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Genetic diversity analysis of common bean (*Phaseolus vulgaris* L.) based on SSR marker

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Abstract
Common bean (*Phaseolus vulgaris* L.) contribute major part of dietary protein for the millions of people across globe, thus making it one of the most legume that can be consumed directly. Study of the genetic diversity of a crop is most important step for understanding genetic variability and its utilization in breeding programmes. Fourteen genomic SSRs were employed for amplifying the genomic regions of 48 common bean (*Phaseolus vulgaris* L.) lines collected from different regions. The discriminatory power of SSRs was very high owing to their high polymorphic nature. A total of 43 alleles ranging from 2 (BM152) to 4(BM157) were amplified in 48 common bean lines with an average of 3.07 alleles per SSR. Un-weighted neighbour joining cluster analysis was performed and the constructed dendrogram divided 48 common bean lines into 5 major clusters having many sub-clusters. High polymorphism was detected among common bean lines. Most of the common bean lines collected from one region came under one group. The effectiveness of SSR markers was once again validated in this study. Diverse pair of common bean lines can be used for development of improved varieties using breeding programs.

Keywords: common bean, genomic SSRs, genetic diversity**,** cluster analysis.

1. Introduction
Common bean (*Phaseolus vulgaris* L.) is one of the most important legume for direct human consumption (Broughton *et al*., 2003) [1]. For the millions of people across globe beans are an important part of the food as they contribute major part of dietary protein (Biswas *et al*., 2010) [2]. Beans in general are plentiful source of many essential components like soluble fibre, starch, phytochemicals, vitamins and minerals, they are also found to have low fat content which increase their popularity (Svetleva *et al*., 2006) [3]. People in many countries depend on beans for around 15% of total daily calories and more than 30% of daily protein intake. Keeping in view their huge importance, common bean are subjected to various programs inorder to improve them (Hanai *et al*.,2010) [4]. It is found that there are huge variations in common beans at genetic level (Biswas *et al*., 2010) [2]. Various molecular markers are used to study genetic diversity among common beans. To construct first molecular linkage map of common bean, RFLP was used (Adam-Blondon *et al*., 1994) [5]. Several other markers mainly, RAPD, SSR's or microsatellite were used to construct their high-density linkage map, SSR markers have also been used to assess intra-specific diversity within the genus of Phaseolus (Gaitan-solis *et al*., 2002) [6]. Out of all the markers, SSR's have been used for population structure studies in various cereals such as rice, maize, wheat as well as legume crops (Liu *et al*., 2010; Zoric *et al*., 2012) [7, 8]. In order to understand population structure of common bean's 349 common bean lines which includes it's cultivated and wild accessions was performed using 26 microsatellite marker (Kwak and Gepts, 2009) [9]. A successful breeding program significantly requires anadequate knowledge of the extent and nature of genetic diversity within the crop species. The availability of genetically diverse landraces of a crop is an important genetic resource that can be used for the improvement of that crop. The evaluation of population structure and genetic diversity of germplasm could also provide valuable information for association mapping, allele mining for novel traits and crop breeding. In the present study we employed SSR markers to evaluate the efficiency of these markers in diversity analysis of common bean collected from foot hills of the Himalayan region of Jammu and Kashmir, some indegenous released and exotic varieties (NBPGR Shimla, ICAR Kanpur). Moreover, we have considered various parameters to elucidate genetic diversity among these common bean lines.

2. Materials and methods

2.1 Source of material

Forty-eightlines of common bean collected from various regions of Jammu and Kashmir specially Bhaderwah which is hot spot of common bean and some indegenous released and exotic varieties (NBPGR Shimla, ICAR Kanpur) were used in this study.

2.2 DNA extraction

Doyle and Doyle (1987) [10], method with little modifications was followed for extraction of genomic DNA from young leaf tissue of common bean lines. The DNA quantity as well as quality was checked by Nanodrop (Eppendorf). Isolated high-quality DNA was diluted to concentration of 25 ng/µL for further use.

2.3 Molecular analysis

14 SSR primers synthesized by IDT (Integrated DNA Technologies, Coralville, Iowa, USA) were used for studying polymorphism among 48 common bean lines listed in Table 1,2. 25 µL reaction mixture containing 3 µL of template DNA (25 ng/µL), 1X PCR Buffer, 2 mM MgCl2, 0.2 mM of each dNTPs (dTTPs, dGTPs, dCTPs, dATPs), 20 pico molar primer concentration, 1 U Taq DNA polymerase (Taq polymerase from *Thermus acquaticus)*, Sigma Aldrich, USA) was amplified in a 96 well Universal Gradient Thermal Cycler (Eppendorf Nexus GX2). Products were separated on a 2.5%. agarose gel along with standard molecular weight marker (100 bp ladder) (Sigma Aldrich, USA). The gel was visually examined under UV and documented using gel documentation system (Biometra). The list of SSR primers used is detailed in Table 2. SSR genotyping 14 SSR selected from Yu *et al*. (2000) [11]; Gaitan-solis *et al*. (2002) [6]; Grisi *et al*. (2007) [12]; Hanai *et al*. (2010) [4]; Cordoba *et al*. (2010) [13], were used for studying polymorphism among common bean lines. PCR products were mixed with loading dye (3–4 µL). The amplified products of some primers were resolved on 2.5% metaphor agarose gel (Sigma Aldrich, USA). PCR products resolved on metaphor agarose gel were visually examined under UV and documented using gel documentation system (Biometra). The clear and reproducible alleles amplified by each SSR among 48common bean lines were scored according to their fragment size (bp) corresponding to the 100 bp molecular weight marker (Sigma Aldrich, USA).

Table 1: Different lines of common bean and their source

Location	Genotype	Colour	Cluster in dendrogram
Bhaderwah, Doda (JK)	BR 2	Dark red	I
	BR 3	Dark red	I
	BR 4	Creamish white	I
	BR 5	Red	I
	BR 6	Painted lady	I
	BR 7	Dark red	I
	BR 8	French yellow	I
	BR 22	Red	I
	BR 31	Red	I
	BR33	Dark red	I
	BR 35	Dark red	I
	BR 39	Dark red	I
	BR 104	Dark red	I
	BR 301	Dark red	I
	BR 303	Red	I
	BR L	Dark red	I
Kupwara, Baramula (JK)	KB 1	Red	I
	KB 7	Black	IV
	KB 8	Brown	I
Shopian (JK)	S1	Dark red	I
	S 2	Dark Red	I
	S 3	Creamish white	I
	S 4	Painted lady	I
	S 5	Painted lady	I
	S 6	Dark red	I
Poonch (JK)	P 14	Painted lady	I
	P 15	Dark red	I
	P 17	Red	I
	P 27	Dark red	I
	P 28	Dark red	II
	P 33	Dark red	II
ICAR Kanpur	ARUN	Dark Red	II
	AMBER	Panited lady	II
	HUR 15	Creamish white	II
	HUR 137	Dark Red	II
	PDR 14	Painted lady	II
NBPGR Shimla (Exotic)	EC 13097	Brown	II
	EC 398527	French yellow	II
	EC 398565	French yellow	II

	EC 398591	Dark red	IV
	EC 405220	French yellow	I
	EC 500505	Black	IV
	EC 500250	Black	IV
	EC 500305	Brown	III
	EC 500507	Black	V
	EC 530898	Brown	III
	IC 199277	Dark red	I
	EC 500374	Black	V

Table 2: Genomic markers used for molecular analysis

S. No.	Primer	Primer sequence 5' →3'	Linkage Group
1	BM200	[F]TGGTGGTTGTTATGGGAGAAG [R]ATTTGTCTCTGTCTATTCCTTCCAC	1
2	BM152	[F]AAGAGGAGGTCGAAACCTTAAATCG [R]CCGGGACTTGCCAGAAGAAC	2
3	BM159	[F] GGTGCTGTTGCTGCTGTTAT [R] GGGAGATGTGGTAAGATAATGAAA	3
4	BM149	[F]CGATGGATGGATGGTTGCAG [R]GGGCCGACAAGTTACATCAAATTC	4
5	BMb742	[F] GTGATGTGATGAATTGGTGA [R] TGTGAACGTGAATGCTATGT	5
6	BM158	[F] CCGAGCGCACCGTAACTGAATGC [R] CGCTCGCTTACTCACTGTACGC	6
7	BM153	[F] CCGTTAGGGACTTGTTGAGG [R] TGACAAACCATGAATATGCTAAGA	8
8	BM154	[F] TCTTGCGACCGAGCTTCTCC [R] CTGAATCTGAGGAACGATGACCAG	9
9	BM157	[F]ACTTAACAAGGAATAGCCACACA [R] GTTAATTGTTTCCAATATCAACCTG	10
10	BM152	[F] AAGAGGAGGTCGAAACCTTAAATCG [R] CCGGGACTTGCCAGAAGAAC	10
11	BM156	[F]CTTGTTCCACCTCCCATCATAGC [R]TGCTTGCATCTCAGCCAGAATC	2
12	PVBR93	[F]TGGGGTGAGAGAGAAAGGTG [R]TACCATAGCAGGCGTTGTTG	7
13	PVBR185	[F] TGGTAAAGCAAAAACGATGG [R] GACAGAAGAGTGAGGGTGTGAA	5
14	PVBR20	[F] TGAGAAAGTTGATGGGATTG [R] TACGCTGTTGAAGGCTCTAC	6

2.4 Data analysis
The profile developed by each marker was scored (1) for the presence and (0) for the absence of a band for each genotype. Average number of polymorphic bands per unit assay were calculated according to Powell *et al*. (1996) [14]. Scored data were used for the estimation of Jaccard's similarity coefficient using NTSYS-pc version 2.02e (Rohlf, 1998) [15] package to compute pair-wise Jaccard's similarity coefficient (Jaccard, 1998) [16] and this similarity matrix was used in cluster analysis using the unweighted pair-group method with arithmetic averages (UPGMA) obtain dendrogram.

3. Results
3.1 SSR genotyping
Fourteen highly polymorphic SSR markers covering all the chromosomes of common bean (detailed in Table 2) were used to amplify genomic DNA of 48 common bean lines by setting the concentration of the components of PCR mixture and thermal profile as mentioned in materials and methods.

The banding patterns of all genotype for all the 14 markers were compared and used to generate allelic data matrix. In the absence of amplification product, data for the relevant genotype was treated as null allele (Plate 1,2,3).

SSRs marker system proved to be highly effective in discriminating the 48 common bean lines. Results obtained are summarized in Table 2. Fourteen SSR markers (genomic) used in the present study were found to be functional as they all amplified PCR product of expected size. A total of 43 alleles ranging from 2 (BM152) to 4(BM157) were amplified in 48 common bean lines with an average of 3.07 alleles per SSR. In this study, PIC value varied from 0.64 (BMb742) to 0.87 (BM159) with an average of 0.71.

3.1.2 Diversity analysis
Diversity analysis was calculated using pair-wise distances between all possible pairs of 48 common bean lines in order to identify genetic relationship among the common bean lines, the dendrogram divided them into 5 clusters (Figure 1,2). Most of the common bean lines grouped in individual cluster belonged to one region (Table 1).

4. Discussion
SSRs marker system proved to be highly effective in discriminating 48 common bean lines. Results obtained are summarized in 1. A total of 43 alleles ranging from 2 (BM152) to 4(BM157) were amplified in 48 common bean lines with an average of 3.07 alleles per SSR. In this study, PIC value varied from 0.62 (BMb742) to 0.87 (BM159) with

an average of 0.692. In earlier studies, an average number of alleles (6.0) in genic and 9.2 alleles in genomic SSR was observed (Blair *et al.*, 2006) [17], 9 alleles per genic SSR and 17 alleles per genomic SSR was observed (Blair *et al.*,2012) [18]. An average of 5.7 and 8.9 alleles per SSR were reported in Nicaragua common bean lines(Jimenez *et al*., 2012) [19]; in another study, an average of 7.14 alleles per SSR was observed and 7 alleles per SSR in common bean landraces of Brazil (Code,2008; Burle*et al.*, 2010) [20,21]. However, an average of 10 alleles per SSR was reported in two different researches on genotypes collected from East and Central Africa (Asfaw *et al.*, 2009; Blair *et al.*, 2010) [22,23]. In a recent study on common bean, a total of 423 alleles with a high mean of 19 alleles per SSR was reported by Okii and coworkers(Okii *et al*., 2014) [24]. In this study, polymorphism information content (PIC) value varied from 0.62 to 0.87 with an average of 0.71 (Table 2). The PIC values in earlier studies ranged from 0.67 to 0.740, with an average of 0.454 (Sharma *et al*., 2013) [25], and 0.30 to 0.89, with an average of 0.67 (Scaranoa *et al.*, 2014) [26].

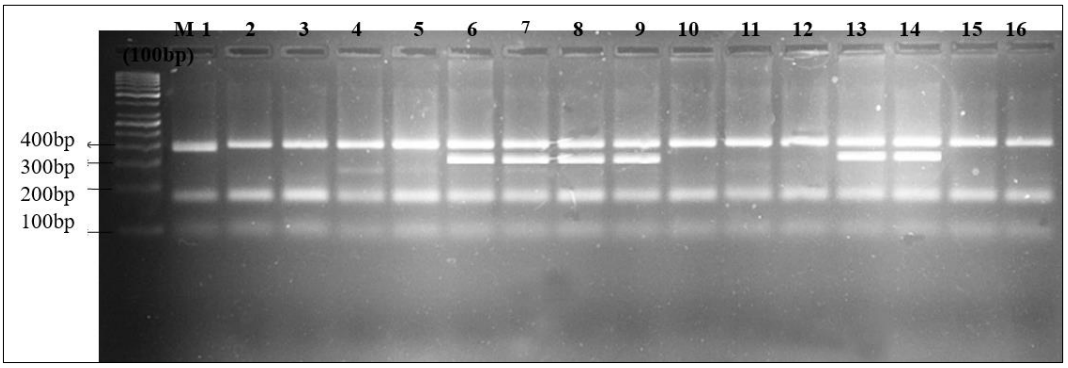

Plate 1: Agarose gel electrophoresis for marker BM 157 in different common bean lines : 100bp marker, lane 1: KB 7, lane 2:EC 500505, lane 3:EC 500250, lane 4:EC 500507, lane 5:EC 500374, lane 6:BR 2, lane 7:BR 3, lane 8: BR 7, lane 9:BR33, lane 10:BR 35, lane 11:BR 39, lane 12:BR104, lane 13: BR 301, lane 14:BR L, lane15:S 1, lane 16:S 2

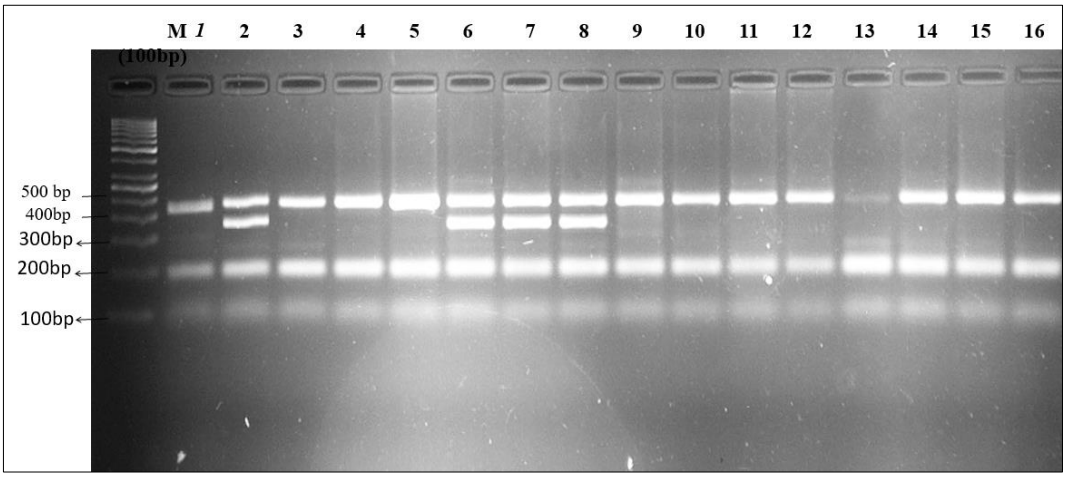

Plate 2: Agarose gel electrophoresis for marker BM 157in different common bean lines : 100bp marker,lane 1:S 6, lane 2: P 15, lane 3:P 27, lane 4: P 28, lane 5:P 33, lane 6:ARUN, lane 7: HUR 137, lane 8:EC 398591, Lane 9:S 4, lane 10:S 5, lane 11:P 14, lane 12: AMBER, lane 13:PDR 14, lane 14:BR 8, lane 15:EC 398527, lane 16: EC 398565.

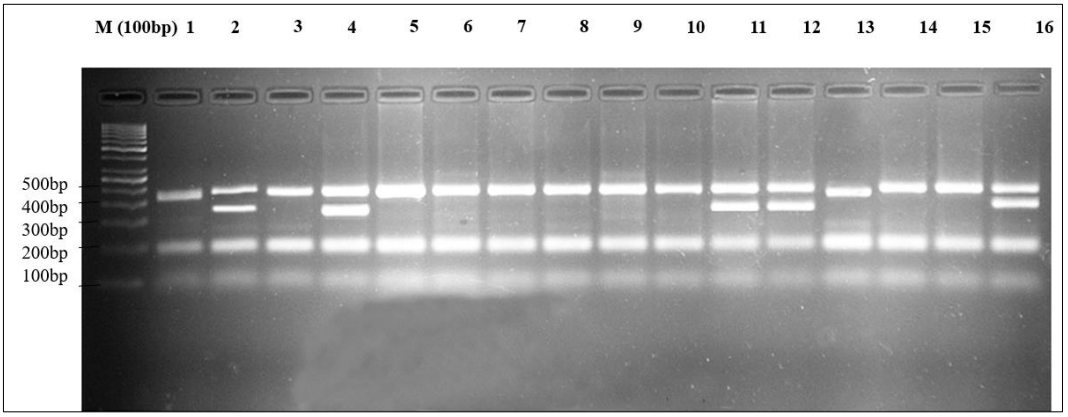

Plate 3: Agarose gel electrophoresis for marker BM 157in different common bean lines : 100bp marker, lane 1:EC 405220, lane 2:BR 4, lane 3:S 3, lane 4: HUR 15. lane 5: BR 301, lane 6:BR L, lane 7:S 1, lane 8:S 2, lane 9:S 6, lane 10: P 15, lane 11:P 27, lane 12: P 28, lane 13:P 33, lane 14:ARUN, lane 15: HUR 137, lane 16:EC 398591

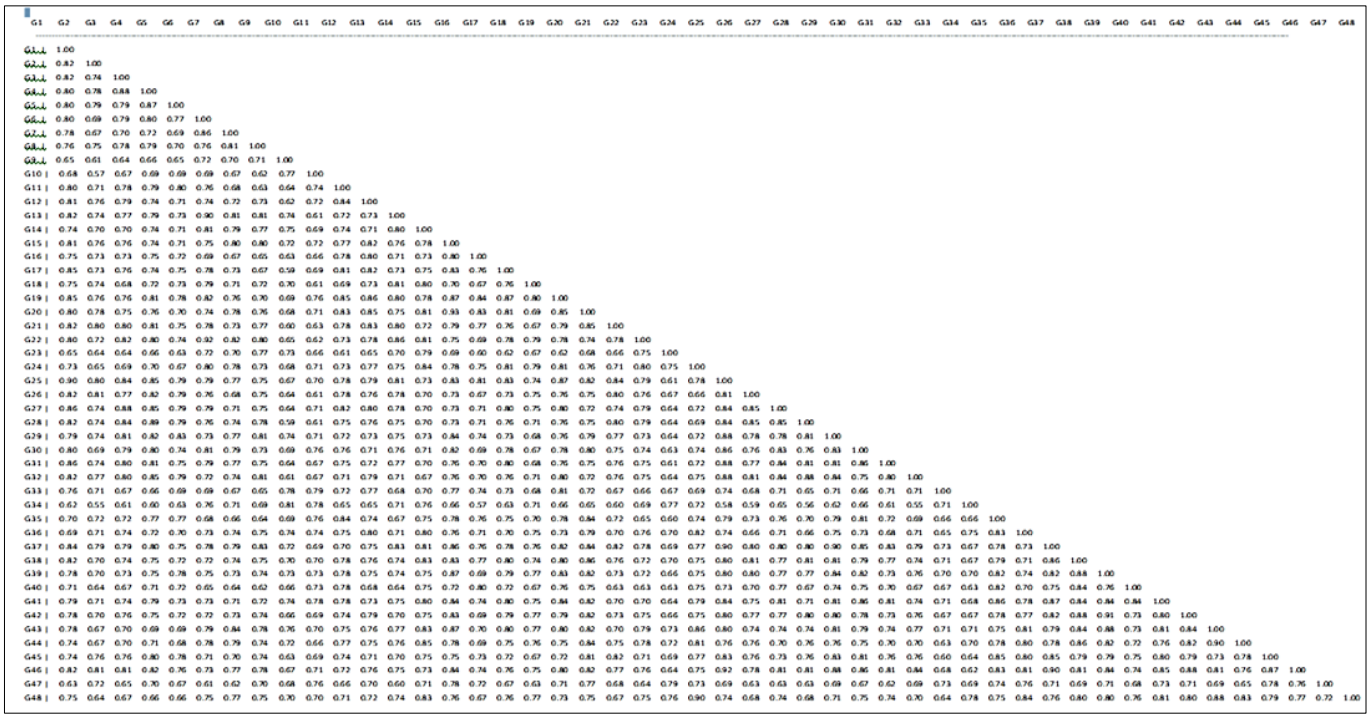

Fig 1: Jacquard's similarity coefficient based on SSR markers in 48 common bean lines

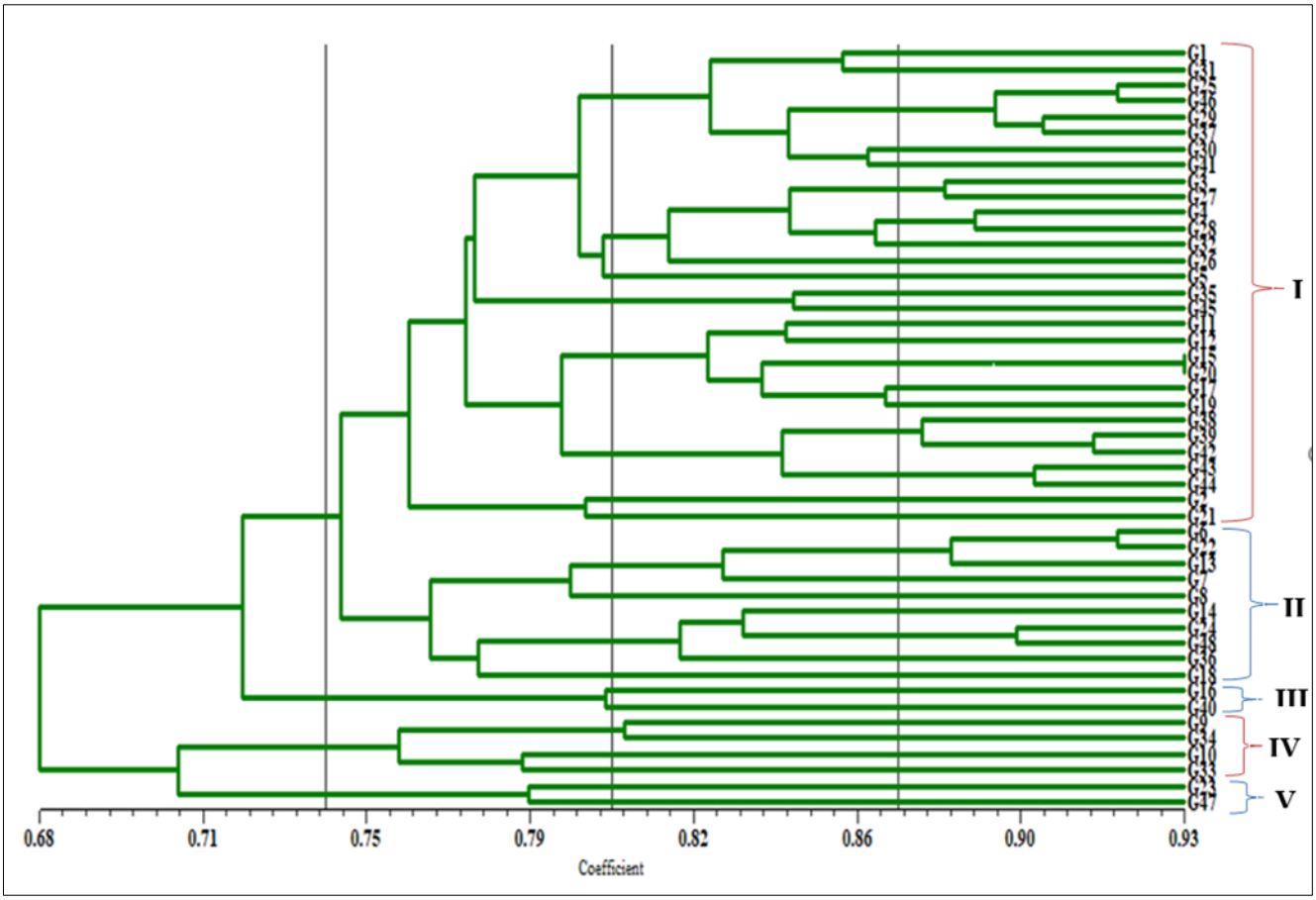

Fig 2: Dendrogram showing clustering of 48 common bean linesconstructed on UPGMA based Jacquard's similarity coefficient obtained from genomic SSR analysis.

5. Conclusion

This study was an effort to study anthocyanin-based diversity among 48 common bean lines using 14 genomic and 6 genic SSR markers. High polymorphism was detected among common bean lines. Most of the common bean lines collected from one region came under one group. The effectiveness of SSR markers for was once again validated in this study. Genome wide association studies can be done with highly

polymorphic markers. Diverse pair of common bean lines can be used for development of improved varieties using breeding programs.

6. Acknowledgment
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7. Conflict of Interest
None

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