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DP Joshi
Department of Genetics and Plant Breeding, C. P. College of Agriculture, Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar, Gujarat, India

LD Parmar
Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar, Gujarat, India

RS Solanki
Department of Genetics and Plant Breeding, C. P. College of Agriculture, Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar, Gujarat, India

MP Patel
Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar, Gujarat, India

Corresponding Author:
DP Joshi
Department of Genetics and Plant Breeding, C. P. College of Agriculture, Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar, Gujarat, India

Assessment of molecular diversity among mungbean [*Vigna radiata* (L.) Wilczek] genotypes using EST-SSR marker

DP Joshi, LD Parmar, RS Solanki and MP Patel

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Abstract
Genetic diversity can be assessed through molecular markers at the DNA level because of their abundance as compared to morphological characters and independence of environmental influences. By using expressed sequence tag-simple sequence repeat (EST-SSR) markers molecular diversity of thirty mungbean genotypes was analysed at S.D.A.U., Sardarkrushinagar (Gujarat) during 2019. DNA was extracted using CTAB method. Total 21 EST-SSR primers were screened across all the genotypes. Amplification of eleven alleles were done and high polymorphism (100.00%) was found from 5 EST-SSRs primers (MB21347, MB11596, MB34120, MB24478 and MB15212). Each locus has 2 to 3 alleles, with 2.2 average alleles per locus. The range pf PIC (Polymorphic Information Content) was 0.204 to 0.580, with 0.348 average. Similarity indices derived from Jaccard's similarity co-efficient were estimated on the basis of five EST-SSR primers ranged from 0.091 to 1.00. Genotype, LM-584 and PIMS 1 showed minimum similarity. Two main clusters and six sub-clusters were formed in a dendrogram prepared using UPGMA analysis. PCA and PCoA plot based on EST-SSR primer study was constructed and genotypes have been distributed into four major groups in each method. Overall results suggested that EST-SSR can be a great tool to explore genetic diversity in mungbean.

Keywords: EST-SSR, mungbean, molecular diversity, dendrogram

Introduction
After the cereals; pulses are the most favoured choice in the Indian vegetarian diet. Pulses can act as a meat for the poor man and a vegetable for the rich man due to its high protein. But *per capita* availability of pulses is 42 g/person/day against the World Health Organization (WHO) recommendation of 80 g/person/day. Among all the pulses in India, mungbean is the most valuable. Scientific classification of mungbean is Kingdom: Plantae; Clade: Rosids in Eudicots of Angiosperms; Order: Fabales; Family: Fabaceae; Sub-family: Faboideae; Tribe: Phaseoleae; Sub-tribe: Phaseolinae; Genus: *Vigna* and Species: *radiata* (Source: USDA ARS, 2018) [21]. Mungbean is a diploid and self-pollinated plant with a chromosome number 2n = 2x = 22 and genome size of 579 Mb/ 1C (0.60 pg/1 C) (Arumuganathan and Earle, 1991; Somta and Srinives, 2007 and Sehrawat *et al*., 2014) [2, 18, 16]. The origin of greengram might be Hindustan and Central Asiatic region. It is also known as moong, mashbean, greengram, goldengram, greenbean and greensoy.

Genetic diversity of the germplasm is the major interest for the plant breeder. So, they are producing high yielding varieties which can tolerate environmental fluctuations for increasing the production and productivity of pulses. Genetic diversity can be assessed through molecular markers at the DNA level because of their abundance as compared to morphological characters and independence of environmental influences. It can give information about genetic diversity and structure of germplasms from various geographical origins (Akkale *et al*., 2010) [1]. In addition, they allow more accurate characterization of the genetic variations and genetic relationships. Thus, molecular markers can successfully provide an alternative tool for genetic analysis.

Variations of the DNA sequences (base-pair additions, substitutions, deletions or sequence patterns) are the bases for molecular marker to detect polymorphisms. List of structural markers is long *viz.*, RFLP (Restriction Fragment Length Polymorphism), RAPD (Random Amplified Polymorphic DNA), AFLP (Amplified Fragment Length Polymorphism), Cleaved Amplified Polymorphic Sequence (CAPS), Microsatellite or Simple Sequence Repeat (SSR), Inter-SSR (ISSR), etc. According to Van *et al*. (2013) [22], an advancement in next generation

sequencing has helped to produce single nucleotide polymorphisms (SNPs) in mungbean.

SSRs are short tandem repeats of 1 to 6 bases that occur randomly and frequently in the eukaryotic genome (Tautz and Renz, 1984) [19]. SSR markers are abundant, locus specific, reproducible, hyper variable, codominant in nature and uniformly present in the nuclear and organelle genome. They are widely distributed in the non-coding regions (genomic SSR) and the transcribed regions [genic or expressed sequence tag (EST)-SSRs] of the genome (Toth *et al*., 2000 and Li *et al*., 2004) [20, 14].

Recently for *Vigna* spp., sequence databases of DNA, cDNA and EST have been generated and are accessible for screening SSRs to make EST-SSRs. Because the identification of genomic SSRs is time-consuming and more costly than EST-SSRs, and the EST-SSRs are physically linked to expressed genes (in which variation may leads to alter the function of gene), they are useful for analyzing the functional diversity in genetic resources. Genic-SSR (coding SSR) are less polymorphic than the non-coding SSRs since, coding region faces extreme selection pressure and slowly evolved. So, EST-SSR has great importance for genomic research. EST-SSR markers also used in marker assisted selection breeding (MAS), gene tagging, molecular mapping and genetic diversity analysis with considering its origin either from a gene of useful agronomic character or co-segregating with the gene of interest. For this type of studies PCR-based techniques are generally used because, it is straight forward, quick to perform and needs only small amounts of DNA.

Materials and Methods

Total genomic DNA Isolation procedure

For the isolation of genomic DNA, thirty diverse mungbean genotypes were grown in the pot. All the procedure start from the DNA isolation to PCR amplification was conducted at Department of Genetics and Plant Breeding, C. P. College of Agriculture, S.D.A.U., Sardarkrushinagar.

CTAB (Cetyl Trimethyl Ammonium Bromide) extraction method (Doyle and Doyle, 1987) [8] with modifications was used to isolate genomic DNA from tender fresh leaves of all the 30 mungbean genotypes. The modifications were; use of PVP in CTAB extraction buffer, extended incubation time for digestion in the CTAB extraction buffer, treatment of phenol: chloroform: isoamyl alcohol (25:24:1), RNase treatment after pheno: chloroform: isoamyl alcohol treatment, use of 3 M sodium acetate along with chilled isopropanol for the better precipitation of nucleic acid. These modifications were suggested by Dharajiya *et al*. (2017) [7].

DNA purity checking and quantification

Purity of extracted genomic DNA were evaluated in 0.8% agarose gel using agarose gel electrophoresis (Primrose and Twyman, 2013) [15]. The quantization of DNA was done with the help of Nano-Drop spectrophotometer, which gave a direct ratio of A_{260}/A_{280}. This ratio was considered for quality and quantification of DNA as ng/µl based on absorbance at 260 nm.

EST-SSR (Expressed sequence tag-Simple sequence repeat) amplification

Total 22 EST-SSR primers were used to get the polymorphism among 30 genotypes. The primers were selected from the publications of Chen *et al*. (2015^b) [5] (Table 1). The extracted genomic DNA were used for PCR amplification with a thermal cycler (Eppendorf).

Table 1: List of EST-SSR primers used for PCR amplification in the present study

Sr. No.	Primer		Sequence	No. of nucleotides	Tm value (ºC)	% GC
1.	MB19823	F	TGCCCTCTGTTATGGTGGAG	20	59.35	55.00
		R	AGTTGCATCGGCCTCATCTC	20	59.35	55.00
2.	MB10675	F	GCAGAAGGAAGCTCAAGATCG	21	59.82	52.38
		R	GCTTCCCACAACTCCCAGAA	20	59.35	55.00
3.	MB11384	F	AACGACTGCAATGCACAACC	20	57.30	50.00
		R	GAGTTGTCTGAGGCTGAGGG	20	61.40	60.00
4.	MB9044	F	TGCCTCATGAGGGAAATCAACA	22	58.39	45.45
		R	ACACACACTTACTGCGAAGT	20	55.25	45.00
5	MB9309	F	AGGAGCCAACAACAAACCCA	20	57.30	50.00
		R	AGCAACGCCATCATCATCCA	20	57.30	50.00
6.	MB14798	F	CGCAAATGGAAACCCACCAG	20	59.35	55.00
		R	TTATGCCACGAGCTTCGAGC	20	59.35	55.00
7.	MB15469	F	TGGACCTTTTTCCCCATTCATCT	23	58.87	43.48
		R	AGTGCAGGCAGGAGCAAATA	20	57.30	50.00
8.	MB21347	F	GCCATCACCAACTACCCCTC	20	61.40	60.00
		R	AGGGGAGGGCGTAGATGTAG	20	61.40	60.00
9.	MB25181	F	ATGTCTGCATCATGGGAGCC	20	59.35	55.00
		R	CCCCAACAACAGCAGCAATC	20	59.35	55.00
10.	MB52717	F	ACGAAATCAACGAGGCATATGA	22	56.53	40.91
		R	ACTTTTGTTGCGGAGGGGAA	20	57.30	50.00
11.	MB27164	F	CTCAACAAGTTCCTCAGCGC	20	59.35	55.00
		R	CCAGAACCGGTGGAAGTCTC	20	61.40	60.00
12.	MB15686	F	CCCAACCTCTCCGCAAAGAT	20	59.35	55.00
		R	ACAGCCAATCCACGTACCTC	20	59.35	55.00
13.	MB22940	F	CCTCCCTCTCCCTCTTCCTC	20	63.45	65.00
		R	TGGGATTCCGGGCAAATCTT	20	57.30	50.00
14.	MB2421	F	AAGAGAAGGGGTTCGTGCTC	20	59.35	55.00
		R	AGGGCGAGAGAATCAAACCG	20	59.35	55.00
15.	MB27721	F	GCTTAACTTCAACGGCACCC	20	59.35	55.00
		R	TTAGCCCTTCCGTTTCGCTT	20	57.30	50.00

16.	MB11596	F	CTCCTGGGCACATTTCCACT	20	59.35	55.00
		R	ACCACCCACATCATTTCCCC	20	59.35	55.00
17.	MB34120	F	ACTGAGTCTCACCAGAGCCA	20	59.35	55.00
		R	ATTCTCCGGCACTCAACAGG	20	59.35	55.00
18.	MB24478	F	TGGCATTCTCCCAATTCCCT	20	57.30	50.00
		R	TCCTCCTGATTGGACCTCTCA	21	59.82	52.38
19.	MB11659	F	CCCTCACAAACTCGAGACCC	20	61.40	60.00
		R	GAAACGAAGGTGGCTGAGGA	20	59.35	55.00
20.	MB15212	F	CATGGGTCATGCACTTTCGT	20	57.30	50.00
		R	CGCATCCATTGAAGACCAAGC	21	59.82	52.38
21.	MB14180	F	CAGATTCCAACCCGAAGCCA	20	59.35	55.00
		R	GCGAAAGAAGCTCGTCCTCT	20	59.35	55.00

Two types of master mix were checked for the better product of the primer. One was the manually prepared master mix, and another was the QIAGEN®TopTaq™ master mix kit. For particular primer, whichever master mix gave the best result was selected.

Preparation of manual master mix is discussed in this paragraph. Amplification (for each genotype) was carried out in 20 µl volume reactions having 40 ng/µl of genomic DNA, 1X KAPA Taq Buffer B (contain 1.5 mM $MgCl_2$), 0.2 mM each dNTPs, 0.4 pmol/µl each forward and reverse primers, 0.5 U KAPA *Taq* DNA polymerase and nuclease-free water.

Preparation of master mix with QIAGEN®TopTaq™ master mix kit is discussed in this paragraph. Amplification (for each genotype) was carried out in 20 µl volume reactions with 40 ng/µl of genomic DNA, 10 µl QIAGEN®TopTaq™ master mix, 7 µl RNase-free water, 1 µl (0.4 pmol/µl) each forward and reverse primers.

For PCR reaction condition, Gradient PCR was used to know annealing temperature for each primer pair and after getting the annealing temperature normal thermal cycler was performed as per following conditions: one cycle of initial denaturation (4 min at 94°C), 35 cycles at 94°C for 1 min, 55.3-59.8°C for 1 min 30 s and 72°C for 1 min. The final extension was performed at 72°C for 7 min.

DNA fingerprinting and documentation

All the PCR amplified products were resolved in 2.5% agarose gel (stained with ethidium bromide) for EST-SSR marker [with some modifications in the study of Gupta *et al.* (2014) [9]] and photographed with FluorChem FC 2 gel documentation system (Alpha Innotech Corporation, U.S.A.).

Analysis of EST-SSR data for molecular diversity study

Data were scored based on the absence or presence of the PCR products. Data were entered into the binary matrix and then analyzed with PAleontologicalSTatistics (PAST)-Version 3.25 (Hammer *et al.*, 2001) [12] for genetic diversity evaluation. Jaccard's similarity co-efficient (Jaccard, 1908) [13] were used to find coefficients of similarity and unweighted pair group method and arithmetic average (UPGMA) function was used for cluster analysis. The relationship between the greengram genotypes was graphically shown in the dendrograms by using the cluster analysis function. The COPH function was used to analyse the cophenetic correlation. In this process, the similarity matrix and dendrogram were correlated to get the goodness-of-fit of the dendrogram made up using the similarity coefficients. The marker data were then standardized for Principal Component Analysis (PCA) and Principal Co-ordinate Analysis (PCoA) to highlight the resolving power of ordination.

The PCA and PCoA were derived and scatters were plotted on the first two components and co-ordinates, respectively. Molecular Weight (MW) of bands separated on the gel was calculated using the software program AlphaEaseFC - version 4.0 developed by Alpha Innotech Corporation, U.S.A. The polymorphism percentage was calculated as per the method suggested by Smith *et al.* (1997) [17]. Formula of Binkowski and Miks (2018) [3] were used to find Polymorphic information content (PIC) and Heterozygosity (H).

Results and Discussion

Genomic DNA

Gel electrophoresis of genomic DNA showed intact bands. For PCR amplification, requirement of A_{260}/A_{280} ratio by quantification of genomic DNA samples is 1.8. Obtained ratios were between 1.73 to 1.97 and average ratio was 1.87. The concentrations of genomic DNA of all the samples were ranged from 45.00 to 410.00 ng/µl and have 194.56 ng/µl average. Overall results indicated good quality of DNA.

Amplification by EST-SSR primers

Out of 21 EST-SSR primers, five primer pairs were found to be polymorphic; two primers found monomorphic while rest of the primers not amplified properly among the greengram genotypes. The result obtained using five polymorphic primers have been presented in Table 2. The banding pattern clearly distinguishes genotypes into various clusters which show the diversity. The EST-SSR amplification profiles obtained in the present study with primers MB24478 and MB21347 are shown in Figure 1 and 2, respectively.

Pooled analysis of EST-SSR amplification

Five polymorphic primers amplified a total of 11 reproducible DNA fragments with an average of 2.2 bands per primer. The results were in accordance with Gupta *et al.* (2012) [10] (2.3 bands per primer), Gupta *et al.* (2014) [9] (3 bands per primer), Chen *et al.* (2015[a]) [4] (2.66 bands per primer) and Chen *et al.* (2015[b]) [5] (2.33 bands per primer). However, Chueakhunthod *et al.* (2018) [6] reported 5 bands per primer. Thus, primers of the present investigation had produced the very similar amplification as compared to most of the studies with EST-SSR primer in mungbean. The highest number of amplified bands (3) was produced by the primer MB24478 while, primer MB21347, MB11596, MB34120 and MB15212 each exhibited two amplified bands. Hundred percent polymorphism were noticed for all 5 primers. Gupta *et al.* (2013) [11] stated that compared to genomic SSR markers, EST-SSRs had higher polymorphisms because of its association with transcribed genes which represent the functional variation. Chueakhunthod *et al.* (2018) [6] also found 96.60% polymorphism in 11 EST-SSR primers.

Range of the PIC value of 5 polymorphic EST-SSR primers were 0.204-0.580 with an average of 0.348. Primer MB24478 gave highest PIC value (0.580). Previous workers also found

similar results in mungbean with EST-SSR primers. Gupta *et al.* (2012) [10] got PIC range of 0.09-0.59 with 0.31 average, Chen *et al.* (2015[a]) [4] got PIC range of 0.06-0.63 with 0.36 average and Chen *et al.* (2015[b]) [5] got PIC range of 0.067-0.613 with 0.344 average.

Range of the size of PCR amplified DNA fragment was 127.26 bp to 263.95 bp. Primers MB21347, MB11596, MB34120, MB24478 and MB15212 which amplify EST-SSRs containing CCATCA, GTG, TCA, TCATCT and TTG repeat motifs, respectively found polymorphic due to their associations with the conserved function of genes. Chueakhunthod *et al.* (2018) [6] found the putative function of primer MB21347 as xyloglucan galactosyltransferase KATAMARI1 homolog (involved in the pathway of protein modification for cell elongation) and the primer MB24478 as pentatricopeptide repeat-containing protein At5g04810, chloroplastic like. These shows their importance for the genetic diversity study of mungbean.

Construction of dendrogram and classification of genotypes based on EST-SSR marker

Jaccard's similarity co-efficient were estimated on the basis of five EST-SSR primers ranged from 0.091(between LM-584 and PIMS 1) to 1.000 (between M.GP-124-B and LAM-GG-127, LM-385 and LM-554, LM-350 and LM-554, GAM-5 and LM-554, LM-7 and LM-2, LM-34 and LM-2, TT8E × 345 and LM-2, PS-10 and LM-359, LM-34 and LM-7, TT8E × 345 and LM-7, TT8E × 345 and LM-34, LM-350 and LM-385, GAM-5 and LM-385, GAM-5 and LM-350). Similarity indices 1.000 indicated most similar genotypes while, most divergence found between LM-584 and PIMS 1 genotypes. Previous workers also found similar results. Gupta *et al.* (2014) [9] found the range of Jaccard's similarity co-efficient between 0.16 to 0.98 and Chueakhunthod *et al.* (2018) [6] found from 0.61 to 1.00.

Table 2: Particulars of polymorphic EST-SSR primers used in the study

Sr. No.	Primer	No. of polymorphic bands	No. of monomorphic bands	No. of total bands	Polymorphism %	PIC value	Range of Molecular weight (bp)	Heterozygosity (H)
1.	MB21347	2	0	2	100	0.291	237.23 to 263.95	0.353
2.	MB11596	2	0	2	100	0.204	127.26 to 139.31	0.231
3.	MB34120	2	0	2	100	0.294	147.96 to 166.92	0.358
4.	MB24478	3	0	3	100	0.580	237.12 to 261.37	0.654
5.	MB15212	2	0	2	100	0.371	152.32 to 164.07	0.491
Total:		11	0	11	-	-	-	-
Mean:		2.2	0	2.2	100	0.348	-	0.417

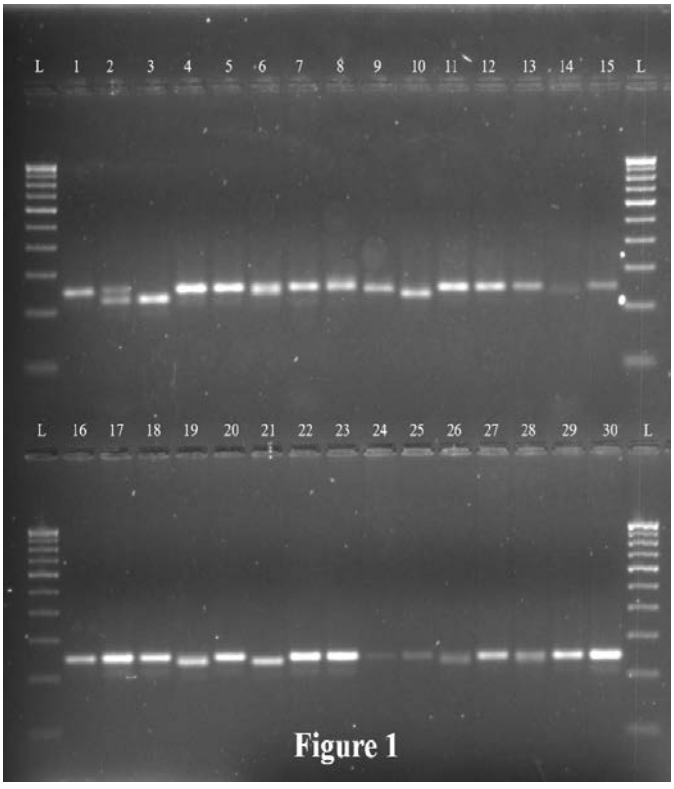

Fig 1: EST-SSR amplification profiles obtained for mungbean genotypes with MB24478

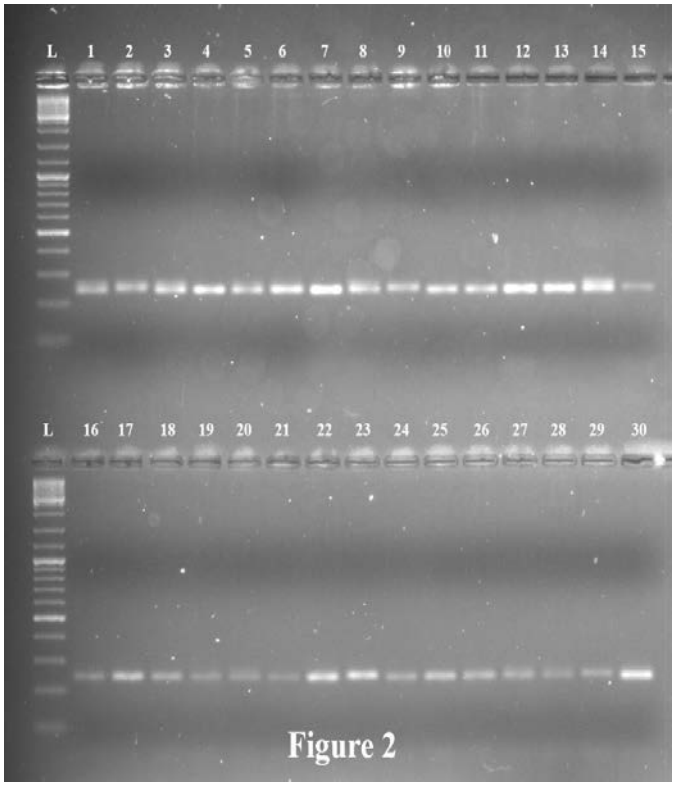

Fig 2: EST-SSR amplification profiles obtained for mungbean genotypes with MB21347

L	100 bp DNA ladder
1.	Guj-1
2.	PIMS-1
3.	A-59-7
4.	A-61-1
5.	LAM-GG-127
6.	GP-229-B
7.	SML-68
8.	M.GP-124-B
9.	LM-141
10.	MBC-5
11.	LM-554
12.	LM-578
13.	No-223(1)
14.	LM-584
15.	CM-512
16.	LM-2
17.	LM-1
18.	LM-389
19.	PS-10
20.	LM-7
21.	LM-359
22.	LM-34
23.	TT8E × 345
24.	LM-385
25.	LM-350
26.	LM-353
27.	Chaklama-2
28.	LM-309
29.	GM-4
30.	GAM-5

The distribution of all the genotypes based on the clusters from UPGMA dendrogram (cophenetic correlation coefficient: 0.7748) is shown in Table 3 and Fig. 3. Clusters A and B were two main clusters with 32% similarity. Cluster A was partitioned into A1 and A2 sub-clusters with 37% similarity between them. Sub-cluster A1 was partitioned into sub-clusters A1:1 (contains five genotypes) and A1:2 (contains three genotypes) with 46% similarity. Cluster B was partitioned into B1 and B2 sub-clusters with 43% similarity.

Table 3: Distribution of genotypes based on the clusters from UPGMA dendrogram from EST-SSR primer

Cluster	Sub-cluster		Genotypes	No. of genotypes
A	A1	A1:1	LAM-GG-127, M.GP-124-B, SML-68, LM-353, MBC-5	05
		A1:2	LM-578, LM-1, LM-389	03
	A2		PIMS-1	01
B	B1	B1:1	A-59-7, LM-309, A-61-1, LM-554, LM-385, LM-350, GAM-5, GM-4	08
		B1:2	LM-141, Guj-1, CM-512, LM-2, LM-7, LM-34, TT8E×345, PS-10, LM-359	09
	B2		GP-229-B, LM-584, No-223(1), Chaklama-2	04

Sub-cluster B1 was further divided into sub-clusters B1:1 (contains eight genotypes) and B1:2 (contains nine genotypes) with 49% similarity between them. Cluster B2 contains four genotypes. The results were in accordance with Gupta *et al.* (2014) [9] (studied 20 genotypes and found 7 clusters) and Chen *et al.* (2015[b]) [5] (studied 31 genotypes and found 2 clusters).

Usefulness of genic SSR markers is more than the genomic SSR markers. They would be directly used in marker trait association because they show true variation within the expressed portion of the genome (Li *et al.*, 2004 and Varshney *et al.*, 2005) [14, 23]. EST-SSR markers may detect important genetic diversity possibly associated with desired characters for breeding due to their location in genes (Chen *et al.*, 2015[a]) [4].

The conducted study revealed that sub-clusters were clearly observed among greengram genotypes so the potential utilization of EST-SSRs for explaining genetic diversity in greengram is justified. It is also noted that some pairs of genotypes had similar genetics (similarity indices 1.000), thus the accurate differentiation of their genetic relationships needs more molecular markers.

Therefore, for further diversity analysis, the markers used in this study will be an important resource together with the markers used by other researchers. Chen *et al.* (2015[a]) [4] was also agreed with this statement for EST-SSRs

Principal Component Analysis (PCA) and Principal Co-ordinate analysis (PCoA) based on EST-SSR: Results of 2-D plot of PCA is showed in Fig. 4 and results of 2-D plot of PCoA is showed in Fig. 5. Analysis of PCA showed four major clusters and some genotypes scattered far from the clusters in the PCA plot. First two most informative principal components with eigenvalues of 0.63 and 0.53 respectively, accounted for 54.21% of the total genetic variation. Genotypes in each cluster of PCA were found in same cluster in dendrogram also. So, it indicates the efficiency to correlate the result of PCA plot with the result of dendrogram.

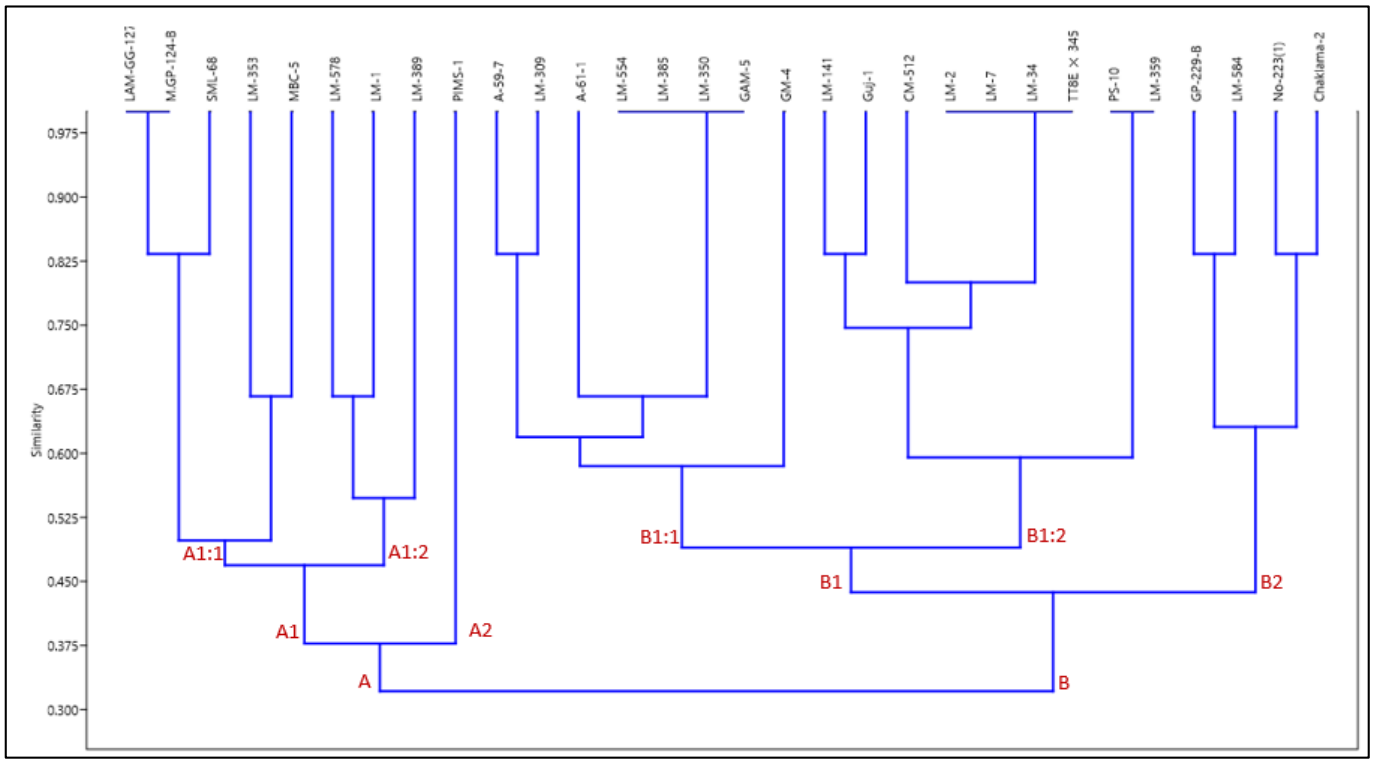

Fig 3: UPGMA dendrogram of thirty genotypes of mungbean based on EST-SSR primers

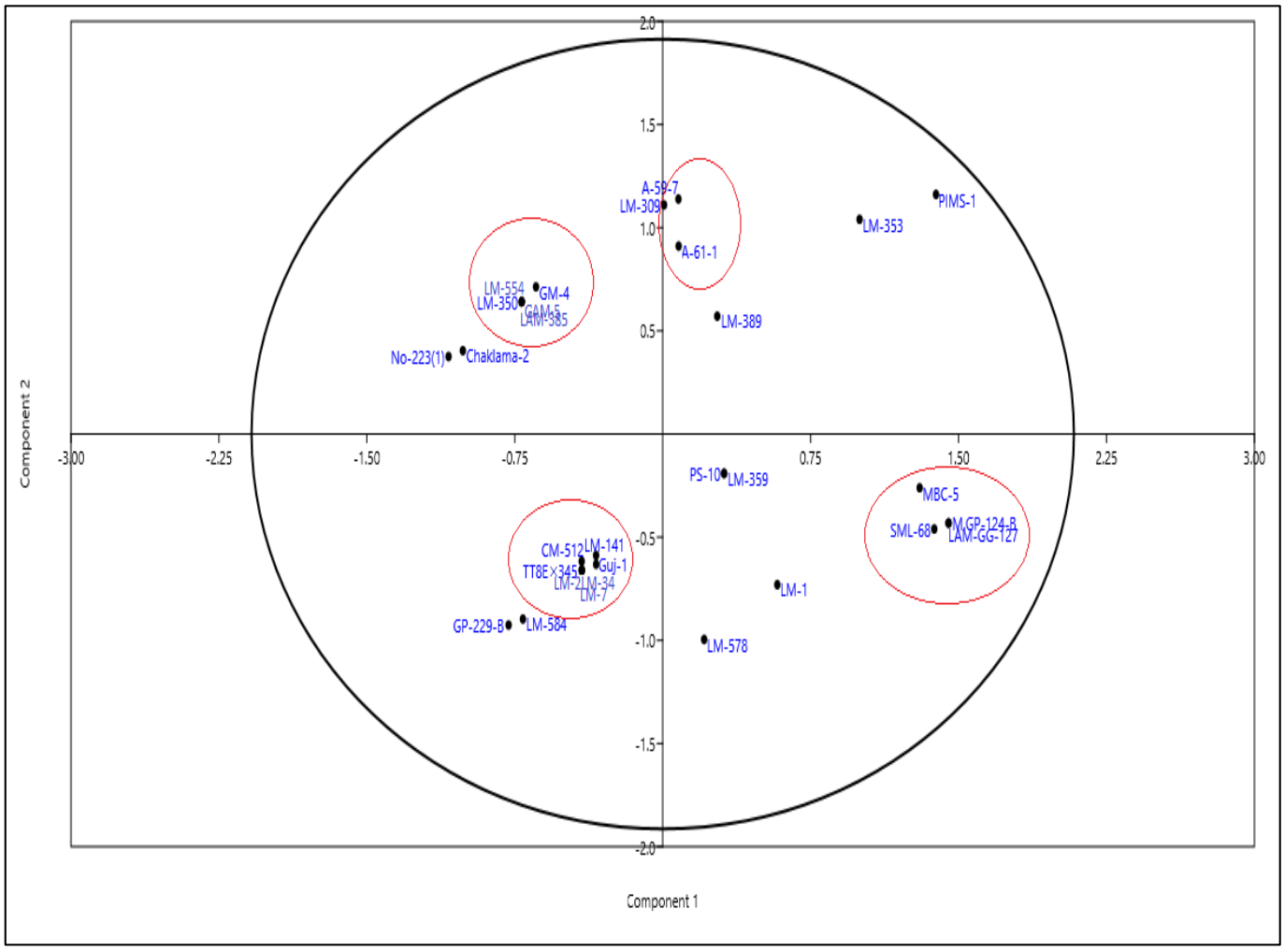

Fig 4: 2-D plot of Principal Component Analysis (PCA) showing the relationship among mungbean genotypes based on EST-SSR markers

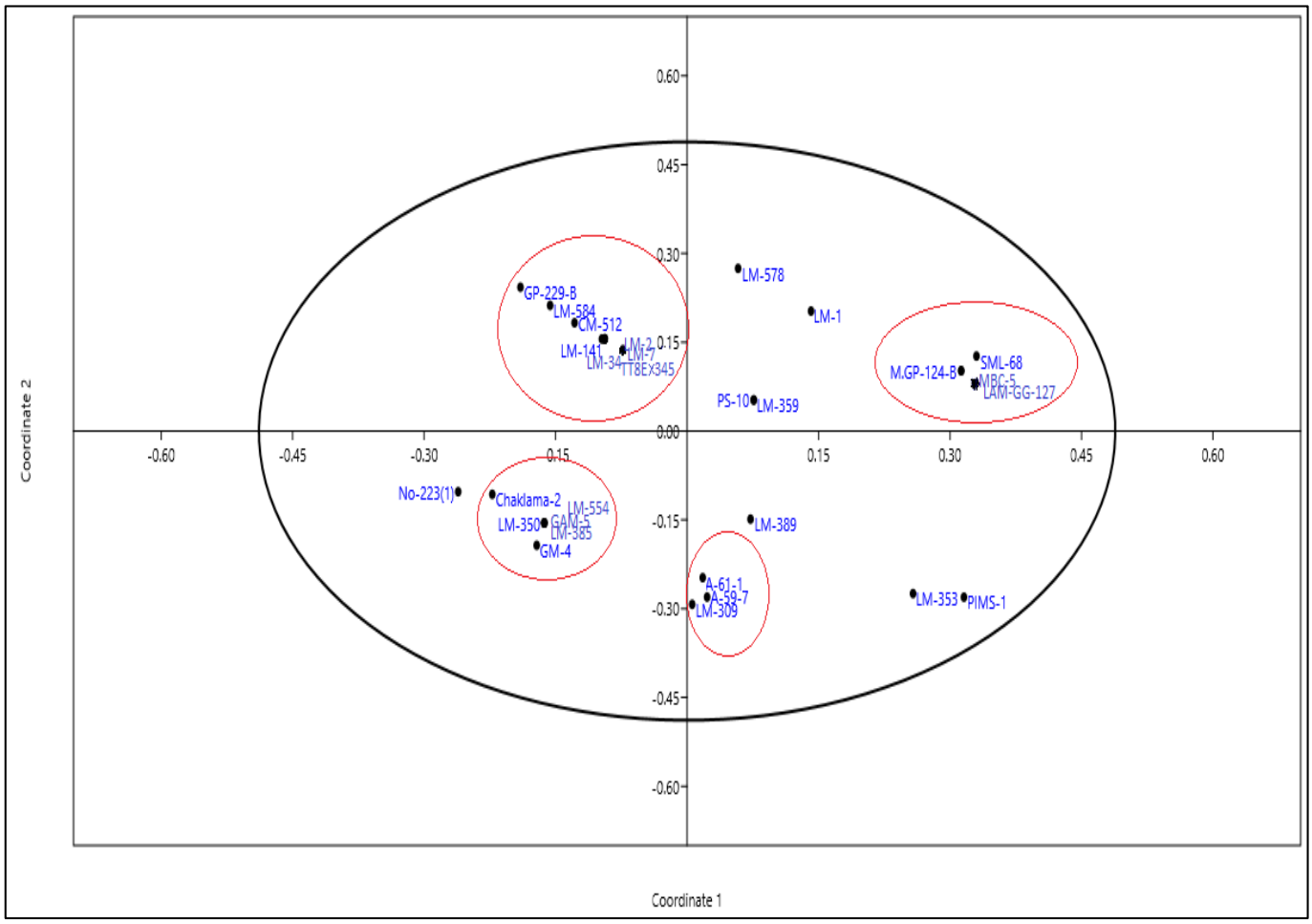

Fig 5: 2-D plot of Principal Co-ordinate Analysis (PCoA) showing the relationship among mungbean genotypes based on EST-SSR markers

Component 1 and component 2 are denoted as X and Y-axis, respectively. PIMS-1 (lowest number of seeds per pod, low number of branches per plant and high protein content) is the only genotype in the cluster A2 of dendrogram which found alone in the PCA plot (+1.12 X-axis, +1.38 Y-axis). All the 4 genotypes of the B2 cluster of the dendrogram were found far from each other. Genotype No-223 (1) found at +0.34 X-axis and -1.09 Y-axis which was early flowering, early maturing and medium heighted. Genotype LM-584 had contrasting traits (late flowering, late maturing and high height) which was found at opposite direction of No-223 (1) at -0.93 X-axis and -0.71 Y-axis. Likewise, GP-229-B (high number of seeds per pod) and LM-353 (high number of pods per plant and high protein content) were found distantly scattered.

On the basis of cluster of PCA

1. LM-554, LM-385, LM-350, GAM-5 and GM-4 falls under the same cluster in dendrogram (B1:1) and PCA (+0.62 to +0.68 X-axis, -0.66 to -0.75 Y-axis). They showed similarity in morphological characters like days to flowering, days to maturity, plant height, number of branches per plant and number of pods per plant.
2. LM-141, Guj-1, CM-512, TT8E × 345, LM-2, LM-7 and LM-34 falls under the same cluster in dendrogram (B1:2) and PCA (-0.59 to -0.65 X-axis, -0.35 to -0.41 Y-axis). They showed similarity in days to flowering, days to maturity, number of branches per plant and 100-seed weight.
3. A-59-7, LM-309 and A-61-1 falls under the same cluster in dendrogram (B1:1) and PCA (+0.90 to +1.12 X-axis, -0.02 to -0.06 Y-axis). They showed similarity in days to flowering, days to maturity and number of seeds per pod.
4. LAM-GG-127, M.GP-124-B, SML-68 and MBC-5 were in same cluster in dendrogram (A1:1) and PCA (-0.25 to -0.50 X-axis, +1.28 to +1.45 Y-axis). They showed similarity in plant height, pod length and protein content.

Some genotypes found scattered from all the group like LM-389 (medium days flowering and less protein content), LM-353 (a greater number of pods per plant and high protein content), PS-10 and LM-359 (medium heighted, less number of branches per plant and less 100-seed weight), LM-1 (early flowering, early maturity, a smaller number of branches per plant, less number of pods per plant and high 100-seed weight), LM-578 (medium heighted, a smaller number of pods per plant and less seed yield) due to their distinct characters.

PCoA is another method, somewhat similar to PCA. Four clusters/groups were formed in the PCoA analysis. Mostly similar results were found in PCoA and PCA analysis. But, Chaklama-2, LM-584 and GP-229-B were found scattered and slightly away from the clusters in PCA analysis and in the specific clusters in the PCoA analysis.

PCA and PCoA analysis showed that large diversity existed in the studied mungbean genotypes. Chen *et al.* (2015[a]) [4] also found the result of PCoA similar to UPGMA clustering.

In conclusion, the use of studied EST-SSR primers would be certainly speed-up the future work of mungbean breeding including genome mapping, varietal identification studies, genetic diversity and marker assisted selection with interesting adaptive characters.

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