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Molecular characterization of Indian bean (*Lablab purpureus* L.) genotypes

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Abstract
Molecular diversity of 20 Indian bean genotypes analyzed using molecular markers *viz.,* Random Amplified Polymorphic DNA (RAPD), Inter Simple Sequence Repeats (ISSR) and Simple Sequence Repeats (SSR). Indian bean genotypes were collected from Vegetable research station, Junagadh agricultural university, Junagadh.13 RAPD, 10 ISSR and 10 SSR primers produced total 78, 61 and 32 bands, respectively. RAPD and ISSR primers share 100% polymorphism, while SSR primer shares 98.33% polymorphism. Mean polymorphic content value highest for RAPD (0.72) and ISSR (0.71) as compared to SSR (0.43). SSR markers exhibit wide range of similarity (0.57-0.96) compared to RAPD and ISSR markers. SSR shows low primer index as compared to RAPD and ISSR primer. In Mental test, combined analysis of RAPD, ISSR and SSR shows r = 0.80 (good fit). Based on molecular data in the present study it can be concluded that the molecular markers could be a better tool for studying the genetic diversity.

Keywords: Indian bean, RAPD, ISSR, SSR

Introduction
Indian bean (*Lablab purpureus* L.; 2n = 2x = 22) is an important herbaceous annual plant and edible food legume in the world representing 50% of grain legumes for direct human consumption (McClean *et al.*, 2004) [17] especially in Latin America and eastern and southern Africa. Indian bean is seed propagated, true diploid (2n = 22) and have a relatively small genome (650 Mb) (Broughton *et al.*, 2003) [7]. Indian beans were domesticated in at least two major centers in Mesoamerica and the Andes (Gepts, 1988) [12] and possibly in a third minor centre in the northern Andes (Islam *et al.*, 1997) [14]. Botanically, the Indian bean is classified as a dicotyledonous legume.

Beans display a wide range of growth habits (Van and Pastor, 1987). Bush types are the most widely grown, and maturing in 60 days from seeding in a tropical climate and yielding from 700 to 2000 kg/ha on average. Climbing beans can mature in 100 to 120 days at mid-elevations and can produce the highest yields for the crop up to 5000 kg/ha.

The Indian bean is highly nutritious source of starch, protein and dietary fiber and is an excellent source of iron, potassium, selenium, molybdenum, thiamine, vitamin B6 and folic acid. The fruit and beans are edible if boiled at 110 ^{0}C. Otherwise; they are toxic due to presence of cyanogenic glycosides, glycosides that are converted into hydrogen cyanide when it consumed. Seed contain flavonoids kievitone and genistein, which play an important role in prevention and treatment of cancer (Guretzki *et al.*, 2014) [3].

Random amplified polymorphic DNA (RAPD) is PCR based which was developed by (Williams *et al.*, 1990) [33]. In the RAPD technique, DNA polymorphisms are produced using a single arbitrary primer that binds to the opposite strands of the genomic DNA template (Tingey *et al.*, 1992) [30]. The major advantages of RAPDs are the utility of universal primers (Williams *et al.*, 1990) [33] and DNA sequence information or radioactive chemicals are not required (Ragot and Hoisington, 1993) [25].

Inter-Simple Sequence Repeat **(**ISSR) involves amplification of DNA segment present at an amplifiable distance in between two identical microsatellite repeat regions oriented in opposite direction. Inter-Simple Sequence Repeat usually 16-25 bp long as primers in a single primer PCR reaction targeting multiple genomic loci to amplify different sizes of inter-SSR sequences. The microsatellite repeats used as primer can be either di-nucleotides or tri-nucleotides. ISSR markers are highly polymorphic ISSR segregate mostly as dominant markers following simple Mendelian inheritance. (Reddy *et al.*, 2002) [27].

Micro-satellite sequences are especially suited to distinguish closely related genotypes; because of their high degree of variability, they are, therefore, favored in population studies (Smith and Devey, 1994) [29] and for the identification of closely related cultivars

(Vosman *et al.*, 1992) [31]. The ISSRs are DNA fragments of about 100-3000 bp located between adjacent, oppositely oriented microsatellite regions. The ISSRs are amplified by PCR using microsatellite core sequences as primers with a few selective nucleotides as anchors into the non-repeat adjacent regions (15-18 bp). These DNA markers offer several advantages over traditional phenotypic markers, as they provide data that can be analyzed objectively.

Materials and Methods

Plant material

The experimental material consisted of total 20 genotypes of Indian bean, which were collected from Vegetable Research Station, Junagadh Agricultural University, Junagadh; which Listed in table-1.

DNA isolation

Total genomic DNA extraction was carried out by CTAB method as described by Doyle and Doyle (1987) [10] with minor modifications as per Chaudhary *et al.* (2008) [9]. The quantity and quality of the isolated DNA was determined by using Nanodrop Spectrophotometer (Thermo Scientific, U.S.A.). Dilutions of 50 ng/µl of each genotype were prepared and stored at 4 °C for further use in PCR analysis.

RAPD analysis

RAPD was performed according to method given by Biswas *et al.* (2010) [6] with some modifications. The PCR master mix (25µl) contained 10x PCR buffer (10 mM Tris-HCl, pH 8.3), 100 mM each dNTPs, 25 pmoles primer, 50 ng of genomic DNA and 3 unit of Taq DNA polymerase. The samples were subjected to 45 repeats of the following cycle: 94 °C 1 min, 37°C for 1 min, 72 °C for 2 min with an initial denaturation of 5 minutes and a final extension of 10 minutes.

ISSR analysis

PCR reactions were performed as per Saravanan *et al.* (2013) [28] with required modifications. The PCR master mix (25µl) contained 10x PCR buffer (10 mM Tris-HCl, pH 8.3), 100 mM each dNTPs, 25 pmoles primer, 50 ng of genomic DNA and 3 unit of Taq DNA polymerase. The samples were subjected to 40 repeats of the following cycle: 94 °C 1 min, 55°C for 1 min, 72 °C for 2 min with an initial denaturation of 5 minutes and a final extension of 10 minutes.

SSR analysis

PCR reactions for SSR were carried out in a reaction volume of 25 µl as per method stated by Cabral *et al.* (2011) [8] with necessary modifications. The PCR master mix (25µl) contained 10x PCR buffer (10 mM Tris-HCl, pH 8.3), 100 mM each dNTPs, 25 pmoles forward and reverse primer, 50 ng of genomic DNA and 3 unit of Taq DNA polymerase. The samples were subjected to 35 repeats of the following cycle: 94 °C 1 min, Tm ± 2 °C for 1 min, 72 °C for 2 min with an initial denaturation of 4 minutes and a final extension of 7 minutes.

All the above PCR amplification (RAPD and ISSR) were performed in 0.2 ml thin-walled PCR tubes placed in a thermal cycler. The products of both RAPD and ISSR were analyzed by electrophoresis in 1.5% agarose gel stained in ethidium bromide (10 mg/ml) and run in 1x TBE buffer at 100 V for 2 h. The separated bands were visualized under UV transilluminator and photographed using a gel documentation system (Alpha Innotech).

Statistical analysis

Clear and distinct bands amplified by RAPD, ISSR AND SSR primers were scored for the presence (1) and absence (0) for the corresponding band among the genotypes. The binary data were subjected to UPGMA (Rohlf 2000) [24] analysis using NTSYSpc version 2.02 (Anderson *et al.* 1993) [4].

Results and discussion

The yield of DNA isolated ranged from 257.2 ng/µl in GP-156 to 1301.6 ng/µl in GP-154 with optical density near about 1.70 (GP-156) to 2.08 (Gujarat Papdi -1) indicated that DNA extracted was pure.

RAPD analysis

Total 30 RAPD primers were used for amplification of Indian bean genotypes. Among 30, 13 primers give a good amplification. The banding patterns of 13 genotypes shown in table 2. All the thirteen RAPD primers amplified a total of 78 bands/alleles. Thirteen RAPD primers produced total nine unique polymorphic bands. Two unique polymorphic bands were observed in genotype GP-153 and GP-164 by OPB-04 primer and in genotype GP-161 and Gujarat papdi-1 by S-67 primer. One unique band was observed in genotype GP-165 by OPB-17 primer (Fig.1); in genotype GP-165 by OPD-20 primer (Fig.2); in genotype GP-164 by OPG-14 primer; in genotype GP-140 by OPY-08 primer; in genotype GP-151 by A-10 primer. From the data, 100% polymorphic bands were observed. The RAPD primer OPB-04 produced highest 12 bands (alleles) followed by 11 bands in OPD-20; nine bands in OPB-17, while OPA-09 produced only two bands. All the RAPD primers exhibited 100% polymorphism.

In present study, the amplified fragments were ranged from 112 bp (OPB-04) to 3847 bp (A-10). Similarly, Fillimon *et al.* (2011) observed amplicon numbers per primer ranged from 6 (OPY-20) to 11 (OPD-08) and varied in size between 378 bp and 3250 bp in common bean.

The highest PIC value of 0.89 was recorded by OPD-20, while lowest PIC value of 0.58 was recorded by OPA-09 and OPA-17. Similarly RAPD primer index (RPI) ranged from 1.00 to 10.56 with an average of 4.52 per primer. The highest RPI value was obtained by OPB-04 and the lowest was obtained by OPA-09 (Table 2).

Jaccard's coefficient of similarity of 20 Indian bean genotypes ranged from 10.0% (between GP-155 and GP-159) to 62.2% (between GP-157 and GP-161). Similarly, Asifa *et al.* (2015) classified fourty-five genotypes of common bean cultivars into seven groups and To test the goodness of fit of the clustering of RAPD data, matrixes of cophenetic values were also computed using the program COPH. In the present study also the Mantel test statistic-Z was normalized and degree of goodness of fit for a cluster analysis (Matrix correlation r = 0.84) was found to fall under the category of "good fit", as categorized by Rohlf (2000) [24].

ISSR analysis

Out of fifteen ISSR primers screened, 10 ISSR primers amplified a total of 61 bands. All bands/alleles were polymorphic. Out of 61 polymorphic bands, 47 bands were shared polymorphic and 14 bands were unique polymorphic bands. Out of 14 unique polymorphic bands, one unique polymorphic band was observed, in genotype GP-154 by ISSR 05 primer; in genotype GP-164 by UBC-888 primer; in genotype GP-156 by Oligo-07 primer. Similarly, two unique bands were recorded in GP-151 and GP-155 by ISSR-836 primer. Likewise, three unique bands were observed in

genotype GP-152, GP-158, GP-159 by ISSR 885 (Fig.4); in genotype GP-157, GP-158 by Oligo-03(Fig.5); In GP-140, GP-156 and GP-164 and by PV gaatt001 primer. From the ISSR data, 100% of polymorphic bands were observed (Table 3). Oligo-03 primer produced highest 10 bands (alleles) followed by eight band in primer PVgaatt001, while ISSR-885 produced only two bands. Similarely, Niharika *et al.* (2010) [19] examined twelve ISSR primers which produced 94 bands in vigna genotypes, of whch 75 were polymorphic with an average of 6.25 fragments per primer. Percentage polymorphism ranged from 55.55% (4824-039) to 90% (4824-047), with an average of 79.5%.

Largest amplified fragment of 2738 bp and the smallest fragment of 204 bp were amplified by PVgaatt001 and UBC-888, respectively. Similarly, Pardhe and Satpute (2011) [21] worked with three ISSR primers which produced 37 bands in the *Vigna* genotypes with size ranged between 125 bp and 1500 bp and amplified product sizes ranged from 100 to 1500 bp.

The highest PIC value of 0.88 was noticed in Oligo-03, while lowest PIC value of 0.49 was noticed in ISSR-888 with an average of 0.7988 per primer. Likewise, ISSR primer index (IPI) ranged from 1.96 to 8.80 with an average of 4.54 per primer. The maximum IPI value was obtained by Oligo-03 and the minimum was obtained by UBC-888 (Table 4.4). Similar findings were also reported by Abadio *et al.* (2012) [1].

Jaccard's coefficient of similarity between 20 Indian bean genotypes ranged from 3.80% (between GP-158 and GP-165) to 57.8% (between GP-153 and GP-154). Similar findings were reported by Aryanegad *et al.* (2013) [5]. They observed Jaccard's coefficient of similarity between *Trifolium* species varied from 28.6% to 77.5%. This supports the data obtained in the present study

In the present investigation, twenty Indian bean genotypes were grouped into two main clusters, cluster I and cluster II with an average similarity of 8.00%.The cluster I consisted of nineteen genotypes and these were further divided into sub cluster IA and IB with eighteen and one genotype, respectively. The sub cluster IA(i) had only two genotype (GP-140 and GP-158). Similarly, sub cluster IA(ii) was again divided into two cluster IA(ii)a with eleven genotypes namely GP-151, GP-152, GP-153, GP-154, GP-156, GP-155, GP-161, GP-160, Gujarat Papdi -1, GP-164 and GP-157, while sub cluster IA(ii)b had five Indian bean genotypes namely GP-163, GJIB-11, GJIB-2, Swarna Utskrit and GP-165.On the other hand, sub cluster IB was solitary cluster with one genotype (GP-162).The cluster II consist only one genotype (GP-159) (Figure 6).

Similar findings was also reported by Perez *et al.* (2015) [22], while analyzing the genetic variability among 39 Faba bean accessions using 12 ISSR markers. They utilized 12 ISSR primers which produced 142 bands, out of which 134 were polymorphic. The values of resolution power (R_p), Polymorphic information content (PIC) and marker index (MI), respectively indicated that primer 848 was most efficient to analyze genetic variability with values of 12.8, 0.40 and 0.86, respectively, followed by primers 857 and ISSR2M. The dendrogram showed six principal groups (I, II, III, IV, V and VI). Groups III and IV were integrated by accessions P25 and P29, respectively from Santiago Tianguistenco and Metepec. Which indicated that both accessions were significantly different at molecular level in relation other the accessions.

To test the goodness of fit of the clustering of ISSR data, matrixes of cophenetic values were also computed using the program COPH. In the present study, the Mantel test statistic-Z was normalized and degree of goodness of fit for a cluster analysis (Matrix correlation: r = 0.76) was found to fall under the category of "poor fit" as categorized by Rohlf (2000) [24].

SSR analysis

Out of twenty SSR primers screened, 10 ISSR primers amplified a total of bands out of which 32 bands/alleles were polymorphic. Out of 32 polymorphic bands, 26 bands were shared polymorphic and five unique polymorphic bands were observed; one unique band observed in genotype by SSR IAC-16, BMd-12, VM 38, AGB 8, BM173 AF483885 primers, respectively. From the data it was observed that 1.67% monomorphic bands and 98.33% polymorphic bands were observed.

AGB 8 primer produced highest six bands (alleles) followed by five bands in primer of VM38 and GATS911 AF48384, while BM142 produced only one band (allele). 100% polymorphism was obtained with nine primers except AGB-8 (83.33%).

The amplified SSR fragments ranged from 100 bp to 945 bp. The largest fragment of 945 bp and the smallest fragment of 100 bp were amplified by AGB-8 and BMd-17, respectively. The polymorphic information content (PIC) was calculated for each primer (Table 4). The polymorphic information content ranged between zero and 0.75. The highest PIC value of 0.75 was noticed GATS911 AF483842, while lowest PIC value of zero was noticed in BM-142 and PVgaat001 with an average of 0.4380 per primer. Likewise, SSR primer index (SPI) ranged from zero to 4.80 with an average of 1.85 per primer. The maximum SPI value was obtained by AGB-8 and the minimum was obtained by BM-142 and PVgaat001.

Jaccard's coefficient of similarity between 20Indian bean genotypes ranged from 57.1% (between GP-140 and GJIB-11) to 96.4% (between GJIB-2 and GJIB-11). Similar finding was reported by Perseguini *et al.* (2011) [23]. They observed Jaccard's coefficient of similarity between common bean accessions varied from 37 to 63%. This supports the data obtained in the present study.

Similar findings were also reported by Neetu and Chawla (2014) [18], studied eleven French bean varieties (*Phaseolous vulgaris* L.) using SSR markers utilizing ten SSR primers. It revealed 32 alleles in 11 varieties with an average of 3.2 alleles per locus. The number of alleles per locus varied from 1 to 5. The size of allele amplified varied from 50 to 500bp. Cluster analysis was done using pairwise Jaccard's similarity coefficient with similarity value ranges from 37% to 94%. The PIC value in the present study ranged from 0 to 0.79, with an average of 0.37 per primer pair. Specific loci were obtained in four local varieties namely Chakrata Local PGR FB 2-07, Bhura Lal Swant, Lal Thumri Swant and Chitkabra Lal Jhulu Swant by 10 SSR primers which could unambiguously discriminate these varieties. This study showed that for establishing distinctiveness in varieties, besides morphological characters, some other DNA based markers might also be considered.

Dendogram cluster I consisted of two genotype namely GP-140 and GP-154 and cluster II consist eighteen genotypes. These genotypes were further divided into sub cluster IIA and IIB with fifteen genotypes and three genotypes (GP-154, Gujarat Papdi-1 and GP-160) respectively. The sub cluster IIA(i) had only seven genotypes and sub cluster IIA(ii) consisted of eight genotypes (Figure 10). Similar finding was also reported by Uma *et al.* (2015) [32]. She studied 64 french bean with 15 SSR primers show high dissimilarity among the

French bean genotypes. Cluster diagram constructed using polymorphic markers identified two major clusters. All exotic collections, landraces, local types and released varieties were grouped in same cluster with some indigenous collection, whereas cluster A comprised only of indigenous collection.

To test the goodness of fit of the clustering of SSR data, matrixes of cophenetic values were also computed using the program COPH. In the present study, the Mantel test statistic-Z was normalized and degree of goodness of fit for a cluster analysis (Matrix correlation: r = 0.77) was found to fall under the category of **"**poor fit" as categorized by Rohlf (2000) [24]. Similarly finding was reported by Akash *et al.* (2017) [2].

Clustering pattern between RAPD, ISSR and SSR combined data

Dendrogram developed by Jaccard's similarity coefficient and UPGMA method showed the highest (66.3%) similarity between GP-157 and GP-161 and the lowest (21.8%) similarity between GP-140 and GJIB-2.The dendrogram consisted of two main clusters I and II with an average similarity of 37%. The cluster I and cluster II consisted of seventeen genotypes and three genotypes, respectively. The cluster I was further sub divided into two sub cluster, cluster IA with fourteen genotypes as well as cluster IB with three genotypes namely GP-162, Gujarat Papdi-1, GP-165. The sub cluster IA was again divided into two clusters, cluster IA (i) with three genotypes namely GP-140, GP-158, GP-159 and IA (ii) with eleven genotypes. The sub sub cluster IB was again divided into two cluster IB (i) with two genotypes *viz*., GP-162 and Gujarat Papdi-1. While sub cluster IB (ii) had one solitary genotypes (GP-165). Likewise, cluster II was again subdivided into two sub cluster IIA with one genotype (GP-155) and IIB with two genotypes namely GP-156 and GJIB-2 (Figure 11).

From the above data it has been concluded that the data revealed by RAPD and ISSR (100.00%) produced higher percentage of polymorphic loci, greater range of genetic distance for discriminating among genotypes than that SSR (98.33%). However, more PIC and slightly higher percentage of polymorphism was amplified by ISSR and RAPD markers as compared to SSR markers. High level of genetic distance means low similarity which is helpful to choose the genotypes with diverse characters for crop improvement programme.

Similarly finding by Saravanan *et al.* (2013) [28]. They studied genetic diversity and identification of superior genotypes are the basic objectives of any crop improvement program. In the present study, two multiloci markers *viz*., RAPD (51), ISSR (15) and single locus marker SSR (10) were used to assess the genetic diversity among 39 genotypes of lablab bean. The analysis based on markers RAPD, ISSR and SSR markers clustered the genotypes into 5, 3, and 6 clusters, respectively. Based on cluster analysis, variety EC7467/1 was highly divergent from the varieties EC10190, EC10192, POOLUVAMPATTI, DINDIGUL LOCAL, and PLS90. Hence, breeding programme involve these genotypes as parent would throw useful recombinants for further exploitation. RAPD dendrogram revealed more relatedness between variety EC7467/1 and EC10192.

Table 1: List of Indian bean genotypes used in present study

Sr. No.	Name of genotypes	Sr. No.	Name of genotypes
1.	GP – 140	11.	GP – 160
2.	GP – 151	12.	GP – 161
3.	GP – 152	13.	GP – 162
4.	GP – 153	14.	GP – 163
5.	GP – 154	15.	GP – 164
6.	GP – 155	16.	GP – 165
7.	GP – 156	17.	GJIB – 2
8.	GP – 157	18.	GJIB – 11
9.	GP – 158	19.	Guj. Papdi – 1
10.	GP – 159	20.	Swarna Utskrist

Table 2: Size, total of amplified bands, per cent polymorphism, PIC and RPI obtained by RAPD primers

Sr. No.	RAPD Primers	5'-3' Sequence	Allele / Band size (bp)	Total No. of Allele / bands (A)	No. of Unique Polymorphic bands (B)	PIC value	RPI (PIC×A)
1	OPA-09	GGGTAACGCC	548-976	2	0	0.5000	1.0000
2	OPA-17	CACCGCTTGT	587-1046	3	0	0.5845	1.7535
3	OPB-04	GGACTGGAGT	112-1038	12	2	0.8804	10.5648
4	OPB-17	AGGGAACGAG	138-3598	9	1	0.8642	7.7778
5	OPD-20	GGATGAGACC	156-3425	11	1	0.8937	9.8307
6	OPG-14	ACCGGCTCAC	344-2736	6	1	0.6873	4.1238
7	OPY-08	AGGCAGAGCA	456-3436	5	1	0.6994	3.4970
8	OPY-20	AAGCGGCCTC	508-2756	4	0	0.6736	2.6944
9	OPZ-13	ACTAAGCCC	354-2243	6	0	0.7842	4.7052
10	A-06	TCCCGCCTCA	113-827	5	0	0.7222	3.6110
11	A-10	GGCTTGGCCT	424-3847	4	1	0.6163	2.4652
12	Q-04	AGTGCGCTGA	448-2356	4	0	0.7049	2.8196
13	S-67	GTCCGACGA	196-789	6	2	0.6097	3.6582
Total				78	9	-	-
Average				-		0.7289	4.9558

Table 3: Size, total of amplified bands, per cent polymorphism, PIC and RPI obtained by ISSR primers

Sr. No.	ISSR Primers	5'-3' Sequence	Allele/ Band size (bp)	Total No. of Allele/ bands (A)	No. of Unique Polymorphic bands (B)	PIC value	IPI (PIC×A)
1	ISSR-05	AGAGTTGGTAGGTCTTGATC	442-1041	7	1	0.8320	5.8240
2	ISSR-17	TCTCTCTCTCTCTCTCRG	344-1472	6	0	0.7388	4.4328
3	ISSR-836	CGTACTCGTGAGAGAGAGAGAGA	289-976	6	2	0.7029	4.2174
4	ISSR-885	CGTACTCGTGAAGAGAGAGAGA	248-407	5	3	0.5950	2.9750
5	UBC-880	GGA GAG GAG AGG AGA	436-1244	4	0	0.6556	2.6224
6	UBC-888	CGTDCGTCACACACACACACA	204-742	4	1	0.4931	1.9724

7	Oligo-03	CTCTCTCTCTCTCTCTG	344-2736	10	3	0.8802	8.8020
8	Oligo-07	TCTCTCTCTCTCTCTCA	496-2346	7	1	0.7675	5.3725
9	BM-154	TCTTGCGACCGAGCTTCTCC	412-723	4	0	0.7096	2.8384
10	PV-gaatt001	AAGGATGGGTTCCGT GCTTG	310-2738	8	3	0.8000	6.4000
Total				61	14		
Average				6.10		0.7174	4.5456

Table 4: Size, total of amplified bands, per cent polymorphism, PIC and RPI obtained by SSR primers

Sr. No.	Markers		Sequence (5' – 3')	Tm (^{0}C)	Allele/ Band size (bp)	Total No. of Allele/ bands (A)	PIC value	SPI Index (PIC×A)
1	SSR-IAC 16*	F	TGTAACGCCCAGATTTG	63.2	296-347	4	0.5680	2.2720
		R	GTTTGCACTCCGACGAT	60.4				
2	BMd 12*	F	CATCAACAAGGACAGCCTCA	66.2	176-445	2	0.1172	0.2344
		R	GCAGCTGGCGGGTAAAACAG	48.2				
3	BMd-17	F	GTTAGATCCCGCCCAATAGTC	63.4	100-192	3	0.5952	1.7856
		R	AGATAGGAAGGGCGTGGTTT	58.3				
4	BM-142	F	TTCCGCTGATTGGATATTAGAG	62.4	164	1	0.0000	0.0000
		R	AGCCCGTTCCTTCGTTTAG	64.6				
5	VM-38	F	AATGGGAAAAGAAAGGGAAGC	57.5	142	5	0.7378	3.6890
		R	TCGTGGCATGCAGTGTCAG	51.8				
6	AGB 8*	F	CACCGGGAGTGGCTGACA	58.5	122-416	6	0.8000	4.8000
		R	GTTTGGGGCGGAGTTCGA	61.9				
7	PV-ctt001*	F	GAGGGTGTTTCACTATTGTCACTGC	67.5	124-195	2	0.4234	0.8468
		R	TTCATGGATGGTGGAGGAACAG	55.8				
8	PV gaat001	F	AAGGATGGGTTCCGTGCTTG	68.1	96-176	1	0.0000	0.0000
		R	CACGGTACACGAAACCATGCTATC	59.7				
9	BM173 AF483885*	F	ATGCGTAAGCGAGGGAGAG	56.9	137-196	3	0.3787	1.1361
		R	CCAGTATATAACCGCTGCTGCTG	59.6				
10	GATS911 AF483842	F	GAGTGCGGAAGCGAGTGAG	53.5	110-362	5	0.7599	3.7695
		R	TCCGTGTTCCTCTGTCTGTG	57.0				
Total						32		
Average						3.20	0.4380	1.8533

PIC = Polymorphism information content; **RPI** = RAPD Primer Index; **IPI =** ISSR Primer Index; **SPI** = SSR Primer Index; *Shows single unique band

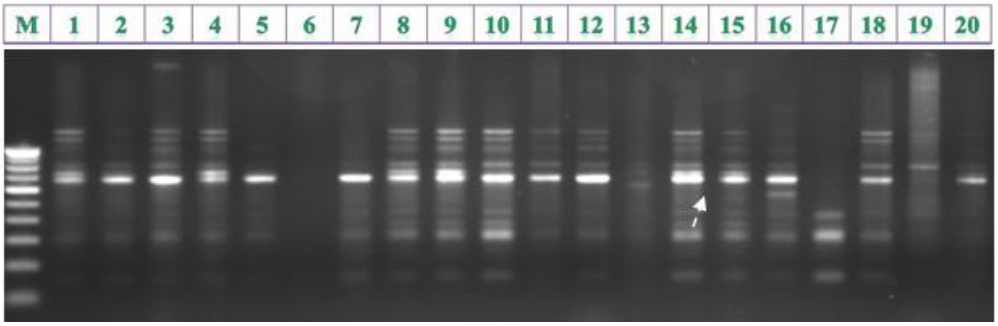

Fig 1: RAPD marker profile amplified by OPB-17 in Indian bean genotypes

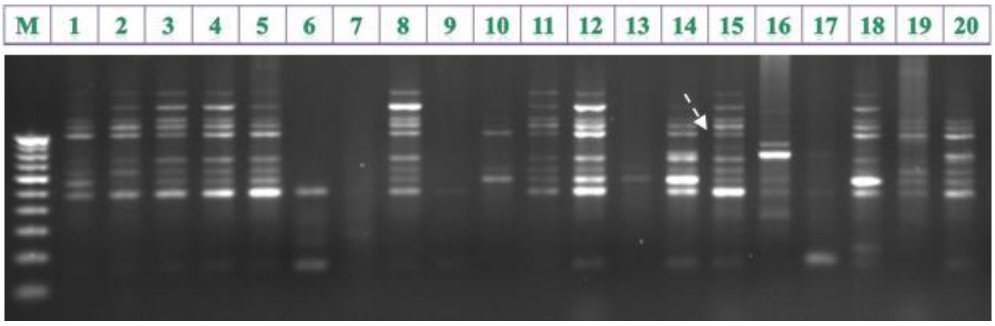

Fig 2: RAPD marker profile amplified by OPD-20 in Indian bean genotypes

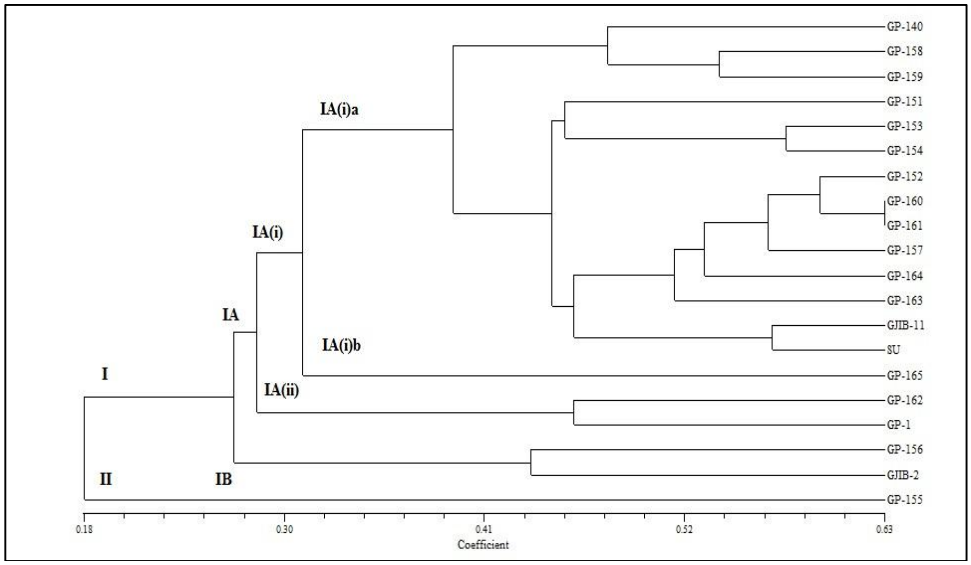

Fig 3: Dendogram depicting the genetic relationship among 20 Indian bean genotypes based on RAPD Marker

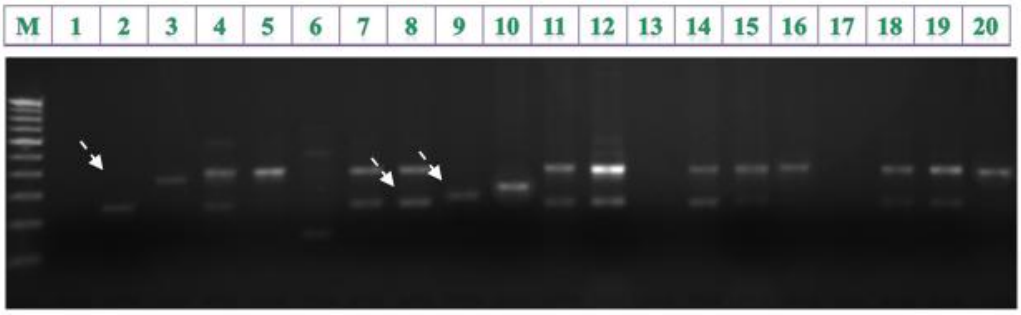

Fig 4: ISSR marker profile amplified by ISSR- 885 in Indian bean genotypes

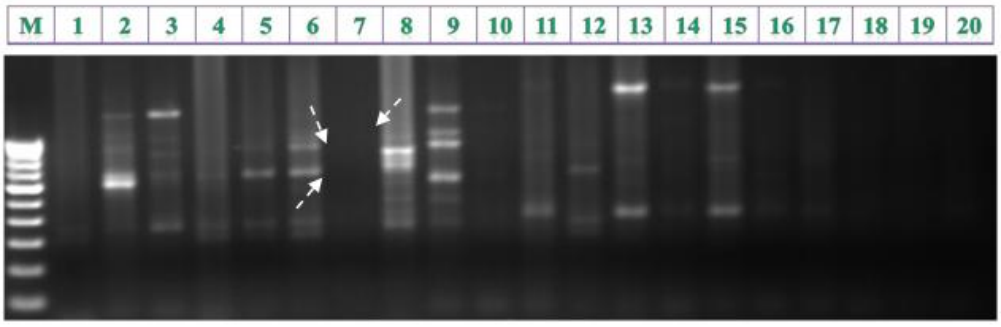

Fig 5: ISSR marker profile amplified by Oligo-03 in Indian bean genotypes

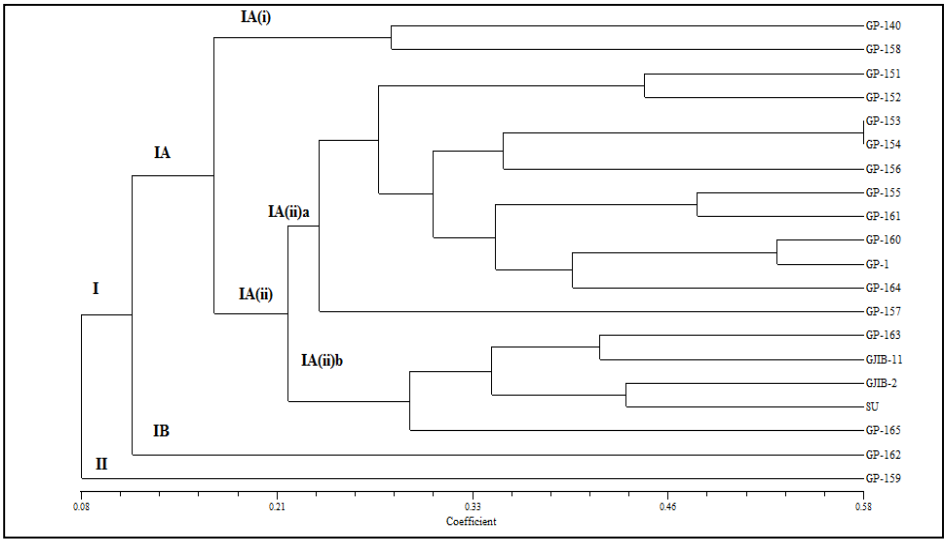

Fig 6: Dendogram depicting the genetic relationship among 20 Indian bean genotypes based on ISSR Markers

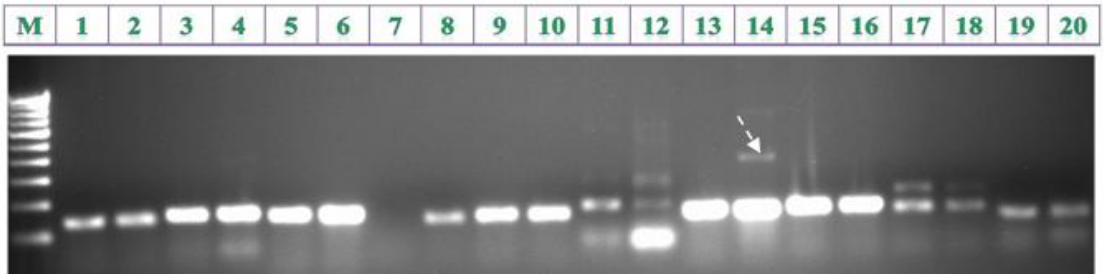

Fig 7: SSR marker profile amplified by VM 38 in Indian bean genotypes

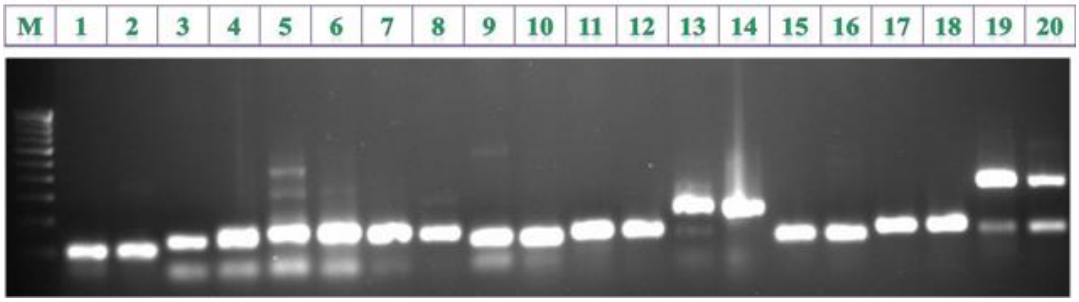

Fig 8: SSR marker profile amplified by GATS911 AF483842 in Indian bean genotypes

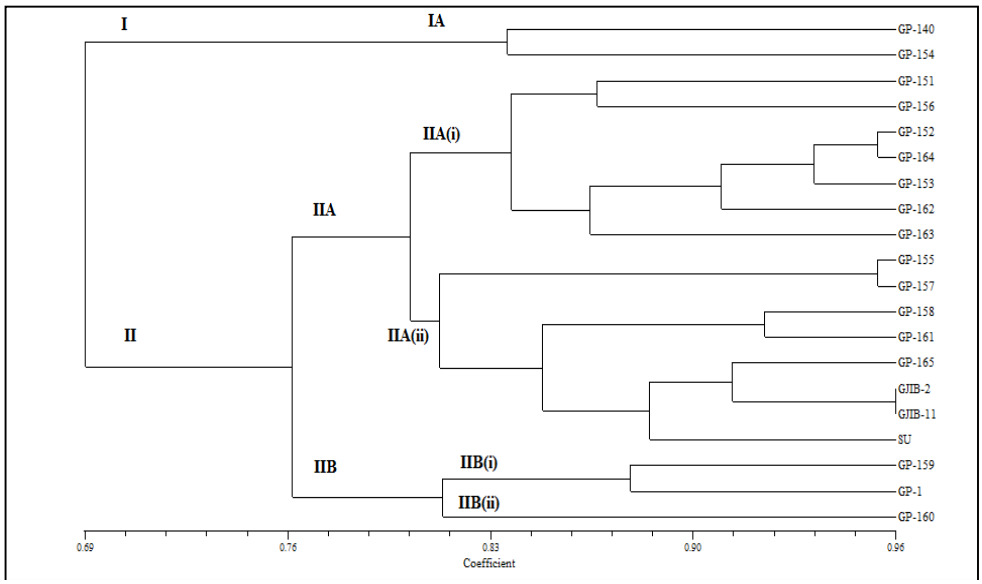

Fig 9: Dendogram depicting the genetic relationship among 20 Indian bean genotypes based on SSR markers

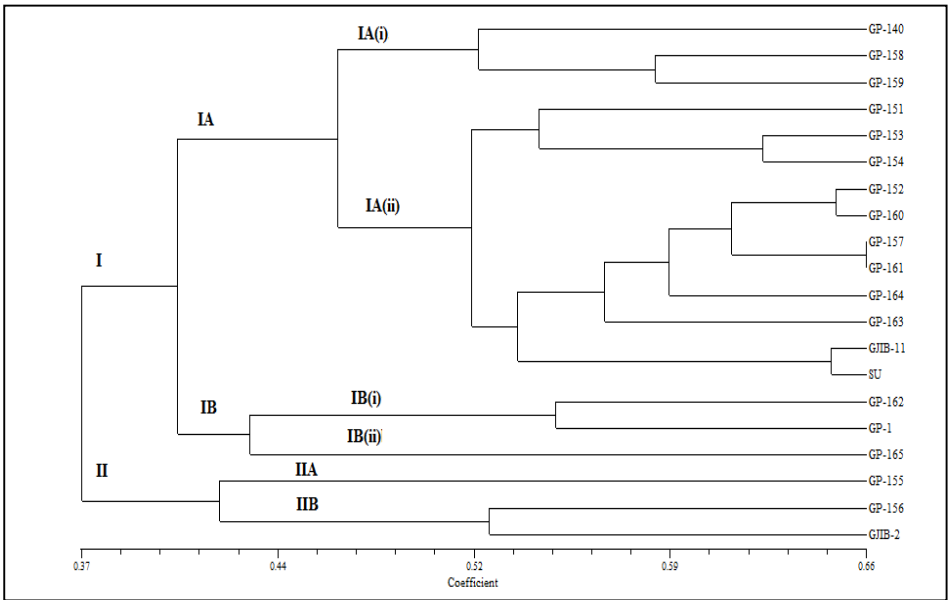

Fig 10: Dendogram depicting the genetic relationship among 20 Indian bean genotypes based on pooled data of RAPD, ISSR and SSR markers

Conclusion

Among the studied techniques, RAPD and ISSR primers gave 100% polymorphism. However, more number of polymorphic fragments, more PIC and higher percentage per primer was amplified by RAPD and ISSR as compared to SSR markers. Both RAPD and ISSR gave distinct clustering patterns. Based on molecular data in the present study it can be concluded that the molecular markers could be a better tool for studying the genetic diversity.

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