in earlier studies (Bhat et al. 2005, Singh et al. 2012). Thus there is a need to screen and identify polymorphic SSR markers in greater numbers to facilitate analyses

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of genetic diversity in mungbean. The process may include transferring the SSR markers from other closely related legumes such as moth bean, adzuki bean, cowpea and rice bean as this will also facilitate correlated studies in across related crops (Datta et al. 2013; Jingade et al. 2014; Shivakumar et al. 2017).

The present study aimed at analysis of genetic diversity in the major released varieties using newly identified as well as earlier reported mungbean specific SSR markers. Further, we have also studied the cross transferability of SSRs to mungbean from other related species *Vigna unguiculata* (cowpea) and *Vigna angularis* (adzuki bean).

Materials and methods

Plant material and primer selection

Total genomic DNA was extracted from the 52 varieties including one accession of the wild progenitor species *Vigna sublobata* using CTAB method (Supplementary Table S1). The quality and quantity of DNA in the samples were determined by gel electrophoresis and Nano Drop 1000 Spectrophotometer readings (Thermo Scientific).

A total of 315 SSR primers were first screened using five mungbean varieties. These included SSR primers from mungbean [58 VR series primers (Dikshit et al. 2007), 11 MB series primers (Kumar et al. 2002) and 200 mgSSR primers (developed in-house from microsatellite enriched library, submitted to NCBI, Accession numbers GI: 406679684 to GI: 406681203)], cowpea [40 VM series primers (Li et al. 2001)] and adzuki bean [six AB series primers (Wang et al. 2004)]. Out of these, 39 primers which provided clear and polymorphic gel patterns in screening were selected and used for further analysis of 52 varieties of *V. radiata* (Table 1).

Polymerase chain reaction (PCR) amplification was done in a thermal cycler (Eppendorf) with 25 µl reaction mixture at temperature conditions of 94°C for 6 min, followed by 35 PCR cycles inclusive of denaturation for 1 min at 94°C, primer annealing for 1 min at 48°C and primer elongation at 72°C for 1 min, followed by last step of extension at 72°C for 10 min. The primer annealing temperature of 48°C was arrived at after elaborate PCR optimization experiments (not reported here). Amplified products were subjected to gel electrophoresis using a 3% agarose gel in 1X TAE buffer and image captured using Syngene Gel Documentation system with Gene Snap software.

Data analysis

The PCR amplified products were scored as a binary matrix and molecular weights of each of the amplicon were estimated using the band position relative to the DNA molecular weight standard. Similarity coefficient and pairwise distances were calculated using the 0-1 binary matrix following Nei and Li (1979). A dendrogram based on Nei & Li genetic distance was constructed using Neighbor Joining procedure with *V. sublobata* as an out-group for rooting the tree. The goodness of fit of the clustering pattern was tested by Mantel's test. Molecular analysis was performed with only the 51 varieties of mungbean excluding wild accession. All varieties were first grouped into 14 populations based on the originating states, followed by further classification into four groups i.e., north, north-west, central and southern groups representing the distinct geographical regions of the country. Principal Components Analysis (PCA) was also carried out to illustrate relationships among the varieties. The software POPGENE ver. 1.31 was used for estimating the genetic diversity parameters, observed (na) and expected (ne) number of alleles, Shannon's information index (I), observed (Ho) and expected (He) heterozygosity. Gene flow (Nm) was calculated according to Nei (1987) for each group. SSR primer informativeness was determined from the Nei's (1973) genetic diversity estimated for each primer. SSR marker selective neutrality was examined by Ewens–Watterson neutrality test and statistic was calculated using 1000 simulations. Further, significance of genetic diversity was explored by carrying out analysis of molecular variance (AMOVA) with software Arlequin 3.5.1.2 (Excoffier and Lischer, 2010). Pairwise comparisons between populations and average pairwise differences between populations were also calculated on the basis of Fst values using Arlequin 3.5.1.2 software.

Results and discussion

SSR analysis

Among 315 SSR primers, 182 (57.8%) primers amplified in mungbean varieties out of which 39 (12%) primers showed polymorphism. In this study, 62.08% of the mungbean specific primers showed amplification and 12.6% primers were polymorphic, which was higher than previous reports (Somta et al. 2008; Seehalak et al. 2009). Earlier, Wang et al. (2009) reported 72% transferability of adzuki bean primers in mungbean. Similarly, Vir et al. (2009) also reported transferability of cowpea primers (20.47%), mungbean

Table 1. Characteristics of amplicons generated by the 39 SSR loci indicating utility of the markers identified

S.No.	Primer ID	Band size	Primer sequence 5' to 3' (F-Forward, R-Reverse primer)	Heterozygosity					
				na	ne	Ho	He	Nei	I
1	mgSSR 25	180-210	F-CCATCATTCTTGCAATTGCGR-AGCAACGAGACCTTGTTGCC	2	1.963	0.000	0.495	0.491	0.684
2	mgSSR 56	170-180	F-CTAAATGCAACAACACATGACACCR-ATTTGTATGGGTGCGACACC	2	1.215	0.000	0.179	0.177	0.321
3	mgSSR 63	180-220	F-TCAGGATATGCTCACCGTGCR-CCACCTCCTAGGGAGTGTCC	2	1.878	0.000	0.472	0.468	0.660
4	mgSSR 142	170-180	F- TTTTGCATTGTTTTGCAGGGR-TAGCCTCTAATCGCTCTGGC	2	1.310	0.000	0.239	0.237	0.400
5	mgSSR 148	180-200	F-AGCTACACAGATCACCTGGTGCR-TCGGAGTGGAGAAGAGAGTCG	3	1.846	0.000	0.463	0.458	0.809
6	mgSSR 157	290-320	F-CCATGGTCATAATTTTGAATGGGR-GCAATTCCGTGTTCGTTGGG	3	2.034	0.569	0.514	0.509	0.877
7	mgSSR 172	140-160	FCGTGCGATCACACATGTGCR-CCTATTTTATTAGTTGCACCACC	3	2.312	0.000	0.573	0.568	0.959
8	mgSSR 170	290-300	F-GCCTTATAAAAAATCGGACGR-TACACGCGTGCACGAACAGG	2	1.360	0.000	0.267	0.265	0.434
9	mgSSR 173	190-210	F-AGCATTGAGAGAGAACGTAGGGR-CTCTTTTCTCTCTTTCTTCCTCC	3	2.272	0.275	0.565	0.560	0.940
10	mgSSR 177	200-210	F-AAAGAGTTGAGTTGACAAAAGCGR-AACACTCTAATTGCTTCTCC	2	1.878	0.000	0.472	0.468	0.660
11	mgSSR 229	300-310	F-TTATGCAGTTCTTGAATGAGGGR-GTAGTCTCTCTTCCTTCTCTCGC	2	1.335	0.020	0.253	0.251	0.418
12	mgSSR 240	390-410	F-ATTTGCACAGTCAGGAAAAGR-GCCTATCCTAAACATCACAAAC	3	1.905	0.137	0.480	0.475	0.824
13	mgSSR 244	170-190	F-ACTGTTATTCCGACAACCTATCR-CTTCGTTTTCTTCTTCCTCAC	3	2.407	0.020	0.590	0.585	0.973
14	VM-24	150-160	F-TCAACAACACCTAGGAGCCAAR-ATCGTGACCTAGTGCCCACC	2	1.486	0.137	0.330	0.327	0.508
15	VM-27	250-270	F-GTCCAAAGCAAATGAGTCAAR-TGAATGACAATGAGGGTGC	3	2.513	0.000	0.608	0.602	1.003
16	VR-040	170-190	F-TGACAACATGGGAAGAAGAAGAR-ACACCAACACAAAAGCAAACAC	3	2.321	0.137	0.575	0.569	0.963
17	VR-095	100-180	F-GAAATGGGAGTTCAAAGAGGAAR-TGGAGAAGTCTGGAAGAGAACC	4	2.847	0.961	0.655	0.649	1.169
18	VR-102	140-160	F-CATGTGAGCTACCTTTCAACAR-CAAGGACTGCTATATCCAAGGC	3	1.830	0.000	0.458	0.454	0.793
19	VR-108	130-150	F-GCTCCAACACTCACTCACAAACR-CAGAAATGCAGGAAAAGAGAGG	3	1.847	0.137	0.463	0.459	0.788
20	VR-111	180-190	F-TGCATCTTTATTGAGTTCCGTGR-GTTTTGGGGTGAATGTTGGATA	2	1.895	0.647	0.477	0.472	0.665
21	VR-140	180-190	F-GGTGTTGTTGTTGAGGAATGAAR-AACATTGAGGACCCACATATCC	2	1.410	0.000	0.294	0.291	0.466
22	VR-147	100-110	F-CCATGTGTGTGAATGTGAGTGAR-CCTTTGATTTTGTGGGATGTGT	2	1.125	0.000	0.112	0.111	0.224
23	VR-256	100-120	F-GCTGTGGTGTATTTACCTTGGGR-ATCCTCCGGTCATTATCTTGTG	3	1.501	0.000	0.337	0.334	0.627
24	VR-303	160-170	F-AGACGAAGAAGAAAACGCAGACR-CCTCACACACAACACAACAGAA	2	1.587	0.490	0.374	0.370	0.557
25	VR-304	170-190	F-GAAGCGAAGAAGCCATAGAAAAR-CCTCACACACAACACAACAGAA	3	2.039	0.137	0.515	0.510	0.881
26	VR-338	180-190	F-ACTGAAGAGAATGGGTTAGGGGR-TCACATTTGTTGGGTTGAAGAG	2	1.511	0.000	0.342	0.338	0.521
27	VR-393	150-160	F-TGGCACTTTCCATAACGAATACR-ATCAGCCAAAAGCTCAGAAAAC	2	1.410	0.000	0.294	0.291	0.466
28	VR-400	140-160	F-ATCATAGATAGGGGACCAACCCR-ATCTTAGGGAGTCTTCGAGGGA	3	1.763	0.000	0.437	0.433	0.767
29	VR-413	100-120	F-GAGAAACCTTGGAGTTGGAGGR-GCCTGTCAAGAAGGAACCTAAA	3	1.911	0.000	0.482	0.477	0.830
30	VR-468	250-300	F-AGCTGCCCCTCTTTACTTAGATTTR-CGTCATTGCATACTTGAATTGG	3	2.631	0.784	0.626	0.620	1.033
31	AB-128100	140-160	F-CATCTTCCTCACCTGCATTCR-TTTGGTGAAGATGACAGCCC	3	2.546	0.059	0.613	0.607	0.996
32	AB-128079	140-150	F-AGCGAGTTTCGTTTCAAGR-GCCCATATTTTTACGCCCAC	2	1.710	0.000	0.419	0.415	0.606
33	AB-128093	190-210	F-CCCGATGAACGCTAATGCTGR-CGCCAAAGAAACGCAGAAC	1	1.000	0.000	0.000	0.000	0.000
34	MB-120 B	200-210	F-AGCCCTTCGTGCTAGGAAATR-CCCTACCGGTTGGTTGGT	2	1.537	0.020	0.353	0.349	0.534
35	MB-122 A	240-250	F-TGGTTGGTTGGTTCACAAGAR-CACGGGTTCTGTCTCCAATA	2	1.821	0.137	0.455	0.451	0.643
36	MB-322 B	190-200	F-TCAGTCAGTGTCGATAGCATAGCR-GACACAGAGAGAGAGAGAGAG	2	1.940	0.000	0.489	0.484	0.678
37	MB-323 B	290-300	F-GCTATGCTATCGACACTGACTGAR-GCGCAAAGAGAGAGAGAGAGA	2	1.973	0.020	0.498	0.493	0.686
38	MB-323 A	290-300	F-TGACGGAGAGAGAGAGAGAGAGR-TGCTTCCTTTTGTCTGAGTTAGAA	2	1.993	0.000	0.503	0.498	0.691
39	MB-738 A	240-260	F-CGCAAAGAGAGAGAGAGAGR-CCCCCATCTGAAAGAAAGAG	2	1.926	0.216	0.486	0.481	0.674
	Mean			2.436	1.841	0.126	0.430	0.425	0.685

na = Observed number of alleles, ne = Effective number of alleles, Ho =Observed heterozygosity, He = Expected heterozygosity, Nei =Nei's (1973) expected heterozygosity, I = Shannon's Information index

(47.18%) and adzuki bean primers (91.18%) in major taxa of Phaseolae tribe. In the present study, 83.3% adzuki bean primers were transferable to mungbean, however, 50% primers were polymorphic. Likewise, 25% cowpea primers were transferable to mungbean and 5% primers were polymorphic. This observation is in agreement with their taxonomical relationships and also showed further possibility of transferring SSR markers from closely related species.

Genetic diversity analysis

A total of 96 alleles with an average of 2.46 alleles per locus were generated from the 39 polymorphic SSR markers. Jaccard's similarity coefficient between the genotypes ranged from 0.133 to 0.750. A few of the polymorphic SSR patterns are presented for the primers VR040, VM-27, MB-322 and mgSSR-63 (Fig. 1). The SSR, VR-095 had highest I (1.169) indicating greater informativeness of this marker for the diversity analyses. The Nei's expected heterozygosity values

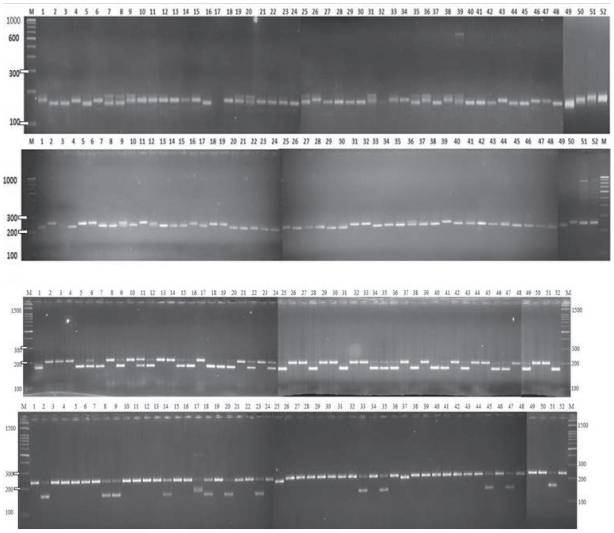

Fig. 1. SSR amplification of *V. radiata* (L.) Wilczek varieties, (a) with primer VR-040 (upper) and VM 27 (lower) and (b) with primer MB-322 (upper) and mgSSR-63 (lower). The lanes 1-52 correspond to varieties as listed in Supplementary Table S1. The lane labelled M is 100 bp DNA ladder [GeNei^{TM}Merck (a), BR Biochem Life Sciences, New Delhi (b)]

for the loci ranged from zero (AB128093) to 0.649 (VR095) with an average of 0.425 (Table 1). The 61.53% SSRs had Nei's expected heterozygosity value greater than 0.425 indicated the effectiveness of primers. The present investigation showed high level of polymorphism (97.44%) with SSR markers among the mungbean varieties consistent with the previous study (Chen et al. 2015). The Ho ranged from zero (AB128093) to 0.961 (VR095) with an average of 0.126 for the polymorphic SSRs, while, He ranged from zero (AB128093) to 0.655 (VR095) with an average of 0.430. We found that all loci analysed were selectively neutral as indicated by Ewens-Watterson test which was consistent with the previous report (Gwag et al. 2010).

This study showed moderate genetic similarity (0.13-0.75) among the varieties analyzed, compared to high genetic similarities reported with RAPD markers, (range 0.65-0.92, Lakhanpaul et al. 2000) and AFLPs, range 0.68-0.92 (Bhat et al. 2005) in our earlier studies using different sets of mungbean varieties. Also, the percentage polymorphism obtained with SSR markers (97.44%) was much higher than RAPD (64.04%) and AFLP (58.67%) markers which indicated higher informativeness of SSR markers in evaluating genetic diversity than RAPD and AFLP markers.

Partitioning of genetic diversity in mungbean varieties

Further, within the region-wise groups, north-west region show higher genetic diversity with a polymorphism estimate of 94.87% followed by south, north-west and least in central region (Table 2). Mean value of Nei's gene diversity and I was 0.374 and 0.598, respectively for the region-wise groups. Genetic differentiation (Fst) values were found in the ranges 0.317 to 0.606 with an average of 0.424 and gene flow (Nm) values ranged from 0.163 to 0.539 with an average of 0.376. Comparatively, low difference was observed between genetic differentiation and gene flow among geographical group, might be due to human activities in different regions resulting in germplasm exchange. The varieties from north-west region including Gujarat, Haryana, New Delhi, Punjab and Rajasthan have mungbean varieties with diverse pedigree resulting higher diversity in these regions. Among the regions, varieties from Telangana and Punjab showed maximum diversity supported by the other diversity parameters (Supplementary Table S2). Further, Nei's genetic distance estimates varied from 0.093 to 0.728 between populations with largest (0.728) and least distance (0.093) were found between Uttarakhand-Bihar and Rajasthan-Punjab populations, respectively (Supplementary Table S3).

AMOVA indicated the molecular variance 62.50% of total variation was residing among populations within the groups followed by 28.68% within individuals

Table 2. Estimation of genetic diversity parameters indicating region-wise differences in genetic makeup of the mungbean varieties analyzed

Group	na	ne	I	Ho	He	Nei	No. of polymorphic loci (%)	Fst	Nm
North	2.256	1.751	0.606	0.114	0.406	0.383	36 (92.31)	0.606	0.163
North-west	2.411	1.794	0.651	0.127	0.408	0.399	37 (94.87)	0.356	0.451
Central	2.000	1.624	0.502	0.103	0.343	0.318	31 (79.49)	0.317	0.539
South	2.333	1.772	0.634	0.144	0.413	0.397	37 (94.87)	0.416	0.351

na = Observed number of alleles, ne = Effective number of alleles, I = Shannon's Information index, Ho = Observed heterozygosity, He = Expected heterozygosity, Nei = Nei's (1973) expected heterozygosity, Fst = Genetic differentiation, Nm = Gene flow estimated from Fst = 0.25(1 - Fst)/Fst

Note: The groups represent: North - Bihar, Uttar Pradesh, Uttarakhand; North-West - Gujarat, Haryana, New Delhi, Punjab, Rajasthan; Central - Madhya Pradesh, Maharashtra; South - Andhra Pradesh, Kerala, Tamil Nadu, Telangana

indicating that the pattern follows the proportions expected in self-pollinated plants. Further, the fixation indices, FCT (0.035) and FSC (0.054) also substantiated these conclusions as the values indicated moderate to high genetic differentiation of groups and populations (Table 3). The high values of FIS (0.686) and FIT (0.713) indicated high differentiation of individual varieties from each state. Pairwise comparison of Fst value between populations indicated high differentiation among varieties from Madhya Pradesh - Gujarat (0.788) and Kerala - Gujarat (0.778) (Supplementary Table S3). This might be due to greater ecological differences between these states which result in higher genetic differentiation among varieties bred for different states. This is substantiated by presence of lowered differentiations among varieties from Punjab-Rajasthan (0.036) and Punjab-Haryana (0.088).

Table 3. Analysis of molecular variance (AMOVA) among/within population in groups and among/within individual in populations in mungbean

Sources of variations	d.f	Sum of squares	Variance components	Variation (%)	F-statistic
Among groups	3	76.789	0.305	3.6	FCT=0.036
Among populations within groups	10	155.297	0.446	5.2	FSC=0.054
Among individuals within populations	37	488.208	5.367	62.6	FIS=0.686
Among individuals	51	125.500	2.461	28.7	FIT=0.713
Mean	101	845.794	8.579		

d.f = Degree of freedoms, FCT = Among group's deviations from Hardy-Weinberg expectations, FSC = Among population within group's deviation from Hardy-Weinberg expectations, FIS = Among individual within populations deviations from Hardy–Weinberg expectations, FIT = Total deviation among individuals from Hardy–Weinberg expectation

Cluster analysis

The rooted neighbor joining tree based on genetic distances grouped the 52 mungbean varieties into four major clusters whereas three cultivated varieties (PDM-11, PDM-54 and Sattya) did not group with any of the clusters (Fig. 2). These cultivated varieties showed variations in allelic pattern with other cultivated varieties but showed some similarities with the wild accession. Similarly, the exotic accessions ET-52201 and ET-62199 showed very low similarity with Indian varieties. Since the local landraces, wild relatives and exotics are adapted to a wide range of habitats, constitute a potential genetic resource for tolerance to biotic and abiotic stresses and therefore can be utilized in genetic enhancement efforts to introduce greater variability in genetic stocks in crop improvement programs (Sehrawat et al. 2013). The composition of the individual clusters indicated that cluster I comprised two sub-clusters with seven and four varieties each. Cluster II also consisted of two sub-clusters with seven and three varieties, respectively. Cluster III was the largest with three sub clusters comprising seven, two and seven varieties. Further, Cluster IV was divided

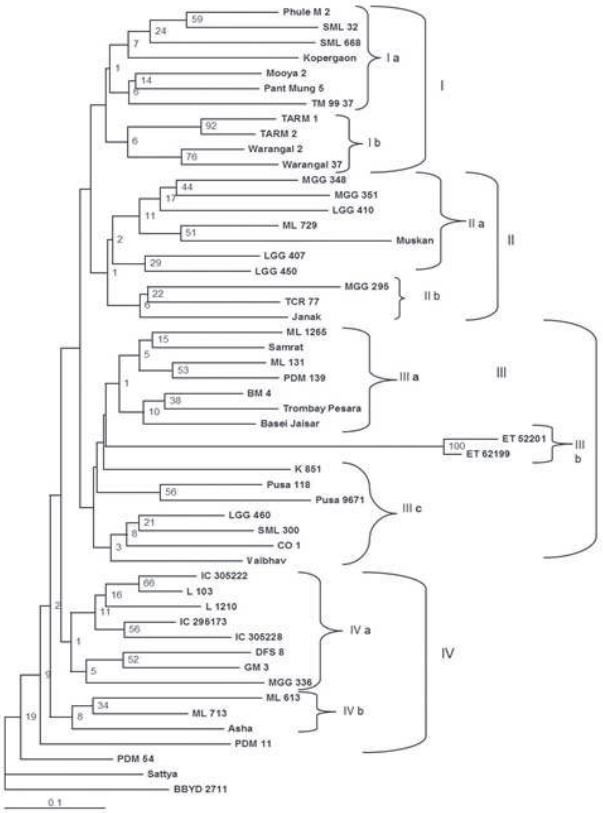

Fig. 2. The Nei & Li dendrogram illustrating the relationships between 52 varieties of *V. radiata* (L.) Wilczek using SSR markers with bootstrap values indicated at each node

into two sub-clusters with eight and four varieties, respectively. However, Mantel's correlation test with a correlation coefficient of 0.63 indicated lack of strong goodness-of-fit for the groupings observed thereby suggesting the need to use greater number of polymorphic markers to obtain a reliable and stable clustering. The PCA revealed that 21.77% of total variance was explained by the first three principal components, with each component accounting for just 8.04, 7.35 and 6.38% of total variability.

The grouping of varieties in the present cluster analysis based on SSR polymorphism did not correlate with the pattern of geographic source. As varieties from same geographic region did not group together even though presence of common parentage for some varieties was frequent. On the other hand, mungbean varieties grown in different agro-climatic regions were grouped in the same cluster, for example, Phule-Mung-2 (Maharashtra), SML-32 (Punjab), Pant Mung-5 (Uttarakhand), Warangal-2 (Telangana) and TM-9937(Madhya Pradesh) grouped in cluster 1. Frequent exchange of breeding stocks among the crop improvement centers might have resulted in greater genetic similarity among varieties. The low bootstrap values for the nodes of clusters are probably due to insufficient number of polymorphic SSRs used or gene flow/recombination between parental materials.

Overall, this study revealed that the microsatellites markers identified here will be valuable genomic resources for genetic diversity assessment, for marker-assisted selection application and also add to the meager number of SSRs so far available in mungbean. Despite the release of several new varieties over years, the observed low level of genetic diversity in the present study might be attributed to severe bottleneck effect during domestication and manual selection during breeding programs. This study emphasized the necessity to broaden the genetic base of the mungbean varieties through genetic enhancement by introgression of genes from closely related progenitor and landrace species and indicate the need to use alternate and intensive hybridization breeding programs in this group of pulses if yield barriers are to be overcome.

Authors' contribution

Conceptualization of research (KVB, ABG); Designing of the experiments (KVB, ABG, AC); Contribution of experimental materials (KVB); Execution of field/lab experiments and data collection (PB, RK, BT, SK); Analysis of data and interpretation (PB, SU, KVB); Preparation of manuscript (PB, RK, AC, ABG, KVB).

Declaration

The authors declare no conflict of interest.

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Supplementary Table S1. List of mungbean varieties used in the present study along with their places of release and characteristics

Varieties/ accessions	Source of variety	Pedigree (Year of release)	Characteristics
Vigna sublobata	Udaipur, Rajasthan	BBYD-2711	Wild progenitor accession
ET-52201	AVRDC, Taiwan	NA	Introduction, germplasm
ET-62199	AVRDC, Taiwan	NA	Introduction, germplasm
Asha	CCSHAU, Hissar, Haryana	K851 × L24-2 (1993)	Medium smooth seeds with shiny color, kharif season, MYMV resistant
BM-4	ARS, Badnapur, Maharashtra	Mutant of No. T-44 by Chemical mutagenesis (1992)	Erect, bushy, green and bold seeds
CO-1	TNAU, Tamil Nadu	Selection from local germplasm (1952)	Small seeds and YMV tolerant
DFS-8	Local, Bihar	Selection from local germplasm	NA
GM-3	S.K. Nagar, Gujarat	ML9 × GM2 (1995)	Erect, shiny green seeds, MYMV resistant
IC-296173	NA	Indigenous collection	NA
IC-305222	Local, Rajasthan	Indigenous collection	NA
IC-305228	NA	Indigenous collection	NA
Kopergaon	Rahuri, Maharashtra	Selection from local bulk germplasm (1976)	Large seeds with shining green seeds, kharif crop
TARM-1	BARC, Maharashtra	Derivatives of cross RUM-5 × TPM-1 (1997)	Small green seed, Pm resistant
TARM-2 tolerant	BARC, Maharashtra	RUM-5 × TPM-1 (1994)	Small and shiny seeds, Pm
Janak	Local, Punjab	NA	NA
K-851	CCSHAU, Hissar, Haryana	Derivatives of the cross 4453-3 × T44 (1982)	Shining green and medium large seeds
Basei Jaisar	Local, Rajasthan	KCGG-89	NA
L-103	PAU, Ludhiana, Punjab	NA	NA
L-1210	PAU, Ludhiana, Punjab	NA	NA
LGG-407	APAU, Andhra Pradesh	Mutant from PM-2 treated with 40 KR gamma radiation (1995)	MYMV resistant
LGG-410	APAU, Andhra Pradesh	Mutant of PM-2 (1995)	Leaf spot resistant, Rabi season
LGG-450	APAU, Andhra Pradesh	Mutant of PM-2 (1995)	Tolerant to MYMV and preharvest sprouting
LGG-460	APAU, Andhra Pradesh	NA (2001)	MYMV resistant
Mooya-2	NA	NA	NA
MGG-295	ARS, Madhira, Telangana	PIMS 4 × CO 3-5-2 (1995)	MYMV tolerant
MGG-336	ARS, Madhira, Telangana	NA	NA
MGG-348	ARS, Madhira, Telangana	NA	NA
MGG-351	ARS, Madhira, Telangana	MGG 332 × LGG 443	NA
Muskan	CCSHAU, Hissar, Haryana	PDM116 × GUJARAT-1 (2004)	MYMV, Anthracnose and Leaf crinkle resistant

ML-131	PAU, Ludhiana, Punjab	ML-1 × ML-23 (1980)	MYMV tolerant and Leaf spot resistant
ML-613	PAU, Ludhiana, Punjab	ML2993 × ML229 (1995)	Shining green and bold seeds, MYMV, CLS and BLS resistant
ML-729	PAU, Ludhiana, Punjab	ML 1 × No.987	Erect, synchronous large seed, MYMV resistant
ML-1265	PAU, Ludhiana, Punjab	ML-613 × K-92-140 (2008)	MYMV tolerant, CLS, BLS and anthracnose
PDM-11	IIPR, Kanpur, Uttar Pradesh	Selection from LM 595 (1987)	Spring season, MYMV resistant
PDM-139	IIPR, Kanpur, Uttar Pradesh	ML 20-19 × ML5 (2001)	Shining green seeds, MYMV resistant, both summer and spring seasons
PDM-54	IIPR, Kanpur, Uttar Pradesh	Germplasm selection, collected from Kandisa (1987)	Erect, bushy, shining green seeds
Phule-Mung-2	MPKV, Maharashtra	J-781 × ML-9 (1992)	MYMV tolerant, seeds small
Phule-Mung-9339	MPKV, Maharashtra	KDM-1 × TARM-18 (2002)	Rain fed season, Pm resistant
Pant Mung-5	GBPAUT, Pantnagar,	VC 6370-92 × VC 6141-96 (2002) Uttarakhand	Both kharif and spring season, CLS and Anthracnose resistant
Samrat	IIPR, Kanpur, Uttar Pradesh	ML20-19 × IV IL5 (1990)	Erect, green, shining seeds, susceptible to CLS
Sattya	CCSHAU, Hissar, Haryana	PDM 116 × GUJARAT-1(2008)	MYMV and Pm resistant, kharif season
SML-668	PAU, Ludhiana, Punjab	Selection from AVRDC Line (2004)	Anthracnose, CLS and MYMV resistant, both spring/ summer season
SML-300	PAU, Ludhiana, Punjab	Selection from AVARDC Line	Released cultivar
SML-32	PAU, Ludhiana, Punjab	T.1 × G.65 (1981)	Dull dark green and bold seeds
TCR-77	Local, Kerala	NA	NA
TM 99-37	BARC, Maharashtra and JNKVV, Madhya Pradesh	Kopergaon × TARM-2 (2005)	Moderate resistant to MYMV, summer season
TM-962	ANGRAU, LAM, Telangana	Kopergaon × TARM-2 (2007)	Pm,CLS resistant, rabi and summer season
Warangal-2	ARS, Warangal, Telangana	W.75-76 × Pusa 101 (1995)	Tolerant to MYMV, suitable for all seasons
Warangal-37	ARS, Warangal, Telangana	LAM M2 × ML.267 (1995)	Erect, medium sized shining green color seeds, MYMV resistant, suitable to all seasons
ML-713	PAU, Ludhiana, Punjab	NA (1995)	MYMV, CLS and BLS resistant
Pusa-118	IARI, New-Delhi	NA	Improved variety
Pusa-9671	IARI, New-Delhi	M 981 × Pusa 105	Improved cultivar, MYMV resistant

NA= Not Available, MYMV= Mungbean Yellow Mosaic Virus, Pm= Powdery mildew, CLS= Cercospora Leaf Spot, BLS= Bacterial Leaf Spot, ANGRAU= Acharya N. G. Ranga Agricultural University, CCSHAU= Chaudhary Charan Singh Haryana Agricultural University, PAU= Punjab Agricultural University, TNAU= Tamil Nadu Agricultural University, IARI= Indian Agricultural Research Institute, BARC= Bhabha Atomic Research Centre, IIPR= Indian Institute of Pulses Research, GBPAUT= Govind Ballabh Pant University of Agriculture and Technology, APAU= Andhra Pradesh Agricultural University, ARS= Agricultural Research Station, MPKV= Mahatma Phule Krishi Vidyapeeth, AVRDC= Asian Vegetable Research and Development Center, JNKVV=Jawaharlal Nehru Krishi Vishwa Vidyalaya

Supplementary Table S2. Estimation of genetic diversity parameters indicating presence of wide variation in genetic diversity among varieties from different populations

Populations	Cultivar number	No. of polymorphic loci	Polymorphic loci (%)	na	ne	Ho	He	h	I
Andhra Pradesh	4	26	66.67	1.821	1.551	0.180	0.325	0.285	0.439
Gujarat	1	4	10.26	1.103	1.103	0.103	0.103	0.051	0.071
Haryana	4	34	87.18	2.077	1.781	0.109	0.441	0.386	0.594
Kerala	1	5	12.82	1.128	1.128	0.128	0.128	0.064	0.089
Madhya Pradesh	1	3	7.69	1.077	1.077	0.077	0.077	0.039	0.053
Maharashtra	6	30	76.92	1.974	1.583	0.107	0.337	0.309	0.486
New Delhi	2	12	30.77	1.308	1.297	0.064	0.201	0.151	0.210
Punjab	13	34	89.74	2.308	1.744	0.140	0.384	0.369	0.599
Rajasthan	2	19	48.72	1.564	1.508	0.154	0.325	0.244	0.354
Tamil Nadu	1	5	12.82	1.128	1.128	0.128	0.128	0.064	0.089
Telangana	7	35	89.74	2.154	1.761	0.128	0.419	0.389	0.599
Bihar	1	7	17.95	1.180	1.180	0.180	0.180	0.090	0.124
Uttar Pradesh	6	34	84.62	1.974	1.639	0.111	0.372	0.341	0.521
Uttarakhand	1	4	10.26	1.103	1.103	0.103	0.103	0.051	0.071
Mean				1.564	1.399	0.122	0.252	0.202	0.307

na = Observed number of alleles per locus, ne = Effective number of alleles per locus, Ho = Observed heterozygosity, He= Expected heterozygosity, h= Nei's genetic diversity, I = Shannon-weaver information index

Supplementary Table S3. Pairwise comparison of Nei's genetic distance (above diagonal) and genetic differentiation index (Fst,below diagonal) among populations

Populations	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	0	0.188	0.218	0.093	0.307	0.431	0.190	0.302	0.223	0.170	0.435	0.172	0.384	0.352
2	0.164	0.000	0.325	0.268	0.554	0.504	0.330	0.596	0.387	0.313	0.686	0.328	0.531	0.294
3	0.134	0.219	0.000	0.123	0.314	0.442	0.212	0.369	0.209	0.189	0.310	0.183	0.302	0.248
4	0.036	0.196	0.088	0.000	0.219	0.321	0.148	0.247	0.164	0.107	0.334	0.137	0.319	0.303
5	0.359	0.625	0.277	0.221	0.000	0.586	0.293	0.424	0.271	0.247	0.516	0.269	0.465	0.490
6	0.443	0.788	0.302	0.246	0.648	0.000	0.259	0.509	0.453	0.349	0.412	0.377	0.350	0.679
7	0.171	0.313	0.189	0.145	0.326	0.250	0.000	0.275	0.154	0.114	0.247	0.138	0.214	0.380
8	0.305	0.760	0.240	0.162	0.554	0.761	0.252	0.000	0.259	0.311	0.462	0.321	0.491	0.639
9	0.216	0.377	0.168	0.152	0.317	0.427	0.167	0.250	0.000	0.151	0.361	0.244	0.343	0.438
10	0.105	0.212	0.130	0.095	0.229	0.248	0.100	0.211	0.121	0.000	0.250	0.198	0.356	0.351
11	0.418	0.778	0.191	0.248	0.600	0.719	0.221	0.688	0.340	0.151	0.000	0.379	0.272	0.693
12	0.109	0.260	0.148	0.107	0.294	0.306	0.138	0.249	0.246	0.160	0.297	0.000	0.295	0.385
13	0.394	0.775	0.188	0.237	0.588	0.736	0.184	0.727	0.360	0.256	0.591	0.258	0.000	0.728
14	0.348	0.556	0.149	0.257	0.591	0.783	0.355	0.732	0.401	0.256	0.744	0.313	0.773	0

1-14 populations are Rajasthan, Gujarat, Haryana, Punjab, New Delhi, Madhya Pradesh, Maharashtra, Tamil Nadu, Andhra Pradesh, Telangana, Kerala, Uttar Pradesh, Uttarakhand and Bihar