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Genetic Divergence in French Bean Accessions

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ABSTRACT

Background: Crop improvement has paramount importance to meet the global demand of food to feed the growing human population. **Methods:** A total of 23 French bean genotypes with diverse genetic background were taken to assess their phenotypic attributes and molecular characterization using inter simple sequence repeat (ISSR) markers. The data collected during two growing seasons over four locations on different quantitative characters were pooled and analyzed under randomized complete block design for deciphering the extent of genetic diversity. Similarity value, based on Jaccard's coefficient and UPGMA (un-weighted pair group method with arithmetic mean) analysis was calculated. The dendrogram was constructed using Jaccard's similarity matrix for above analysis. **Result:** The genotypes showed a wide variation in morphological characters like plant height, pods per plant, pod weight, pod length and pod yield per plant. The genotype HAFB-5 showed the maximum potential for pod yield per plant and long pods. Four ISSR primers used in this study amplified 27 reproducible bands with 18 (66.66%) polymorphic. The genotype Kashi Param and HAFB-4 were found diverse among the selected genotypes on the basis of molecular markers.

Key words: Dendrogram, French bean, ISSR primer, Jaccard's coefficient, Polymorphism, UPGMA analysis.

INTRODUCTION

The French bean (*Phaseolus vulgaris* L.) is one of the most important annual diploid leguminous vegetables in India. It is rich source of essential amino acids like lysine in contrast to animal protein. It is also rich in carbohydrates, dietary fibre minerals, antioxidants like polyphenols and vitamins (Broughton *et al*. 2003). Due to its high nutritional value, importance was given to enhance its yield and conservation by adopting better management practices and thorough understanding of genetic variation among different genotypes of French bean. Genetic diversity has the evolutionary significance for the survival and adaptation of species in different agro-climatic conditions. If there is not enough genetic diversity among genotypes, it is practically not possible to increase the yield and other desirable characters of the crop. A complete understanding of the genetic diversity and population structure of the French bean is essential for its conservation and management.

Hence, it is important to know the extent of existing genetic variations in the plant material. Many studies have been devoted to assess genetic diversity within and among cultivated and wild French bean genotypes based on phenotypic traits, isozymes (Belletti *et al*. 1996) and seed protein analysis (Lioi *et al*. 2005). Moreover, during the last few decades, the use of different molecular markers was used to study the genetic diversity of plants. Random Amplified Polymorphic DNA (RAPD) and Amplified Fragment Length Polymorphism (AFLP) have been used for genetic characterization in many crop species including common bean (Duran *et al*. 2005; Tiwari *et al*. 2005; Kumar *et al*. 2008; Hanai *et al*. 2007; Kumar *et al*. 2014).

Because of the limitation of morphological and biochemical markers, efforts were being directed to use Simple Sequence Repeats (SSRs) to study the genetic diversity of common beans (Hamman *et al*. 1995; Gopinath *et al*. 2013; Zargar *et al*. 2016; Jannat *et al*. 2022). SSRs were used to study the allelic variation within a locus. However, Inter Simple Sequence Repeat (ISSR) is an alternative marker system for RAPD and it is used to study the allelic variation among multi locus. But few studies were conducted to assess the genetic diversity of French bean by using ISSR markers (Galvan *et al*. 2003, Vyas *et al*. 2018, Sharma *et al*. 2020, Janghel *et al*. 2021). So, in the present investigation, an attempt was taken to study the genetic variation among French bean genotypes collected from different agro-climatic zones of India by ISSR markers.

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MATERIALS AND METHODS

Plant materials

The plant materials used in this investigation consisted of twenty-three french bean genotypes including released varieties collected from various parts of India. The list of the genotypes along with their source is presented in Table 1.

Experimental procedure

The experiment was conducted during the year of 2015-16 at department of Agriculture Biotechnology, College of

Agriculture, OUAT, Bhubaneswar. The data of two growing seasons over four locations were collected, pooled and statistically analyzed under randomized complete block design by SARS software. Planting was done at a spacing of 30 cm between the rows and 10 cm between plants. The experimental fields were well prepared and all the recommended package of practices were followed in raising the crop. The laboratory experiment was conducted in the Department of Agriculture Biotechnology, OUAT, Bhubaneswar, Odisha. Four to five young and fresh leaves of each genotype were collected from field and wrapped in aluminium foil and brought to the laboratory for DNA extraction.

Observations recorded

The observations were recorded in both the growing seasons on different morpho-physiological characters in the field as well as in the laboratory after the crop harvest. Five randomly selected plants were tagged and observations were recorded on these plants for different characters in each replication. The data recorded on five plants per treatment were averaged over locations over years.

DNA extraction

Total DNA of a single leaf was isolated according to the protocol established by previous investigators Doyle *et al*. (1997). The isolated total DNA was air-dried at room temperature and re-suspended in 200 µl of 1 X Tris EDTA buffer and stored at -0.20°C for further use.

Polymerase chain reaction (PCR) amplification using ISSR markers

A total four nos. of markers (USB-807 [17bp;AG(8)T], USB-810 [17bp;GA(8)T], USB-835 [18bp;AG (8)AC], USB-836 [18bp;AG(8)TC]) were employed for PCR amplification. The PCR reaction was carried out in a thermal cycler (Peglab, Germany) with 45 cycles (94°C-5 minutes, 94°C-1 minute, 50°C-1 minute, 72°C-2 minute, extension at 72°C for 2 minutes and stored at 4°C). The amplified products were separated by electrophoresis on 2% agarose gel with ethidium bromide and photographed under UV light using transillumination system (GelDoc. System, UNIVTECH, Cambridge).

Scoring and data analysis

Data collected during the two growing seasons on these traits were pooled and analysis of variance was done as suggested by Panse and Sukhatme (1985). Genetic diversity was studied using D^2 statistics of Mahalanobis (1928) and populations were grouped into clusters by following the method suggested by Rao (1952). Cluster analysis was done using the Sequential Agglomerative Hierarchical Nested (SAHN) cluster analysis procedure of NTSYS software version 2.1 which uses the unweighted pair group method with arithmetic averages (UPGMA) to obtain genetic relationships and to cluster varieties described by Sneath and Sokal (1973). The results were used to construct a final dendrogram showing 23 accessions.

Table 1: Genotypes along with source of collection.

Name of genotypes	Source
FAB Var-3	UAS, Dharwad
HAFB-5	ICAR RES.COMPLEX FOR EASTERN REGION, Ranchi
HAFB-6	ICAR RES.COMPLEX FOR EASTERN REGION, Ranchi
PDR-14	IIVR, Varanasi
FB-53	UAS, Dharwad
Arka Anoop	IIHR, Bangalore
Arka Suvidha	IIHR, Bangalore
VL Bean-2	VPKAS, Almora
Pant Anupam	GBPUAT, Pantnagar
VL Bean-1	VPKAS, Almora
Contender	VPKAS, Almora
Almora Local -1	VPKAS, Almora
VL Bean-3	VPKAS, Almora
HAPB-4	ICAR RES.COMPLEX FOR EASTERN REGION, Ranchi
Swarna Lata	ICAR RES.COMPLEX FOR EASTERN REGION, Ranchi
HAFB-4	ICAR RES.COMPLEX FOR EASTERN REGION, Ranchi
Swarna Priya	ICAR RES.COMPLEX FOR EASTERN REGION, Ranchi
HAFB-3	ICAR RES.COMPLEX FOR EASTERN REGION, Ranchi
Arka Sharat	IIHR, Bangalore
Kashi Param	IIVR, Varanasi
IIHR-4	IIHR, Bangalore
HUR-137	IIVR, Varanasi
Arka Komal	IIHR, Bangalore

Table 2: Mean values of 23 genotypes for 13 quantitative characters of French bean in four locations.

Genotype	Days to 50% flowering	Days to 1st green pod picking	Plant height (cm)	Number of primary branches per plant	Number of secondary branches per plant	Basal inter-nodal length (cm)	Basal inter-nodal diameter (cm)	Green pod length (cm)	Green pod width (cm)	Pod breadth (cm)	Number of pods per plant	Single per weight (g)	Pod yield pod plant (g)
FAB VAR-3	36.53	57.88	54.30	3.30	6.43	2.13	0.64	13.51	0.66	0.88	11.09	7.86	83.05
HAFB-5	31.14	52.17	65.41	4.38	9.30	2.59	0.74	16.89	0.91	1.15	25.27	7.10	158.61
HAFB-6	38.24	61.48	73.06	5.26	9.49	3.29	0.75	16.46	1.02	1.13	18.97	9.33	154.95
PDR-14	54.24	76.28	57.04	3.22	4.48	1.83	0.38	10.74	0.55	1.03	6.98	7.68	47.75
FB-53	39.33	59.35	66.72	4.33	6.47	1.88	0.68	14.74	0.93	1.02	19.67	6.40	108.85
Arka Anoop	37.17	65.13	67.27	4.25	7.08	2.63	0.81	13.41	0.73	1.03	17.75	8.27	128.48
Arka Suvidha	42.75	62.00	52.05	4.37	6.82	1.94	0.67	16.48	0.92	1.04	10.55	9.33	84.67
VL Bean-2	42.70	61.72	66.27	4.16	7.58	2.54	0.71	12.58	0.90	1.02	21.87	7.26	136.44
Pant Anupam	46.26	70.12	46.97	3.61	5.98	1.79	0.48	11.55	0.76	0.94	10.27	6.41	59.09
VL Bean-1	37.07	57.26	45.80	3.32	5.80	1.72	0.44	12.37	0.72	1.12	7.18	8.69	62.04
Contender	41.28	62.91	61.18	4.06	7.42	2.02	0.44	15.08	0.85	1.12	13.56	8.51	99.23
Almora Local -1	46.35	68.21	48.32	3.33	4.39	1.84	0.45	12.34	0.63	0.95	8.43	7.50	58.31
VL Bean-3	41.78	63.08	64.17	3.46	6.66	2.54	0.58	13.56	0.65	1.00	12.52	8.71	85.25
HAPB-4	52.10	69.62	51.88	3.90	6.12	2.07	0.52	14.39	0.75	0.96	8.26	7.92	58.31
Swarna Lata	55.59	79.29	121.76	3.79	6.73	6.66	0.66	16.88	0.81	1.11	13.90	8.83	108.83
HAFB-4	53.02	81.33	108.28	4.56	6.67	7.73	0.70	15.36	0.77	0.99	12.76	7.50	83.82
Swarna Priya	44.88	71.39	46.20	3.87	5.56	1.92	0.40	14..40	0.62	0.96	13.22	6.75	78.88
HAFB-3	44.28	68.17	42.03	3.34	4.83	1.31	0.34	10.17	0.57	1.05	12.42	6.46	72.77
Arka Sharat	52.03	67.56	46.67	3.96	5.48	1.58	0.44	15.15	0.65	0.95	14.80	5.73	75.07
Kasi Param	45.10	64.89	62.37	3.88	5.60	1.33	0.49	13.78	0.60	0.87	22.58	4.78	95.08
IIHR-4	44.07	66.70	35.94	2.95	4.67	1.22	0.75	10.43	0.51	0.87	10.38	4.67	44.26
HUR-137	35.89	59.00	61.47	3.82	6.83	1.49	0.63	14.89	0.82	1.01	17.68	7.66	120.74
Arka Komal	37.23	60.21	62.97	4.72	9.23	2.24	0.72	15.89	0.93	1.11	19.91	8.70	154.84
Grand mean	43.43	65.46	61.22	3.90	6.50	2.44	0.58	13.93	0.75	1.00	14.34	7.48	93.88
Range	31.14-55.59	52.17-81.33	35.94-121.76	2.95-5.26	4.39-9.49	1.22-7.73	0.34 - 0 .81	10.17-16.89	0.51-1.02	0.87-1.15	6.98-25.27	4.67-9.33	44.26-158.61
CD AT 5%	0.89	1.43	2.02	0.13	0.21	0.35	0.03	0.50	0.02	0.03	1.20	0.40	9.11
CV (%)	3.61	3.88	5.85	5.91	5.52	6.07	8.04	6.25	4.60	4.40	14.73	9.34	16.82

RESULTS AND DISCUSSION

Yield and yield attributes

The data obtained in the study were pooled and analyzed. The mean performance of 23 genotypes in respect of different quantitative characters is presented in Table 2. In respect to 50% flowering, the genotypes exhibited a wide range of variation from HAFB-5 (31.14) to Swarna Lata (55.59) with an overall mean of 43.43. Days to 1st green pod picking varied from FAB var-6 (52.17 days) to HAFB-4 (81.33 days) with an overall mean of 65.46 days. The magnitude of variability of plant height varied from IIHR-4 (35.94 cm) to Swarna Lata (121.76 cm) with an overall mean of 61.22 cm. The number of primary branches per plant was maximum in HAFB-6 (5.26) and minimum in IIHR-4 (2.95) with an average value of 3.90. Number of secondary branches per plant ranged from Almora Local-1(4.39) to HAFB-6 (9.49) with an average of 6.50. The variation in basal internodal length ranged between IIHR-4 (1.22 cm) to HAFB-4 (7.73 cm) with an average value of 2.44 cm.

Table 3: ANOVA for 13 quantitative characters of 23 genotypes of French bean.

Characters	Mean sum of square				
	Replications (16)	Genotype (27)	Environment (7)	GXE (189)	Error (433)
Days to 50% flowering	3.793**	938.829**	17.571**	6.483**	2.440
Days to 1st green pod picking	7.978**	1239.006**	38.643**	11.528**	6.294
Plant height	20.470**	8063.105**	1971.786**	23.740**	12.671
Number of primary branches per plant	0.121	8.617**	9.665**	0.354	0.053
Number of secondary branches per plant	0.262	50.772**	8.379**	0.287	0.131
Basal internodal length	0.227	51.361**	6.9068**	0.433	0.384
Basal internodal diameter	0.002	0.451	0.114	0.012	0.002
Green pod length	1.484	103.978**	29.477**	2.708**	0.760
Green pod width	0.001	0.443	0.030	0.007	0.001
Green Pod breadth	0.002	0.186	0.067	0.006	0.002
Number of pods per plant	5.326**	673.080**	51.670**	9.874**	4.425
Single pod weight	0.787	39.161**	28.879**	2.040**	0.498
Pod yield per plant	423.313**	35486.990**	14622.290**	1019.277**	256.593

Figures in parentheses indicate the degree of freedom (df).

*Significant at 5% level of probability, **Significant at 1% level of probability.

Table 4: Distribution of French bean genotypes in various clusters based on D^2 analysis.

Clusters	Number of genotypes in the cluster	Genotypes included in the cluster	Source
I	6	VL Bean-3, FAB Var-3, Pant Anupam, HAPB-4, Almora Local-1, VL Bean-1	UAS, Dharwad, G.B.P.U.A. and T, Pantnagar,HARP, Ranchi, VPKAS, Almora
II	7	HUR-137, FB-53, VL Bean-2, Contender, Arka Anoop, Arka Suvidha, Arka Komal	UAS, Dharwad, VPKAS, Almora, IIHR, Bangalore, IIVR Varanasi
III	4	Swarna Priya, Arka Sharat, HAFB-3, Kashi Param	HARP Ranchi, IIHR Bangalore, IIVR Varanasi
IV	2	HAFB-5, HAFB-6	HARP, Ranchi
V	2	HAFB-4, Swarna Lata	HARP, Ranchi
VI	1	PDR-14	IIVR, Varanasi
VII	1	IIHR-4	IIHR Bangalore

Table 5: Average intra-cluster (diagonal) and inter-cluster distance (D^2 values) among 7 clusters of 23 French bean genotypes.

Cluster	I(6)	II(7)	III(4)	IV(2)	V(2)	VI(1)	VII(1)
I	71.762	175.341	90.845	448.164	627.415	158.765	157.308
II		67.446	248.683	149.606	544.938	451.540	372.457
III			67.359	557.371	712.793	127.296	140.245
IV				70.704	624.710	862.562	751.796
V					52.259	623.664	1022.579
VI						0.000	233.201
VII							0.000

The variability in basal internodal diameter ranged from 0.34 cm (HAFB-3) to 0.81cm (Arka Anoop) with an average value of 0.58cm. The magnitude of green pod length ranged from HAFB-3 (10.17 cm) to HAFB-5 (16.89 cm) with an average of 13.93 cm. The extent of variability with respect to green pod width ranged from 0.51 cm (IIHR-4) to 1.02cm (HAFB-6) with average value of 0.75 cm. The magnitude of green pod breadth ranged from 0.87cm (Kashi Param) to 1.15 cm (HAFB-5) with average value of 1.00 cm. The mean value for number of pods per plant was 14.34 with a range of 6.98 (PDR-14) to 25.27 (HAFB-5). The genotype Arka Suvidha exhibited maximum single pod weight (9.33 g) with an average value of 7.56 g. The green pod yield per plant ranged between 44.26 g (IIHR-4) to 158.61 g (HAFB-5) with an average value of 93.82 g.

Analysis of variance

The analysis of variance for thirteen different quantitative characters was carried out and the results are presented in Table 3. It revealed that there was significant difference among genotypes for all the characters under investigation except basal internodal diameter, green pod width, green pod breadth. The environmental differences were significant for all the character except basal internodal diameter, green pod width, green pod breadth. However, the genotype × environment interactions were significant for the traits such as days to 50% flowering, days to 1st green pod picking, plant height, green pod length, number of pods per plant, single pod weight and green pod yield per plant.

Genetic diversity

Genetic diversity was studied using D^2 statistics of Mahalanobis (1928) and populations were grouped into clusters by following the method as suggested by Rao (1952). The genotypes were grouped into seven clusters. Among the clusters, maximum number of genotypes (7) were included in cluster II followed by cluster I (7), cluster III (4), cluster IV (2), cluster V (2). Cluster VI and VII were mono-genotypic cluster consisting of one genotype each indicating their independent identity and importance due to various unique characters possessed by them (Table 4). The formation of largest cluster II comprising 7 genotypes might be due to a free flow (or) exchange of breeding material from one place to another.

The present pattern of grouping of genotypes indicated that the genotypes have grouped or diverged into different clusters irrespective of their geographical origin which means that the genetic constitution of the varieties was more dominant than their geographical origin while forming a cluster (Choubey *et al*. 2003, Singh, 2006). This kind of genetic diversity was recorded among the genotypes belonging to the same geographic origin might be due to differences in adoption, selection pressure and selection criteria and environmental condition as suggested by

Table 6: Amplification of ISSR markers in twenty-three genotypes of French bean.

Primer	Total number of bands amplified	Number of polymorphic bands	Percentage of polymorphism	Percentage of polymorphic bands (PPB) relative to total
USB-810	7	4	57.14	14.81
USB-835	8	5	62.5	18.51
USB-836	5	3	60	11.11
USB-807	7	6	85	22.22

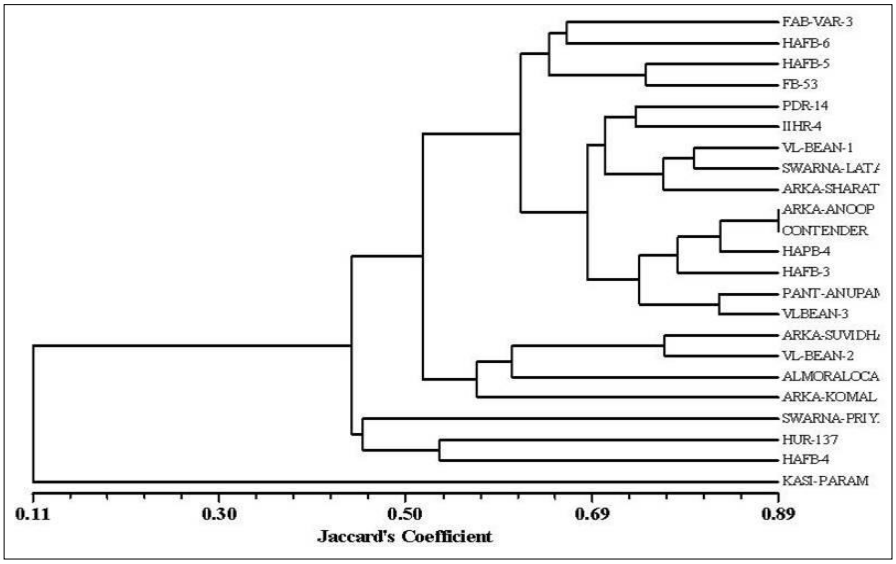

Fig 1: Dendogram illustrating genetic relationships among 23 French bean genotypes, generated by the UPGMA cluster analysis (NTSYS).

Table 7: Similarity matrix table among 23 genotypes of French bean.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
1	1.00																						
2	0.59	1.00																					
3	0.66	0.66	1.00																				
4	0.58	0.57	0.57	1.00																			
5	0.66	0.75	0.66	0.72	1.00																		
6	0.62	0.50	0.55	0.60	0.67	1.00																	
7	0.47	0.62	0.52	0.61	0.55	0.35	1.00																
8	0.52	0.68	0.58	0.66	0.60	0.44	0.76	1.00															
9	0.68	0.48	0.68	0.66	0.68	0.75	0.43	0.47	1.00														
10	0.62	0.54	0.70	0.76	0.69	0.70	0.50	0.63	0.78	1.00													
11	0.62	0.50	0.62	0.68	0.69	0.88	0.40	0.50	0.84	0.79	1.00												
12	0.52	0.57	0.50	0.57	0.52	0.50	0.52	0.68	0.48	0.61	0.50	1.00											
13	0.66	0.45	0.59	0.65	0.60	0.67	0.47	0.52	0.82	0.77	0.76	0.59	1.00										
14	0.61	0.60	0.68	0.60	0.75	0.81	0.43	0.54	0.76	0.78	0.84	0.60	0.68	1.00									
15	0.60	0.68	0.77	0.66	0.68	0.62	0.55	0.70	0.69	0.80	0.70	0.68	0.60	0.77	1.00								
16	0.40	0.52	0.52	0.45	0.47	0.35	0.46	0.53	0.37	0.42	0.34	0.44	0.34	0.43	0.47	1.00							
17	0.66	0.42	0.53	0.53	0.60	0.79	0.38	0.37	0.80	0.62	0.81	0.42	0.66	0.74	0.55	0.33	1.00						
18	0.60	0.59	0.59	0.72	0.73	0.67	0.47	0.60	0.75	0.77	0.76	0.52	0.66	0.68	0.76	0.40	0.60	1.00					
19	0.10	0.13	0.13	0.11	0.10	0.07	0.08	0.16	0.09	0.10	0.08	0.06	0.10	0.09	0.11	0.18	0.03	0.10	1.00				
20	0.55	0.54	0.54	0.73	0.75	0.75	0.50	0.47	0.76	0.70	0.76	0.42	0.68	0.69	0.62	0.32	0.67	0.75	0.04	1.00			
21	0.39	0.35	0.42	0.50	0.39	0.44	0.43	0.50	0.47	0.47	0.44	0.42	0.52	0.47	0.45	0.43	0.37	0.39	0.16	0.47	1.00		
22	0.37	0.64	0.47	0.55	0.50	0.37	0.60	0.56	0.40	0.52	0.42	0.55	0.50	0.45	0.57	0.41	0.35	0.50	0.07	0.52	0.38	1.00	
23	0.47	0.44	0.44	0.52	0.40	0.40	0.57	0.64	0.43	0.50	0.45	0.44	0.47	0.37	0.47	0.46	0.38	0.47	0.18	0.37	0.53	0.41	1.00

Gokulkrishnan *et al*. (2012). According to Nancee *et al*. (2013) choice of the parents for hybridization should be decided on the basis of genetic diversity rather than geographic diversity. Inter cluster distance values were greater than intracluster distance value suggesting heterogeneous and homogeneous nature of the strains between and within the clusters respectively (Table 5). The highest inter cluster distance value was observed between cluster V and VII, followed by IV and VI. Based on the above studies, it could be suggested that crosses involving genotypes from divergent clusters (V and VII, IV and VI, IV and VII, III and V) are likely to exhibit high heterotic expression for yield component and wider segregation in filial generations. Similar lines of observations were obtained by Patel *et al*. (2011).

Morphological diversity by hierarchical cluster

An un-weighted pair group method with arithmetic mean (UPGMA) analysis was made and a dendrogram was constructed using Jaccard's similarity matrix involving data generated from four ISSR primers on 23 genotypes of French bean (Fig 1). These genotypes were grouped into five clusters as observed in the dendrogram. One genotype, Kashi Param was completely isolated from all other clusters. If Kashi Param is excluded, Swarna Priya and IIHR4 were the most distantly related parental pair as evident by lowest similarity value: 0.32 (Table 6). The genotypic pairs- Swarna

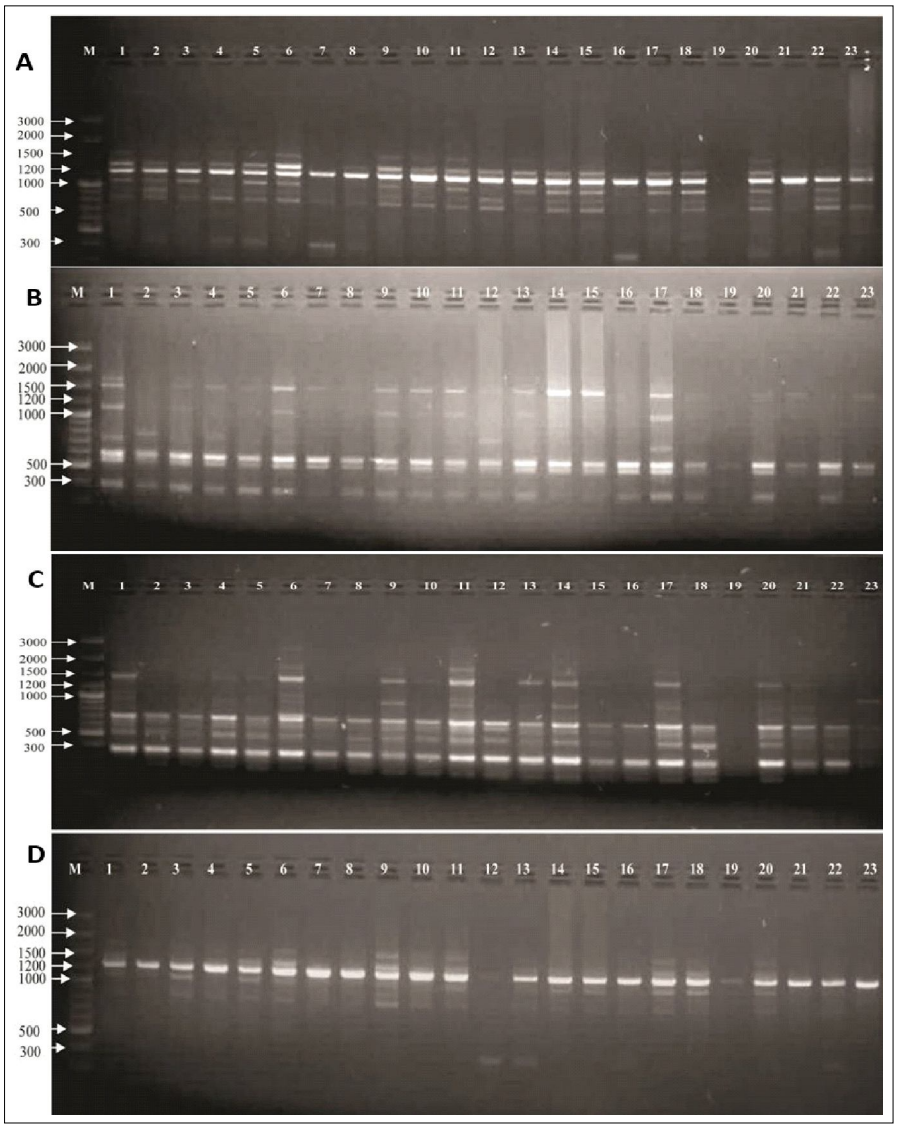

Fig 2: Amplification of French bean genotypes with USB-807 primer (A), USB-810 primer (B), USB-835 primer (C), USB-836 primer (D). From A-D, Lane 1: FAB VAR-3, 2: HAFB-5, 3: HAFB-6, 4-PDR-14, 5: FB-53, 6: ARKA ANOOP, 7: ARKA SUVIDHA, 8: VL BEAN-2, 9: PANT ANUPAM, 10: VL BEAN-1, 11: CONTENDER, 12: ALMORA LOCAL-1, 13: VL BEAN-3, 14: HAPB-4, 15: SWARNALATA, 16: SWARNAPRIYA, 17: HAFB-3, 18: ARKA SHARAT, 19: KASI PARAM, 20: IIHR-4, 21: HUR-137, 22: ARKA KOMAL, 23: HAFB-4. M-1000bp ladde.

Priya and VL Bean-3; Swarna Priya and HAFB-3; Swarna Priya and Contender; Swarna Priya and Arka Anoop; HUR 137 and HAFB-5; Arka Komal and FAB Var-3 also appeared to be fairly distant from all other genotypes. By excluding Kashi Param, at a similarity index value of 0.52, the remaining genotypes were divided into two cluster *viz*. I and II. The first cluster include 15 cultivars which was further divided into two sub-clusters *viz*. I-A and I-B. The first sub-cluster (I-A) contained four French bean cultivars (FAB Var-3, HAFB-6, HAFB-5 and FB-53). The second cluster (I-B) having eleven French bean cultivars was further grouped into two sub-clusters (I-B-1 and I-B-2) at a similarity value 0.65. Cluster I-B-1 contained five cultivars (PDR-14, IIHR-4, VI bean-1, Swarna Lata and Arka Sharat); whereas cluster I-B-2 contained six cultivars (Arka Anoop, Contender, HAPB-4, HAFB-3, Pant Anupam and VL Bean 3). In this sub cluster, cvs. Arka Anoop and Contender clustered at a similarity value 0.88 depicting very close ancestry relationships. In cluster II, cv. Arka Komal cluster at a similarity value of 0.55, appeared to be distant from the other three accessions (Almora Local 1, Arka Suvidha and VL Bean 2). The cluster III was made up of three phenotypically divergent genotypes viz. Swarna Priya, HUR137 and HAFB-4, although Swarna Priya separated itself from the rest of the two genotypes.

Polymorphism of genotypes based on ISSR markers

Divergences among genotypes were analyzed by ISSR markers. The average numbers of clear bands generated per polymorphic primer were 7, with a maximum of 8 for primer USB-835 (Table 7). The four di-nucleotide motif primers under the current study resulted in the amplification of a total of 27 bands and among them 18 (66.66%) were found to be polymorphic. The percentage of polymorphic bands (PPB) relative to the total number of amplified bands, ranged from 11.11% (USB-836) to 22.22% (USB-807). Out of four primers used, primers USB-835 and USB-810 provided the clearest banding pattern. Although most of the bands generated with primer USB-836 were monomorphic, some diversity between and within cultivars was observed (Fig 2).

Our study showed that a high degree of genetic diversity existed within French bean germplasm tested, (64.28% bands are polymorphic). In previous survey of Galvan *et al*. (2001) in French bean, 53% of the ISSR makers generated with tri-nucleotide motif primers were polymorphic. However, our results suggest that ISSR- PCR products generated with di-nucleotide motif primers can be useful markers for analyses of French bean germplasms. Similarity value based on Jaccard's coefficient clearly demonstrates that overall similarity between genotypes was low, reflecting strong genetic differentiation among the lines tested and our results were in accordance with previous investigators (Alghamdi *et al*. 2011; Immaculee *et al*. 2015, Jannat *et al*. 2022).

CONCLUSION

The genotypes used in the present studies showed a wide variation in morphological characters like plant height, pods per plant, pod weight, pod length and yield per plant. The genotype HAFB-5 had shown the maximum potential for pods yield per plant due to their high number of pods per plant and high pod length. ISSR analysis revealed high levels of genetic variation, even with the use of a limited set of primers. The present study also shows that ISSR markers can be a useful tool for the assessment of genetic diversity among French bean genotypes for the efficient utilization of germplasms in the breeding programme.

Conflict of interest: None.

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